Use of Stable Isotopes and Otolith Micro-Chemistry to Evaluate Migration in Male Chinook Salmon, *Oncorhynchus tshawytscha*, from an Alaskan River

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Use of Stable Isotopes and Otolith Micro-chemistry to Evaluate Migration in Male Chinook Salmon, *Oncorhynchus tshawytscha*, from an Alaskan River

Abstract

In salmonid fishes, males display much more variation in age and size at maturity than females, including a greater proportion of non-anadromous individuals, and those spending fewer years at sea than females. The life history of Chinook salmon is especially variable among Pacific salmon species, including non-anadromous (precocious parr) and early maturing anadromous males (jacks) but these have been studied primarily in populations towards the central and southern part of their range. In this study we investigated reports of small and putatively non-anadromous male Chinook salmon in Lake Creek, Alaska, using otolith microchemistry and stable isotopes. Small males (ca. 300–350 mm fork length) displayed otolith Sr:Ca ratios and \( \delta^{15}N \) values consistent with anadromy; indeed, the \( \delta^{15}N \) values of these “mini-jacks” that had spent a year at sea and larger jacks (ca. 500 mm) were more enriched than those of the larger, older conspecifics. Thus the multiple alternative anadromous male life history patterns reported in southern populations (and often associated with rapid pre-smolt growth in hatcheries) are present in more northerly wild populations of Chinook salmon as well. Moreover, variation in stable isotopes indicated differences in marine distribution related to age (with younger fish closer to the coast), and otolith microchemistry suggested that some of the young males may have moved to low salinity water during their period of marine residence.

Keywords: anadromy; life history; male reproductive patterns; migration

Introduction

Although salmonid fishes are famous for their anadromous migrations, the family is also characterized by considerable variation in anadromy within and among species and populations (Hendry et al. 2004; Quinn and Myers 2004; Rounsefell 1958). In addition, the absence of male parental care results in alternative male life history traits, including greater prevalence of non-anadromy and reduced duration of marine residence compared to the patterns shown by females (Fleming and Reynolds 2004). Males that mature as parr are known in a number of salmonid species, including but not limited to Atlantic salmon, *Salmo salar* (Myers et al. 1986), brown trout, *S. trutta* (Bohlin et al. 1994; L’Abée-Lund et al. 1990), and masu salmon, *Oncorhynchus masou* (Koseki and Maekawa 2000; Morita and Nagasawa 2010).

Chinook salmon, *O. tshawytscha*, achieve the largest maximum size of the Pacific salmon but they also vary greatly in life history (Roni
and Quinn 1995). Variations include sexually mature male parr (Gebhards 1960; Mullan et al. 1992; Rutter 1902) and anadromous males that spend one or two fewer years at sea than most females—referred to as jacks and mini-jacks, respectively (Johnson et al. 2012; Vøllestad et al. 2004). Such small adult salmon are likely to be under-sampled in the field (Zhou 2002), so their true prevalence may be difficult to determine. Under artificial conditions, some of the mature male parr can survive after spawning (Robertson 1957) and reproduce in successive years (Mayer 2002; Unwin et al. 1999).

Non-anadromous mature male Chinook salmon parr tend to occur in stream-type populations (Mullan et al. 1992; Taylor 1989) rather than ocean-type populations, as the latter migrate to sea in their first year of life and so would not typically be found in streams in the fall when spawning occurs. In contrast, Chinook salmon showing the stream-type life history (Healey 1991) emerge from gravel nests in spring and some are large enough to spawn that fall as parr in the upper Columbia River system, for example. Very little information is available on the incidence of non-anadromy and other alternative male Chinook salmon life history patterns in more northerly systems, where fry emerge in early summer, so close to the spawning period that it seems unlikely that they could breed that season. There was an anecdotal report of apparently residual (non-anadromous) Chinook salmon with an average fork length of 344 mm in Wishbone Lake, in the Togiak River system of Bristol Bay, Alaska but the author surmised that they “became locked in this lake during a recent high water event rather than a self-sustaining population of landlocked salmon” (Nelle 2002).

In addition to possible non-anadromy, the variation in duration of marine residence seen in male salmon allows them to forage in different ways, and in different areas. It has long been recognized that early-maturing salmon tend to be larger for their age than those remaining at sea (LaLanne 1971; Parker and Larkin 1959). They may have been larger as smolts or have grown faster while at sea, in which case their foraging may have differed from that of older but slower-growing members of the population. It was recently reported that stable isotopes of nitrogen varied among sockeye salmon, *O. nerka*, as a function of age; fish that matured at younger ages were more enriched in $^{15}$N compared to older fish (Johnson and Schindler 2013). The authors interpreted this result to indicate variation in foraging areas by salmon of different ages but the generality of this pattern is unclear, and the authors noted that differences in prey could also contribute to a linkage between age and $\delta^{15}$N. In Chinook salmon, recoveries of fish marked with coded wire tags indicated that younger fish were closer to their natal river than older fish (Weitkamp 2010), but analysis of the relationship between age at maturity and return timing led to the conclusion that Chinook salmon of different ages shared a common marine distribution (Bracis and Anderson 2013).

This study investigated reports and observations of small and unusually-colored male Chinook salmon in Lake Creek, flowing south from Lake Chelatna to the Yentna River, and thence into Cook Inlet near Anchorage, in south-central Alaska. Adult male Chinook salmon in this and other areas of Alaska are commonly very red in color but anglers and guides reported catching small males with olive-brown coloration. These small males (Figure 1) were observed feeding on insects and drifting eggs (Slater, personal observa-
as rainbow trout and other resident fishes commonly do (Denton et al. 2010; Scheuerell et al. 2007), leading to speculation that they might be non-anadromous. This would be a rare case of non-anadromy in such northerly populations and seemed worthy of investigation. Accordingly, we obtained samples from Chinook salmon over a range of body sizes and analyzed otolith microchemistry (Zimmerman et al. 2003) and muscle tissue stable isotope ratios (Johnson and Schindler 2009) to determine whether or not the small males had migrated to sea, and if so, whether they differed in $\delta^{15}\text{N}$ in a manner consistent with that reported in sockeye salmon.

**Methods**

From 15–24 June 2012, 40 Chinook salmon captured in Lake Creek by licensed recreational anglers were retained and measured for fork length. Samples of dorsal muscle were stored in 95% ethanol, and otoliths were removed, cleaned, and individually stored dry for later analysis. During this period there was no spawning by salmon in Lake Creek and fish would not have had the opportunity to eat drifting eggs, so stable isotope values were expected to reflect marine foraging or foraging in the stream on locally-produced prey if the fish were non-anadromous. The sample was augmented by an additional 10 salmon sampled as carcasses from 7–16 August. The sampling was not designed to fully represent the size and age distribution of the population but, rather, to encompass the range of sizes and emphasize the smaller, putative resident males. Twenty-two samples were selected for isotopic analysis, from 292–1170 mm fork length.

The otoliths from five putative resident individuals (mean: 346 mm, range: 317–385 mm) were embedded in epoxy blocks, and transverse sections containing the core were prepared with an Isomet lapidary saw and mounted on glass slides. Sections were ground until the core was exposed, and then rinsed in milli-Q ultrapure water. Otoliths were aged by counting the number of annular (hyaline) regions associated with slow winter growth, and then analyzed at the University of Washington Earth and Space Sciences electron microprobe facility with a four spectrometer JEOL Model 733 electron microprobe. Prepared sections were coated with a 250 Å layer of conducting carbon. Strontium to calcium ratios were evaluated at 15 equidistant points from the core (natal) to the edge (recent) along the ventral lobe. Data were collected using an accelerating voltage of 15kV, a beam current of 5 nA, and a beam diameter of 10 μm. Counting time for strontium was 120 s on peak, while calcium X-rays were collected until 0.4% relative statistical error was reached (70–75 sec). Background intensities were collected only on the first analysis and these count rates were retained for all subsequent analyses. This is permitted as the background count rate is dominated by CaCO$_3$, and is insensitive to variations in minor element concentrations. Raw X-ray intensities were corrected for matrix effects using the CITZAF routine (Armstrong 1995). Elemental ratios were calculated as mmol of strontium per mol of calcium, and compared graphically to a reference of 1.5 mmol Sr · mol Ca$^{-1}$ as a threshold for marine residence (Miller et al. 2010).

In some streams, Sr:Ca values are sufficiently high that otolith chemistry indicative of movement to marine waters may be detected in non-anadromous fish (Kraus and Secor 2004). To evaluate the possibility of anomalous marine migration signals in our sampled fish, we compiled stream Sr:Ca values for two stations in the Susitna River (Susitna Station and Sunshine), and one station in the Yentna River (Susitna Station), from the USGS National Water Quality Information System (nwis.waterdata.usgs.gov). Using the otolith-water Sr:Ca partition coefficient (0.305) from Phillis et al. (2011), we determined the expected otolith Sr:Ca values for fish living in the Susitna-Yentna system.

Fish tissues for isotope analysis were initially dried by evaporating the ethanol from each storage capsule. Once no liquid remained samples were freeze dried in a lyophilizer for 36 h, then ground to a fine powder and packed into tin capsules. Stable isotope analyses were conducted at the University of Washington Isolab with a Costech elemental analyzer (Analytical Technologies Inc., Valencia, CA) coupled to a Finnigan MAT-253.
stable isotope-ratio mass spectrometer (Thermal Electron Corporation, Bremen, Germany). The isotopic ratio of nitrogen ($\delta^{15}$N) was expressed in standard notation of per mil differences from the standard atmospheric nitrogen gas, and carbon ($\delta^{13}$C) as the per mil difference from Vienna Pee Dee Belemnite (Fry 2006). Although there is some concern about the effects of ethanol preservation on the resulting isotopic signatures (Hobson et al. 1997, Sarakinos et al. 2002), most work has found minimal effects when samples are fully dried in the original collection vessel. Additionally, samples from the putative anadromous and resident individuals were treated in the same manner. Values of $\delta^{13}$C were corrected for lipid concentration (Post et al. 2007) to remove the bias that depleted lipids introduce as erroneous variation in muscle $\delta^{13}$C.

Results and Discussion

Otolith microchemical analysis indicated that each of the five putative resident fish analyzed for Sr:Ca had values exceeding the conservative threshold for residence in marine waters (1.5 mmol · mol$^{-1}$, Figure 2). Low Sr:Ca values (< 1.5 mmol · mol$^{-1}$) were observed for the first 3 sample points on all otoliths, and the first 6 points (40% of otolith width) in three of five sampled otoliths. We observed low Sr:Ca values for a substantial portion of each otolith, indicating extended freshwater residence. Additionally, visual examination of these otoliths revealed two clear annuli for each fish, corresponding to the first winter in freshwater, followed by a large region corresponding to marine growth, and a second annulus near the otolith’s edge. Therefore, both chemical analysis and visual examination indicated a stream type life history and a single year at sea, consistent with previous data on the age composition of this population (Roni and Quinn 1995). However, all individuals exceeded our threshold for marine residence for a portion of otolith growth following initial freshwater values. In three individuals, Sr:Ca values were consistently high throughout the period, consistent with one full year in marine waters expected of a jack life history. However, the Sr:Ca values in the other two individuals declined below the marine threshold near the midpoint in the marine growth period, then increased before declining to freshwater levels before capture in the river. This bimodal pattern indicated possible use of nearshore or estuarine waters of reduced Sr:Ca, suggesting an unusual migration pattern by these fish.

The average observed values of $\delta^{15}$N and $\delta^{13}$C for all size groups ($\delta^{15}$N = 14.60, and $\delta^{13}$C = -19.79; Table 1) were similar to the averages reported for anadromous Alaskan Chinook salmon (Johnson and Schindler 2009). The smallest fish were more enriched and less variable in $^{15}$N than the larger fish but the $\delta^{13}$C values did not vary with fish size (Table 1; Figure 3). These results were consistent with anadromy and therefore inconsistent with the hypothesis that the smallest fish had resided exclusively in fresh water. Bilby et al. (2001) reported stable isotope values for juvenile salmon feeding in streams with high carcass density that were substantially less enriched than those
in our samples, so the isotopic signatures were almost certainly not derived from any combination of freshwater prey items. However, the two fish with otolith chemical signatures indicative of variable or more nearshore marine residence were also the most enriched in $\delta^{13}$C, consistent with a different, more coastal marine residence than the other small males. Indeed, $\delta^{15}$N was especially enriched among the smallest fish. This might be interpreted to indicate that they were feeding at higher trophic levels in the marine environment than the larger fish but this seems unlikely. Similar results were reported for sockeye salmon; jacks had the highest $\delta^{15}$N values, followed by ocean-age 2 fish, and the lowest values were seen in ocean-age 3 (Johnson and Schindler 2013). The relationship between age and $\delta^{15}$N was interpreted by Johnson and Schindler (2013) as evidence that the younger sockeye salmon had a more coastal distribution than the older fish, consistent with the generally lower $\delta^{15}$N values in zooplankton offshore (Schell et al. 1998). It is therefore likely that the $\delta^{15}$N in Chinook salmon of different ages reflects stable isotope signatures associated with different feeding areas rather than trophic levels. Combined with the otolith microchemistry from the two small fish that apparently resided in water of reduced salinity (as inferred from Sr:Ca) in the middle of their year at sea, these results suggest considerable variation in distribution patterns among these males.

In conclusion, we found no evidence of non-anadromy in the male Chinook salmon sampled but the results indicated interesting aspects of the migration patterns of early maturing males in this species. Jacks (males spending one full year at sea) are known in many populations (Roni and Quinn 1995). In ocean-type Chinook salmon, some males, known as mini-jacks, leave freshwater in spring and return at maturity that fall, having spent only a summer at sea. In some cases this constitutes a very short migration (Vøllestad et al. 2004) but in other cases the fish complete return migrations of $>1600$ km (Zimmerman et al. 2003) and $>2600$ km (Johnson et al. 2012). This life history would be impossible in northerly populations because adults are returning (e.g., caught in Lake Creek in June) at about the time juveniles are emerging from the gravel, and so only a full year of life prior to maturation would be possible. There may be non-anadromous male Chinook salmon in northern populations but in general non-anadromy in this species is associated with lakes. For example, self-sustaining popula-

### TABLE 1. Stable isotope values for male Chinook salmon categorized by size (N in parentheses, mean fork length reported) that were sampled at maturity in Lake Creek, Alaska.

<table>
<thead>
<tr>
<th>Size</th>
<th>Fork length (mm)</th>
<th>Average $\delta^{15}$N (SD)</th>
<th>Average $\delta^{13}$C (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very large (4)</td>
<td>1110</td>
<td>13.89 (0.77)</td>
<td>-19.94 (0.26)</td>
</tr>
<tr>
<td>Large (4)</td>
<td>710</td>
<td>13.74 (1.02)</td>
<td>-19.98 (0.38)</td>
</tr>
<tr>
<td>Medium (2)</td>
<td>555</td>
<td>15.35 (0.32)</td>
<td>-19.34 (0.20)</td>
</tr>
<tr>
<td>Small (12)</td>
<td>338</td>
<td>15.00 (0.31)</td>
<td>-19.74 (0.33)</td>
</tr>
<tr>
<td>Total (22)</td>
<td>566</td>
<td>14.60 (0.82)</td>
<td>-19.79 (0.35)</td>
</tr>
</tbody>
</table>

Figure 3. Distribution of A) $\delta^{13}$C, and B) $\delta^{15}$N values for adult Lake Creek Chinook salmon as a function of body length.
tions of non-native Chinook salmon are found in Lake Chelan and Lake Cushman, Washington (Wydoski and Whitney 2003), the Great Lakes (Crawford 2001), and some New Zealand lakes (McDowall 1990).

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