Differential growth in estuarine and freshwater habitats indicated by plasma IGF1 concentrations and otolith chemistry in Dolly Varden *Salvelinus malma*

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This study employed a combination of otolith microchemistry to indicate the recent habitat use, and plasma concentrations of the hormone insulin-like growth factor 1 (IGF1) as an index of recent growth rate, to demonstrate differences in growth and habitat use by Dolly Varden *Salvelinus malma* occupying both freshwater and estuarine habitats in south-west Alaska. Extensive sampling in all habitats revealed that fish had higher IGF1 levels in estuarine compared to lake habitats throughout the summer, and that the growth rates in different habitats within the estuary varied seasonally. In addition, otolith microchemistry indicated differentiation in estuarine habitat use among individual *S. malma* throughout summer months. Although growth in the estuary was higher than in fresh water in nearly all sites and months, the benefits and use of the estuarine habitats varied on finer spatial scales. Therefore, this study further illustrates the diverse life histories of *S. malma* and indicates an evaluation of the benefits of marine waters needs to include sub-estuary scale habitat use.

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Key words: anadromy; diadromy; endocrine; hormone; migration; salmonids.

INTRODUCTION

The use of both marine and freshwater habitats by migratory fishes (*i.e.* diadromy) is a strategy adopted by many different species to exploit the different rates of growth and probabilities of survival in each habitat (Northcote, 1978; Gross, 1987; McDowall, 1988). Anadromous species (those breeding in fresh water and migrating to sea to feed) are proportionally more common than catadromous species (breeding in the sea and feeding in fresh water) in high latitude catchments that are nutrient-poor compared to adjacent marine waters (Gross *et al*., 1988). In contrast, size-dependent mortality in marine waters (Sogard, 1997) often favours growth in freshwater environments prior to ocean entry (Rounsefell, 1958; Quinn & Myers, 2004). The advantages of inhabiting multiple habitats at various life stages are balanced by the energetic cost of flexible osmoregulatory ability and the potential arduousness of migration (Northcote, 1978). Although many salmonids are commonly described as anadromous, considerable variation in the scale of anadromy exists within and among species (Quinn & Myers, 2004).

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For example, all pink salmon *Oncorhynchus gorbuscha* (Walbaum, 1792) are anadromous, spending little time in freshwater habitats, maximizing growth potential at sea and maturing at a young age (Quinn, 2005). In contrast, sockeye salmon *Oncorhynchus nerka* (Walbaum 1792) often rear for 1 or 2 years in lakes, trading-off relatively poor growth for higher survival at sea at a somewhat larger size than the juvenile *O. gorbuscha*. In some freshwater habitats, fully resident *O. nerka* forms (i.e. kokanee) have developed, trading smaller body size at maturation for increased survival (Wood, 1995; Quinn, 2005). In comparison to semelparous salmonids, however, facultatively anadromous species such as brown trout *Salmo trutta* L. 1758, Arctic char *Salvelinus alpinus* (L. 1758), bull trout *Salvelinus confluentus* (Suckley 1859), Dolly Varden *Salvelinus malma* (Walbaum 1792), cutthroat trout *Oncorhynchus clarkii* (Richardson 1836), and rainbow trout or steelhead *Oncorhynchus mykiss* (Walbaum 1792) add additional complexity by varying widely in their age at migration, length of time spent at sea and habitats used (Armstrong, 1974; Armstrong & Morrow, 1980; McDowall, 1988; Thorpe, 1994; Klemetsen *et al.*, 2003). Consequently, individuals from a given cohort may be spread among habitats, experiencing markedly different environmental conditions affecting growth, survival and ultimately fitness. Despite the diversity in movement patterns for facultatively anadromous individuals, few studies have attempted to evaluate the growth trade-offs involved in movement to alternative habitats, particularly for populations that may simultaneously inhabit marine and freshwater environments.

Although growth rates of fishes are often used as a proxy for habitat quality, in semelparous anadromous species, direct comparisons between marine and freshwater growth are difficult because movement often results from an ontogenetic shift and individuals in each habitat may be of different sizes or life stages. In facultatively anadromous species, however, individuals of a similar size and age may simultaneously occupy marine, estuarine and freshwater habitats, facilitating comparisons of growth among habitats. Many studies have shown rapid growth of salmonids in estuarine environments (Reimers, 1973; Healey, 1979; Tschaplinski, 1987). Few studies have compared growth in estuaries to growth of conspecifics at an equivalent life stage in freshwater environments; a comparison that is necessary to understand the relative benefit of migratory and resident behavioural strategies (Kjelson *et al.*, 1982; Cunjak, 1992; Hayes *et al.*, 2008). In addition, large differences in habitat type, size and fish movement and density among estuarine, marine and freshwater environments may present logistical challenges to making comparable measurements. As a consequence, growth comparisons are usually attempted with small sections of habitat or with caging studies, leading to difficulties in scaling results to larger portions of the habitat and population (Macdonald *et al.*, 1988; Miller & Simenstad, 1997).

There are myriad techniques to evaluate growth, from individual mark-and-recapture, to changes in the population’s size modes through time. Each of these techniques is challenging to apply to wild fishes, where a large population size and habitat volume may be coupled with extensive movements that reduce the effectiveness or confidence in methods that require multiple sampling events. Low statistical power can limit the ability to compare growth rates between habitats, even when the mean change in size indicates growth (Cunjak, 1992). To make habitat-growth comparisons more effective, a single-capture growth measure is required. Few techniques have proven effective in indicating growth in wild fishes in a single capture event, but laboratory research in multiple species indicates that plasma
insulin-like growth factor 1 (IGF1) concentration can be a quantitative metric for recent somatic growth (Beckman, 2011). IGF1 is a hormone produced by the liver in response to changes in growth hormone, consumption rate and diet quality (Beckman, 2011). IGF1 directly stimulates cell division and growth; therefore, IGF1 concentration may directly reflect growth and is not merely a growth correlate. Several studies in salmonids, including *S. alpinus* (Cameron et al., 2007), have indicated strong correlations between plasma IGF1 concentration and changes in fish length (Beckman et al., 2004a, b).

In free-ranging fishes, knowledge of the movement history of an individual is important to place the observed growth rates in a spatial context. Identifying the movement history of individuals using traditional methods (e.g. tagging and serial sampling), however, requires repeated sampling of all possible habitats, a challenging task in large-scale natural environments. Otolith microchemistry has been used to determine habitat use within and among habitats in a variety of species (Brown, 2006; Miller, 2007; Bradbury et al., 2008; Macdonald & Crook, 2010). Otoliths are calcium carbonate components of the teleost inner ear, used for balance and orientation (Campana, 1999). During otolith construction, fishes regularly accrete calcium carbonate in a protein matrix, enlarging otoliths with age (Campana, 1999). Other elements may replace calcium in proportion to their concentration in the saccule fluid, reflecting the concentration of those elements in the aqueous environment (Elsdon et al., 2008). Several elements, in particular Sr and Ba, vary widely in concentration between marine and freshwater environments (Kraus & Secor, 2004). The movements of diadromous fishes are therefore detectable by analysing the concentration of elements at discrete regions in the otolith that correspond to life events (e.g. age and migration) (Elsdon & Gillanders, 2003; Zimmerman, 2005).

*Salvelinus malma* are facultatively anadromous species, ideally suited to comparisons of growth among habitats. In many populations, *S. malma* make their first marine migration at age 3 or 4 years, although where appropriate habitat exists, individuals of a range of size and age classes may be found in both marine and fresh waters simultaneously (Armstrong & Morrow, 1980; Morrow, 1980). In addition, *S. malma* probably make extensive use of estuarine waters and remain in near-shore coastal waters, as they generally occupy marine environments in the summer and return to fresh water in late summer or autumn for reproduction and overwintering (Armstrong, 1974; Bernard et al., 1995; Bond & Quinn, 2013). An analysis of plasma IGF1 concentrations from free-ranging *S. malma* captured in a range of freshwater and estuarine environments was paired with chemical analysis of regions of the otolith formed in the weeks preceding capture. Together, these analyses allowed for comparisons of growth and habitat use in both freshwater and estuarine habitats throughout the summer months. In addition, these analyses were used to infer the extent of movement among habitats within the estuary, as well as movement between the estuary and lake habitat. It was hypothesized that growth in estuarine environments would be higher than that in lake habitats. Because *S. malma* are abundant throughout the sampling region (Narver & Dahlberg, 1965), individuals may make regular movements throughout the estuary as the tidal cycle allows, weakening any site-specific signals of growth unless the fish’s recent movement history was considered. Therefore, IGF1 may indicate either average growth of the estuarine environment or site-specific growth depending upon the scale and timing of *S. malma* movement.
**FIELD COLLECTIONS**

The Chignik Lakes catchment in south-western Alaska drains a 1536 km² basin and includes 25 km² Chignik Lake and a 33 km² semi-enclosed lagoon (Fig. 1; Narver & Dahlberg, 1965; Simmons *et al.*, 2013). Chignik Lagoon loses c. 50% of its available surface area during extreme tidal exchanges (c. 4 m) (Narver & Dahlberg, 1965; Simmons *et al.*, 2013) and varies in salinity (Fig. 1) by tidal height, river flow and distance from the sand spit that separates the lagoon from the more marine Chignik Bay, ranging from 0 to 34 (Simmons *et al.*, 2013).

*Salvelinus malma* were collected with a 35 m beach seine (3 mm mesh, 4 m bag tapering to 1 m wings) used to encircle a standard portion of the near-shore habitat (c. 190 m²) at six sites in Chignik Lake and five sites in Chignik Lagoon (Fig. 1) every 10–14 days from June to August in 2009 and 2010. Lagoon sites are named according to long-term sampling convention: Peahi: Chignik River and estuary ecotone, Alpha and Pillar Rock: Inner lagoon, Hume Point: Mid lagoon, Spit: Outer lagoon. At each site, all *S. malma* were counted, and a sub-set was measured for fork length (*L*ₕ) and mass (*M*). For IGF1 analysis, target sample sizes were 10 randomly selected individuals; however, to ensure enough plasma for hormone analysis, individuals >120 mm *L*ₕ were sampled from each site at each interval. Each fish was sacrificed in MS-222 anaesthetic, and following measurement of *L*ₕ and *M*, immediately bled from the caudal vein with a heparinized syringe. Whole blood was stored in individually labelled microcentrifuge tubes on ice. Within 6 h of collection, blood tubes were centrifuged at 3000 g for 5 min. Plasma was removed and stored at −20°C until the end of each field season and −80°C thereafter. Each
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Fish was examined for gonadal development and visually categorized as immature or maturing at the time of capture by the developmental stage of the gonads; mature males and females had enlarged gonads compared to immature individuals. In addition, sagittal otoliths were removed from each sampled individual, rinsed in de-ionized water and stored dry in microcentrifuge tubes.

BLOOD PLASMA LABORATORY ANALYSES

Blood plasma samples were analysed at the National Marine Fisheries Service, Northwest Fisheries Science Center, using an immunoassay to measure the concentration of IGF1 [as described by Shimizu et al. (2000)]. Briefly, IGF1 was isolated from plasma with an acid-ethanol extraction, and measured by time-resolved fluoroimmunoassay immunoassay using a modification of methods described by Small & Peterson (2005). Each sample was analysed in duplicate, and samples with low (<30%) or high (>85%) average binding, as well as those with a coefficient of variation (c.v.) exceeding 10% were re-analysed or excluded. The interpretation of IGF1 concentration depends upon the maturational state of each individual as gonadal steroids may either stimulate or inhibit IGF1 independent of growth rate (Beckman, 2011). IGF1 is, therefore, most reliable as a growth index for juvenile fishes. Two methods were used to discriminate whether fish had initiated maturation. Gonad mass was measured for each individual; however, early in the summer gonad mass did not provide distinct discriminatory power as clear gonad development may not occur until closer to the autumn spawning period (Blackett, 1968). To further identify individuals preparing to mature in the year of capture, plasma concentrations of 11-keto testosterone (11-KT, male) and 17B-oestradiol (E2, female) steroids were measured in all individuals sampled in 2009. Plasma 11-KT concentrations were measured by an enzyme-linked immunosorbent assay following Cuisset et al. (1994). Plasma E2 was analysed with a double ether extraction followed by radioimmunoassay using the methods of Sower & Schreck (1982). The size and maturation relationships established in the 2009 collection were used to determine which fish to include in IGF1 analyses from the 2009 and 2010 collections.

OTOLITH LABORATORY ANALYSES

Otoliths were cleaned by triple rinsing in nanopure water and sonicating in an ultrasonic bath for 10 min during the final rinse to remove any residual biological material. To create sagittal sections, otoliths were glued sulcus side up, to standard biological slides with crystalbond 409 thermoplastic resin. The sulcus side was polished with successively finer polishing film until the otolith core could be resolved. Each slide was then re-heated to soften the resin and the otolith was inverted. The remaining side was ground until the core was exposed and polished with 0.3 μm polishing film. Polished otoliths were triple rinsed in nanopure water, sonicated for 15 min and dried in a class 100 clean room. Edge chemistry for each otolith was analysed with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) at the Keck Collaboratory, Oregon State University. A VG PQ ExCell ICPMS (www.thermoscientific.com) with a New Wave DUV193 excimer laser (www.esi.com) was used for all analyses of 2009 otoliths and a Thermo X-Series II ICPMS (www.thermoscientific.com) and Photon Machines Analyte G2 193 nm laser (www.cetac.com) were used to analyse 2010 otoliths. The laser was set at a pulse rate of 7 Hz with a 20 μm ablation spot and travelled at 5 μm s⁻¹ to measure Sr, Ba, Mn, Mg, Cu, Ca, Pb and Ca. NIST-612 glass was used to transform ion ratios to elemental ratios. These were converted to molar ratios using the molar mass of each element. Final analyses were computed on the element:Ca in mmol mol⁻¹. A calcium carbonate standard with known concentration (USGS MACS-2) was used to determine the accuracy of the element:Ca and correct final element: Ca values.

DATA ANALYSES

The relationship between sex steroid concentration and Lₙ for each sex was evaluated graphically by comparing Lₙ and either 11-KT (male) or E2 (female) concentrations to identify a size
threshold for maturation in each sex that was used for both 2009 and 2010 samples. All fish above the size thresholds for maturation were removed from further analyses. In addition, some precocious males had elevated gonado-somatic index values ($I_G$) at very small sizes, indicating maturation below the size threshold derived in 2009. Therefore, all males with an $I_G > 3\%$ were also removed from further analyses. The resulting dataset was first tested for year, month and sex effects on IGF1 concentration and $L_F$, and then analysis of variance (ANOVA) as well as Tukey HSD post hoc tests were performed to determine whether there were significant differences among freshwater and lagoon sites.

Edge otolith chemistry evaluates the portion of the otolith formed over some period of time prior to capture. Because the width of annual bands generally decreases with age as the per cent change in fish size decreases each year, the amount of time incorporated into a given distance of otolith transect varies among individuals. Measurements on a finer scale than the width of the ablation trough (20 μm), however, are too small to overcome the coarse averaging of the laser. To balance the time of otolith formation with the analytical constraints of LA-ICP-MS, the otolith width from the last annulus to the edge was evaluated with $L_F$, age and capture day of the year ($D$) as predictors in a series of linear models. To determine residency at each collection site, discriminant function analysis (DFA) was performed to classify individuals from each site with the average edge ratios of Sr:Ca, Ba:Ca, Mn:Ca, Mg:Ca, Cu:Ca and Pb:Ca at four different edge distances (10, 20, 30 and 40 μm). This technique allowed for the use of all elements simultaneously to test whether fish collected at a site would assign back to the capture location. Strong assignment to the capture location would indicate extended residence at a site with similar chemistry, whereas poor assignment would indicate little difference in chemistry among sites, or high rates of movement throughout the estuary. Because otoliths were analysed over several years, and element:Ca vary greatly among elements, data for each year and element were rescaled by subtracting the mean and dividing by the s.d.

Permutational multivariate analysis of variance (perMANOVA) was performed to determine the effects of year, site and sampling month on the resulting groupings. For multivariate analyses, individuals from Peahi were not used due to low sample size. Individuals captured in the Chignik River may have been recent migrants from other habitats; therefore, recent habitat use for those individuals was assigned with a DFA created from otoliths collected in both marine and fresh waters. Additionally, all fish captured at Chignik Lake sites were grouped into a single freshwater category for otolith analyses. IGF1 and otolith chemistry data were normally distributed and did not violate the assumptions of parametric tests, which were performed with a critical $\alpha$ of 0.05 using the R statistical software (R Development Core Team; www.r-project.org).

RESULTS

Across all samples, there was no difference in IGF1 concentration between 2009 (mean ± s.d. = 27.22 ± 17.43, n = 213) and 2010 (mean ± s.d. = 28.50 ± 19.39, n = 428; $t_{465} = -0.851, P > 0.05$) so the data from both years were combined for all further analyses. A visual estimation of gonadal development was used as an indicator of maturation in the season of capture for both males and females. For each visually estimated individual, blood plasma 11-KT and E2 concentrations were evaluated for males and females, respectively. In agreement with previous research (Narver & Dahlberg, 1965), in males $\geq 275$ mm $L_F$ and females $\geq 300$ mm $L_F$ marked evidence of maturation was observed in some but not in all individuals as early as June. Males as small as 130 mm $L_F$ displayed increased gonad mass consistent with maturation, but females did not (Fig. 2). No significant site effect on IGF1 concentration within Chignik Lake ($F_{6.133} = 1.39, P > 0.05$) was observed, and in subsequent analyses all six Chignik Lake sites were pooled. There were significant effects of site ($F_{5.378} = 15.81, P < 0.001$), month ($F_{2.378} = 4.36, P < 0.01$) and a site × month interaction ($F_{7.378} = 5.07, P < 0.001$) on the $L_F$ of individuals included in final IGF1
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analyses. A series of Tukey HSD post hoc tests was performed to determine which sites differed in mean \( L_F \) each month [Fig. 3(a)].

There was no significant difference in IGF1 concentration between sexes in the immature individuals (\( t_{394.3} = 0.23, P > 0.05 \)) but there were significant effects of site (\( F_{16,378} = 11.43, P < 0.001 \)), month (\( F_{2,378} = 4.66, 0.05 > P > 0.01 \)) and a site \( \times \) month interaction (\( F_{10,378} = 4.12, P < 0.001 \)) on IGF1 concentration. That is, there were significant differences in growth rates among sites, but those differences varied by month. Post hoc comparisons among all sites using the Tukey HSD test for differences in means among sites within each month indicated significant differences among sites in each month [Fig. 3(b)]. In June, only fish at mid and outer lagoon sites had growth rates significantly higher than Chignik Lake. By July, however, inner, mid and outer lagoon fish all showed higher growth than fish from Chignik Lake. Additionally, during the same time period, the mid lagoon had a higher concentration than either the inner or outer lagoon sites. Finally, by August fish from the Chignik River site as well as from all lagoon sites were significantly higher in IGF1 concentration than Chignik Lake fish, although there was no difference among the lagoon or river sites.

In a series of linear models, the additive effects of fish age (\( A \)) and \( D \) best predicted ln-transformed edge otolith band width (\( \mu m \)) (Table I) with the equation:

\[
O_w = e^{(1.83 - 0.152A + 0.0157D)},
\]

where \( O_w \) is the otolith width and age is the number of post-emergence winters alive. Using this relationship, some older fish sampled in June may have insufficient otolith growth at the time of capture to accurately reflect summer habitat residence; therefore, only July and August samples were used in DFAs. Assignment of individuals to their collection sites with DFA of margin otolith chemistry was driven almost entirely by differences in Sr:Ca, and the effect of other
Fig. 3. (a) Mean ± s.e. fork length ($L_F$) of *Salvelinus malma* included in the insulin-like growth factor 1 (IGF1) analysis at each sampling location by month (June, ☐; July, ☐; August, ☐), arranged from fresh water to outermost in the estuary. Lowercase letters (June), uppercase letters (July) and numbers (August) indicate significant differences among sites within each month (Tukey HSD pair-wise comparisons, *P* < 0.05). (b) Mean ± s.e. plasma IGF1 concentration of fish sampled at each site. Lowercase letters (June), uppercase letters (July) and numbers (August) indicate significant differences among sites within each month (Tukey HSD pair-wise comparisons, *P* < 0.05). NA, no fish were captured.

Elements was minimal (Table II). There was a high degree of variability in correct assignment (26–96%; Table III) among sites, but overall assignment was highest with 30 μm averaging width (correct assignment: 10 μm, 77.55%; 20 μm, 77.55%; 30 μm, 78.57%; 40 μm, 77.55%), and 30 μm averaging width was employed for all assignments. Comparison of plasma IGF1 concentrations for individuals correctly assigned to their capture location and those misclassified (Table IV) indicated that IGF1 was more similar among fish from a given capture location than from assigned locations. Percentage correct assignment to freshwater, inner lagoon, mid lagoon and outer lagoon sites was 96, 29, 73 and 54%, respectively. PerMANOVA indicated significant differences in group placement in multivariate space in all but one pair-wise comparison (inner lagoon and outer lagoon), while homogeneity of dispersion tests indicated no significant differences in dispersion in all cases but one (inner lagoon and outer lagoon; Table V). Testing for the significance of a month effect was not possible due to insufficient sample size at each sampling station in each month.

Assignment of *S. malma* collected in the Chignik River to freshwater, inner lagoon, mid lagoon and outer lagoon sites indicated that in June most individuals were of recent freshwater origin, but that proportion decreased in each month thereafter (Table VI). By August, the
Table I. List of models evaluated for success in predicting the ln-transformed width of the otolith from the last annulus to the edge of the otolith in Chignik Lakes Salvelinus malma, including the number of parameters in each model (K), the change in AICc ordered from lowest to greatest AICc (ΔAICc), the Akaike weights for each model (AICcWt) and the log-likelihood (LL) of each model. Smaller ΔAICc scores indicate a better fit of the data to the model. Tested parameters include the number of annuli (age, A), day of the year (D) in which the fish was captured and the fork length (L_F) of each individual.

<table>
<thead>
<tr>
<th>Model</th>
<th>K</th>
<th>ΔAICc</th>
<th>AICcWt</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A + D</td>
<td>4</td>
<td>0.000</td>
<td>0.506</td>
<td>-51.515</td>
</tr>
<tr>
<td>A D</td>
<td>5</td>
<td>1.836</td>
<td>0.202</td>
<td>-51.288</td>
</tr>
<tr>
<td>L_F + D</td>
<td>4</td>
<td>2.833</td>
<td>0.123</td>
<td>-52.931</td>
</tr>
<tr>
<td>A L_F + D</td>
<td>6</td>
<td>3.528</td>
<td>0.087</td>
<td>-50.957</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>3.626</td>
<td>0.083</td>
<td>-54.441</td>
</tr>
<tr>
<td>A L_F</td>
<td>5</td>
<td>26.450</td>
<td>0.000</td>
<td>-63.595</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>28.861</td>
<td>0.000</td>
<td>-67.059</td>
</tr>
</tbody>
</table>

vast majority Chignik River fish were unassigned to sampled habitats, probably indicating either recent residence in coastal marine waters that were not directly sampled or an otolith chemical signal created from movement through several distinct habitats.

**DISCUSSION**

Plasma IGF1 concentrations allowed for the comparison of S. malma growth within and among freshwater and marine habitats simultaneously, and revealed comparatively low growth of S. malma in Chignik Lake in all months, with no differences among collection sites. Growth rates in estuarine habitats, however, varied widely by site and month, and otolith chemistry indicated significant seasonal differences in habitat use within estuarine waters. The apparent spatial segregation of individuals separated by only a few km of open estuary was unexpected, given both the long-range migration potential of S. malma (DeCicco, 1992) and the necessary daily movement that accompanies tidal fluctuations in the estuary. In addition, some S. malma remained in

Table II. Coefficients of linear discriminants (LD) 1–3 for all element-to-Ca ratios included in discriminant function analysis (DFA) of Salvelinus malma otoliths. Variance explained is the proportion of variance explained by each discriminant function.

<table>
<thead>
<tr>
<th>Element</th>
<th>LD1</th>
<th>LD2</th>
<th>LD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr:Ca</td>
<td>1.673</td>
<td>0.105</td>
<td>-0.189</td>
</tr>
<tr>
<td>Ba:Ca</td>
<td>0.024</td>
<td>0.115</td>
<td>-0.648</td>
</tr>
<tr>
<td>Mg:Ca</td>
<td>0.097</td>
<td>-0.223</td>
<td>-0.237</td>
</tr>
<tr>
<td>Mn:Ca</td>
<td>0.087</td>
<td>0.029</td>
<td>-0.526</td>
</tr>
<tr>
<td>Cu:Ca</td>
<td>-0.120</td>
<td>-0.229</td>
<td>0.026</td>
</tr>
<tr>
<td>Pb:Ca</td>
<td>-0.032</td>
<td>1.512</td>
<td>0.284</td>
</tr>
<tr>
<td>Variance explained</td>
<td>0.966</td>
<td>0.026</td>
<td>0.008</td>
</tr>
</tbody>
</table>
mid lagoon habitats throughout the summer growing season, and achieved similar or higher growth rates compared to individuals in more marine-influenced outer lagoon site, in contrast to the patterns observed for estuary-rearing _O. clarkii_ (Krentz, 2007). Fish captured at the outer lagoon may have been using the lagoon as a migratory corridor between freshwater and marine habitats. DFA often assigned fish from the outer lagoon to freshwater sites rather than mid lagoon habitats, indicating recent arrival at the outer lagoon from Chignik Lake. This suggests the possibility of at least two distinctly different behaviours; some individuals move to mid lagoon habitats for the duration of the summer growing season, while others move rapidly to outer lagoon or coastal waters, a strategy that has been suggested in anadromous _S. trutta_ (Jonsson & Jonsson, 2011), as well as in some coho salmon _Oncorhynchus kisutch_ (Walbaum 1792) (Rohde _et al._, 2013) and Chinook salmon _Oncorhynchus tsawytscha_ (Walbaum 1792) (Chamberlin _et al._, 2011) populations. In _S. trutta_ (Jonsson & Jonsson, 2011), however, the migratory strategy adopted may be the result of an ontogenetic shift, possibly from increased seawater tolerance and marine reliance with increasing size. In the Chignik Lagoon, however, there was little difference in _L_f of fish from mid and outer lagoon sites. This may result from rapidly growing individuals achieving a threshold size at which individuals leave the lagoon to return to fresh water for

**Table III.** Percentages of individuals assigned to each site by discriminant function analysis (DFA) with Sr:Ca, Ba:Ca, Mn:Ca, Mg:Ca, Cu:Ca and Pb:Ca in the outer 30 μm of each otolith from _Salvelinus malma_ collected in July and August of 2009 and 2010. Site names along the vertical axis indicate capture location; sites on the horizontal axis indicate assigned location with DFA. Bold numbers indicate the percentage of individuals correctly assigned to their collection site

<table>
<thead>
<tr>
<th>Capture location</th>
<th>Fresh water</th>
<th>Inner lagoon</th>
<th>Mid lagoon</th>
<th>Outer lagoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water</td>
<td>96</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Inner lagoon</td>
<td>29</td>
<td>29</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>Mid lagoon</td>
<td>0</td>
<td>5</td>
<td>73</td>
<td>23</td>
</tr>
<tr>
<td>Outer lagoon</td>
<td>27</td>
<td>4</td>
<td>15</td>
<td>54</td>
</tr>
</tbody>
</table>

mid lagoon habitats throughout the summer growing season, and achieved similar or higher growth rates compared to individuals in more marine-influenced outer lagoon site, in contrast to the patterns observed for estuary-rearing _O. clarkii_ (Krentz, 2007). Fish captured at the outer lagoon may have been using the lagoon as a migratory corridor between freshwater and marine habitats. DFA often assigned fish from the outer lagoon to freshwater sites rather than mid lagoon habitats, indicating recent arrival at the outer lagoon from Chignik Lake. This suggests the possibility of at least two distinctly different behaviours; some individuals move to mid lagoon habitats for the duration of the summer growing season, while others move rapidly to outer lagoon or coastal waters, a strategy that has been suggested in anadromous _S. trutta_ (Jonsson & Jonsson, 2011), as well as in some coho salmon _Oncorhynchus kisutch_ (Walbaum 1792) (Rohde _et al._, 2013) and Chinook salmon _Oncorhynchus tsawytscha_ (Walbaum 1792) (Chamberlin _et al._, 2011) populations. In _S. trutta_ (Jonsson & Jonsson, 2011), however, the migratory strategy adopted may be the result of an ontogenetic shift, possibly from increased seawater tolerance and marine reliance with increasing size. In the Chignik Lagoon, however, there was little difference in _L_f of fish from mid and outer lagoon sites. This may result from rapidly growing individuals achieving a threshold size at which individuals leave the lagoon to return to fresh water for

**Table IV.** Plasma insulin-like growth factor 1 (IGF1) concentrations (ng ml⁻¹) of _Salvelinus malma_ by capture location (vertical) and otolith chemistry discriminant function analysis (DFA) assignment location (horizontal). IGF1 values for fish assigned to the capture location are shown in bold

<table>
<thead>
<tr>
<th>Capture location</th>
<th>Fresh water</th>
<th>Inner lagoon</th>
<th>Mid lagoon</th>
<th>Outer lagoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water</td>
<td>25.8</td>
<td>21.0</td>
<td>18.0</td>
<td>21.9</td>
</tr>
<tr>
<td>Inner lagoon</td>
<td>33.0</td>
<td>25.3</td>
<td>15.0</td>
<td>20.5</td>
</tr>
<tr>
<td>Mid lagoon</td>
<td>N/A</td>
<td>38.9</td>
<td>44.0</td>
<td>41.6</td>
</tr>
<tr>
<td>Outer lagoon</td>
<td>21.3</td>
<td>57.7</td>
<td>28.0</td>
<td>31.4</td>
</tr>
</tbody>
</table>

N/A, no fish assigned.

feeding, reproduction and overwintering. In this scenario, the modal size in each habitat may not change despite different growth rates. Although no length measurement of migratory individuals has been made, *S. malma* ascend Chignik River throughout the summer (Bond & Quinn, 2013). Small scale (≤10 km²) estuarine site fidelity has been reported for other *Salvelinus* species, including *S. confluentus* (Hayes et al., 2011), as well as for *O. clarkii* (Johnston, 1982; Krentz, 2007). In Chignik Lagoon, however, a distinct otolith chemical signature of the mid lagoon habitat is surprising, as the large tidal exchange forces fish into a central channel with each tidal cycle, facilitating mixing among sites. It would appear that fish returned repeatedly to shallow feeding sites as they became inundated by rising tides.

Growth rates of *S. malma* were lower in freshwater habitats in all months, despite late summer warming of Chignik Lake and low densities of conspecific competitors. It is likely, therefore, that the lake resident fish are trading lower growth for increased survival in the lake habitat. While there are avian predators in Chignik Lake (*e.g.* Pacific loon *Gavia pacifica*), none of the large-bodied predatory fishes commonly found in other Alaskan lake systems [*e.g.* pike *Esox lucius* L. 1758, *S. alpinus*, lake trout *Salvelinus namaycush* (Walbaum, 1792) and *O. mykiss*] have been observed in Chignik. Survival of lake resident fishes may, therefore, be relatively high. In contrast, lagoon resident fishes displayed higher growth rates despite contending with the energetic demands of osmoregulation in marine waters, higher densities of conspecifics and the need to avoid a suite of predatory fishes [*e.g.* Pacific halibut *Hippoglossus stenolepis* Schmidt 1904, fourhorn sculpin *Myoxocephalus quadricornis*]
(L. 1758), starry flounder *Platichthys stellatus* (Pallas 1787) and Pacific cod *Gadus macrocephalus* Tilesius 1810, and marine mammals (*e.g.* harbour seal *Phoca vitulina*) which may result in changes in feeding behaviour and lowered foraging efficiency (Werner & Hall, 1988).

These analyses revealed significant differences in growth among *S. malma* from freshwater and lagoon sites throughout the summer months. In early summer sampling, however, there was little difference between freshwater, inner-estuary and ecotone sites. This may be due to either lower growth of fish at the river-estuary ecotone and inner lagoon sites or recent entry by fish into the lagoon environment from lake habitats. Peak *S. malma* downstream migration occurs at least 1 month prior to the earliest sampling event in this study (Bond & Quinn, 2013); therefore, individuals captured at ecotone and inner lagoon sites are probably resident at those sites, and not recent emigrants from the lake. The differences in growth among lagoon habitats may be attributable to differences in marine residence time or foraging opportunities that each habitat affords. In previous work in Chignik Lagoon, Narver & Dahlberg (1965) found distinct suites of prey items in *S. malma* at the mid and outer lagoon sites; in all months, the diet of mid lagoon fish was dominated by invertebrate prey, particularly amphipods. Earlier studies also indicate that diets of fish at the outer lagoon, however, were more variable but composed mainly of fishes including sand lance *Ammodytes hexapterus* Pallas 1814 and capelin *Mallotus villosus* (Müller 1776). The mid lagoon site may therefore afford a more optimal mix of intermediate salinities, lower competition and fewer marine predators, allowing fish to achieve the highest measured growth in the study in 2 of the 3 months, despite a less energy dense prey base. In addition, the invertebrate prey in the mid lagoon may be more readily available, while the distribution of *A. hexapterus*, *M. villosus* and Pacific herring *Clupea pallasi* Valenciennes 1847 at the outer lagoon may be more episodic. In Atlantic salmon *Salmo salar* L. 1758 populations, downstream migrating individuals move rapidly to sea, making little use of mid-estuary habitats (Thorstad et al., 2004; Finstad et al., 2005; Gudjonsson et al., 2005). In some cases, a portion of the population remains resident in estuarine waters, and may experience higher growth and lower mortality rates than those in marine waters (Thorpe, 1994), similar to *S. malma* in the Chignik system.

There was a marked increase in the growth rates of fish in the Chignik River during August, but only a small percentage were assigned to freshwater habitat. While few of the remaining individuals assigned to lagoon sites, variability in the duration of residence in fresh water after migration from marine or lagoon habitats, or prior residence outside of the lagoon probably caused otolith chemical signals not indicative of any group included in the DFA. It is possible for the measured otolith chemistry values to be an average of signatures created in several environments; however, previous research indicates that individuals ascending the Chignik River generally move rapidly to upstream habitats, and residence time in the river is short. Therefore, it is likely that *S. malma* moving upstream in August months carried an otolith chemical signal from more coastal waters not well represented by the lagoon otolith chemistry baseline. These data are consistent with the large numbers of *S. malma* returning to fresh water in mid summer after c. 70 days in marine waters, based on sonic tracking and counts at a weir on the Chignik River (Bond & Quinn, 2013). Therefore, the large increase in growth rates in Chignik River fish between June and August, along with a marked decrease in otolith chemistry-based habitat assignment, probably resulted from movement of fish from high-growth marine to low-growth freshwater habitats.
in the late summer, rather than changes in the growth rate of freshwater resident individuals.

Growth of *S. malma* was more similar among individuals at a capture location than among individuals assigned to the same location with otolith chemistry and DFA, indicating the temporal differences between the formation of otolith structure and changes in plasma IGF1 concentration. Detection of otolith chemistry among sites requires that an individual remain resident in waters of differing chemistry long enough to incorporate that chemistry into its otoliths, and create enough structure that is detectable with analytical techniques. In other species, the minimum time for detection of habitat is c. 2 weeks, although this depends upon the age and growth rate of the fish as well as the difference in water chemistry among habitats (Miller, 2009, 2011). In other fishes, IGF1 concentrations indicate growth as a seven-day moving average (Beckman, 2011). Even a near-complete cessation of feeding, as in the case of *S. malma* ascending Chignik River in August, will not show a significant decrease in IGF1 for at least several days (Pierce *et al.*, 2005). Likewise, IGF1 is indicative of the long-term nutritional status of the individual, and single bouts of consumption are unlikely to induce an increase in IGF1 and growth (Shimizu *et al.*, 2009). Therefore, dissimilarity in IGF1 among habitats reflects the state of individuals at that site and their capacity for growth, and is robust to recent differences in short-term foraging success.

The observed differences in growth rates among habitats within the Chignik system were apparently the result of high site fidelity by some individuals, and regular seasonal movement patterns by others. While studies of fish growth in estuarine habitats have traditionally been constrained by the inherent difficulties in mark-and-recapture studies (*e.g.* poor recapture rates reducing statistical power), the combined use of two independent single-capture techniques in this study revealed differences in growth and habitat use. Published studies of the use of estuarine (Narver & Dahlberg, 1965) and marine habitats (Morita *et al.*, 2009) by *S. malma* are rare; nearly all studies infer the use of marine environments through migration timing at weirs (Armstrong, 1974; Bernard *et al.*, 1995), and recapture of tagged fish in distant locations (DeCicco, 1992). Indeed, for most facultatively anadromous fishes, the growth effects of migration and residency are often inferred or assumed, but rarely demonstrated (Thorpe, 1994; Northcote, 1997).

This study provided strong evidence that *S. malma* were not only using estuarine and marine waters that afforded higher growth rates than freshwater habitats, but they were also spatially segregated in their use of those estuarine and marine habitats. Some individuals appeared to reside in mid-estuary waters while others used more marine habitats. Questions remain about what factors determine whether an individual remains in fresh water, moves to estuarine habitats or to coastal ocean habitats. Tagging and tracking of *S. malma* in estuarine and near-shore ocean habitats would allow for determination of the scale of residence and home range during the summer growing season. Although the estuary provided superior growth opportunities throughout the summer months compared to freshwater sites, the benefits of smaller-scale habitat units within the estuary may vary widely. Within anadromous populations of *S. malma*, fully resident phenotypes have been demonstrated, and the different growth and residence patterns of *S. malma* within lagoon waters may indicate that additional migratory ecotypes exist.

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