EFFECT OF REARING LOCATION ON ESCAPEMENT OF COHO SALMON (*ONCORHYNCHUS KISUTCH*) AT FRESHWATER CREEK, CALIFORNIA

by

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by

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ABSTRACT

Effects of rearing location on escapement of coho salmon (*Oncorhynchus kisutch*) at Freshwater Creek, California

Barbara L. M^{C}Coy

Scales of juvenile and adult coho salmon (*Oncorhynchus kisutch*) from Freshwater Creek, California were analyzed to determine: 1) if growth of wild juvenile coho salmon differed between the upper and lower portions of the watershed; and 2) if individuals that reared in the upper and lower portions of the watershed were equally represented in adult escapement. The upper portion of the basin was freshwater, while the lower portion was tidally influenced. Growth differences of juvenile coho salmon were assessed by analyzing circulus spacing on scales of individuals whose rearing grounds were known. The distance between the first two circuli was greater for individuals that reared in the upper watershed, suggesting that these individuals exhibited greater growth than juveniles rearing in the lower stream-estuary ecotone. However, results from scale analysis of adult coho salmon were inconsistent with this finding. The first 17 intercircular spaces in juvenile growth portion of scales from adult coho salmon did not differ between the two rearing locations, suggesting that juvenile growth did not detectably differ between the two portions of the watershed. Inference was limited by a small sample size of adult scales. Because differences could not be consistently detected in juvenile growth of coho salmon between the two portions of the watershed, it was not possible to determine if individuals rearing in these locations were equally represented in adult escapement. Scale analysis revealed the existence of a summer growth check prior to “ocean entry” as well as minimal utilization of the lower reach by juveniles immigrating from the upper reach.

ACKNOWLEDGEMENTS

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INTRODUCTION

Positive relationships have been established between marine growth of juvenile salmon and survival of adult salmon, and between marine survival of juvenile salmonids and size at ocean entry. For example, Moss et al. (2005) found evidence that pink salmon (*Oncorhynchus gorbuscha*) escapement was related to faster ocean growth in juveniles during the first summer at sea. Friedland and Haas (1996) found that returns of 1-seawinter Atlantic salmon (*Salmo salar*) were correlated with late summer growth of post-smolts. Peyronnet et al. (2007) determined that recruitment of Atlantic salmon was linked to late summer and early winter growth during the first year at sea. First fall and winter growth at sea have also been found to affect marine survival of coho salmon (*O. kisutch*) (Beamish et al. 2004). Marine survival of juvenile salmonids is in turn related to size at ocean entry, as larger salmonid smolts have a better chance of surviving size-selective predation in the marine environment (Mathews and Buckley 1976, Bilton 1978, Olson 1978, Bilton et al. 1982, Holtby et al. 1990, Koenings et al. 1993).

Growth potential of juvenile salmonids can vary between rearing habitats located within the same watershed. Dempson et al. (1996) found that Atlantic salmon parr reared in lakes were larger than those reared in streams within the same watershed. Erkinaro et al. (1997) found that Atlantic salmon parr reared in tributaries were larger than their mainstem counterparts. Ebersole et al. (2006) found that coho salmon smolts that reared in a tributary of a coastal Oregon watershed were larger than their counterparts that reared in other tributaries within the same watershed. Hayes et al. (2008) found that juvenile steelhead trout (*O. mykiss*) that reared in the estuary-lagoon portion of a central

California watershed composed the majority of steelhead trout reaching ocean entry sizes (~ 150-250 mm fork length) for that watershed. This critical size for marine survival, they hypothesized, influences adult escapement. Therefore, adult escapement may be ultimately affected by rearing location within a watershed.

A population of coho salmon in Freshwater Creek (Humboldt County, California) has been monitored by the California Department of Fish and Game (CDFG) since 2002. Monitoring data suggest that approximately 40 percent of smolt production for the watershed has originated from the lower stream-estuary ecotone, based on mark/recapture production estimates for downstream migrants (Wallace 2008, personal communication). Juvenile coho salmon have also been observed to vary in morphology between rearing locations within this watershed. In comparison with fish that reared in the lower reach of Freshwater Creek, coho salmon smolts that reared in the upper watershed were smaller in size at the onset of winter as well as during spring migration (Ricker 2008, personal communication). Wallace (2006) reported that young-of-the-year coho salmon from the lower reach, in early August, had an average fork length of 68 mm, while their upstream counterparts averaged 56 mm.

Apparent growth differences between juvenile coho salmon from the upper and lower watershed may reflect differing riparian habitats. The upper watershed of Freshwater Creek is forested, while the lower watershed has been cleared for pastures and rural development allowing more sunlight to reach the stream channel. According to the River Continuum Concept (Vannote et al. 1980), characteristics for the upper reaches of a river include a denser canopy leading to more shading of the channel and inputs of allochthonous material as well as lower stream temperatures. Lower reaches are characterized by a more open canopy that allows more sunlight to reach the channel leading to warmer stream temperatures and greater in-stream primary production. Temperature and food consumption strongly affect growth of cutthroat trout (*O. clarki*) (Martin 1985), and are likely to affect growth of coho salmon as well.

Differences in growth of juvenile coho salmon between the upper and lower reaches may be reflected in the circulus spacing of scales collected from individuals in each location. Circulus spacing has been shown to be correlated with body size in various species of fishes (Doyle et al. 1987, Fukuwaka and Kaeriyama 1997, Ramstad et al. 2003). For coho salmon, greater growth of an individual has been correlated with wider circulus spacing on scales (Fisher and Pearcy 1990, Holtby et al. 1990, Beamish et al. 2004). When wider circulus spacing on scales occurs after a narrowing of circulus spacing, the alternating banding pattern creates a growth check (Bilton and Robins 1971). Growth checks on scales can result from physiological changes or stresses that slow growth (i.e., changes in food availability, spawning, temperature changes, habitat shifts, and exposure to pollution) (Jearld 1983). Therefore, circulus spacing analysis of juvenile coho salmon scales from Freshwater Creek may substantiate suspected differences in growth between smolts reared in the upper watershed and those reared in the lower stream-estuary ecotone.

The objectives of this project were to determine: 1) if the growth of juvenile coho salmon differed between the upper watershed and the lower stream-estuary ecotone of Freshwater Creek; and 2) if individuals that reared in the upper and lower reaches of this stream were equally represented in adult escapement. Juvenile growth was assessed by measuring spacing of circuli on scales, with wider spacing assumed to represent greater growth. The first objective was based on analysis of scales that were collected from fish whose rearing grounds were known, while the second objective was based on analysis of scales collected from fish of unknown rearing grounds.

STUDY SITE

Freshwater Creek drains a catchment of approximately 92 km^2 into Humboldt Bay in coastal northern California (Figure 1). The 23 km-long mainstem of Freshwater Creek is a fourth order stream. In the headwaters, where the elevation maximum is 825 m, rainfall averages approximately 150 cm/yr. At the mouth of Freshwater Creek, rainfall averages approximately 100 cm/yr (Ricker 2006a). The upper watershed consists of rocky substrate, while the lower portion of the creek is composed of fine sediments. The creek empties into approximately 4.16 km of Freshwater Slough, which drains into approximately 2.91 km of Eureka Slough before entering Humboldt Bay. Tidal influence from the bay affects both sloughs and the lower portion of Freshwater Creek.

For the purposes of this study, Freshwater Creek was classified into two reaches: upper and lower (Figure 2). The upper reach consisted of all waters upstream from the Howard Heights Bridge (40^o 45’ 52.49” N, 124^o 03’ 52.41” W), approximately 12.89 km upstream of the mouth of Eureka Slough. The 5.82 km lower reach, representing the lower stream-estuary ecotone, flowed downstream from the Howard Heights Bridge to its entrance into Freshwater Slough (40^o 48' 7.41" N, 124^o 07' 0.81" W). The upper reach was composed entirely of freshwater, whereas, the lower reach experienced varying degrees of tidal influence, depending on tide and rainfall. Wallace (2006) measured salinities of 7.6 ppt about 8.5 km upstream of the slough mouth, at the location of the permanent weir (40^o 47' 1.97" N, 124^o 04' 54.07" W) during late August high tides. The maximum extent of tidal influence has been determined to be at Howard Heights Bridge (Humboldt Bay Watershed Advisory Committee 2005). Stratification of the water

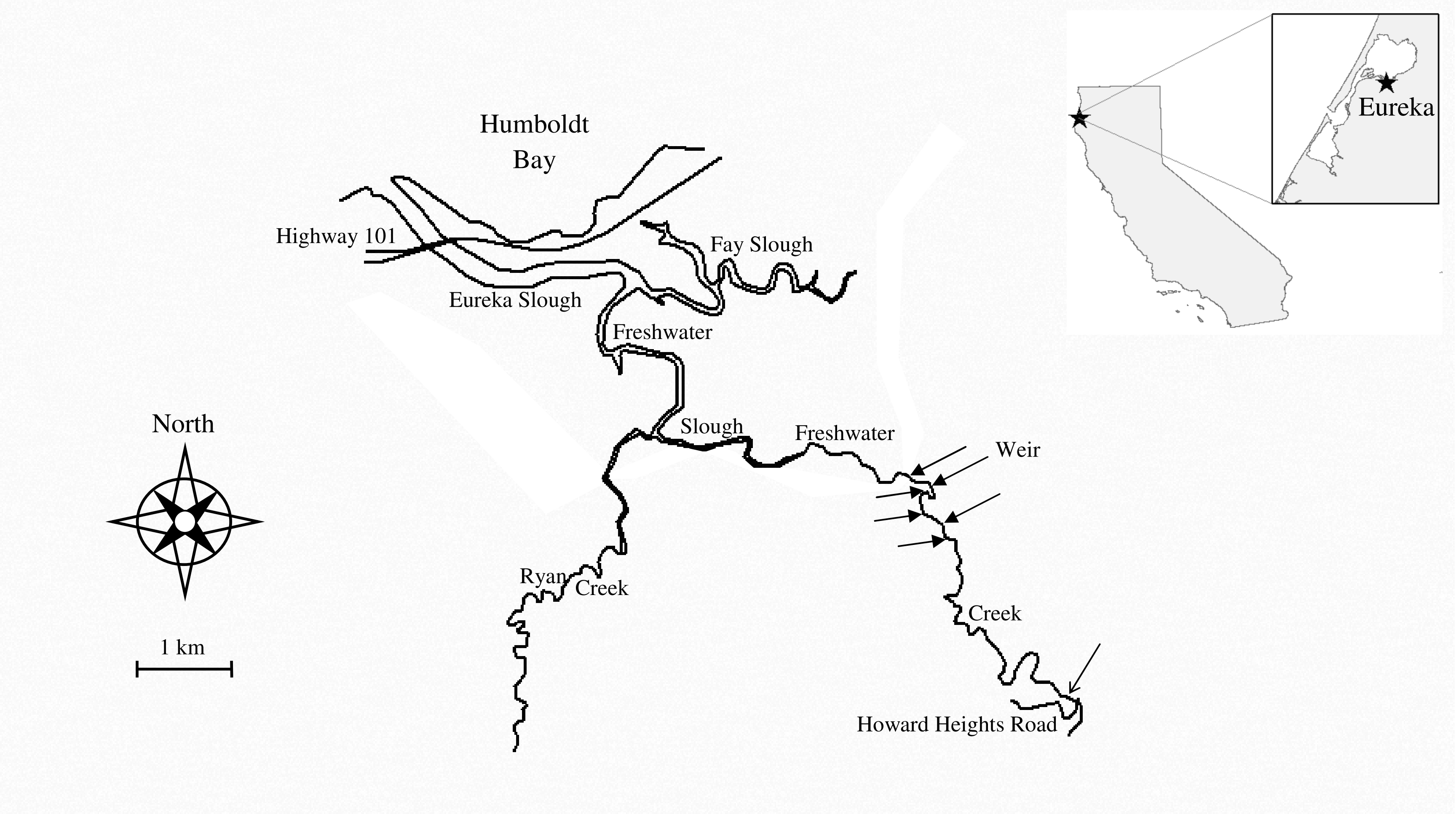

Figure 1. Map of Freshwater Creek, tributary to Humboldt Bay, in northern California. Closed arrows indicate sample sites in the lower stream-estuary ecotone of Freshwater Creek. Open arrow indicates location of floating inclined-plane trap. The maximum extent of tidal influence is Howard Heights Road (modified from Wallace 2006).

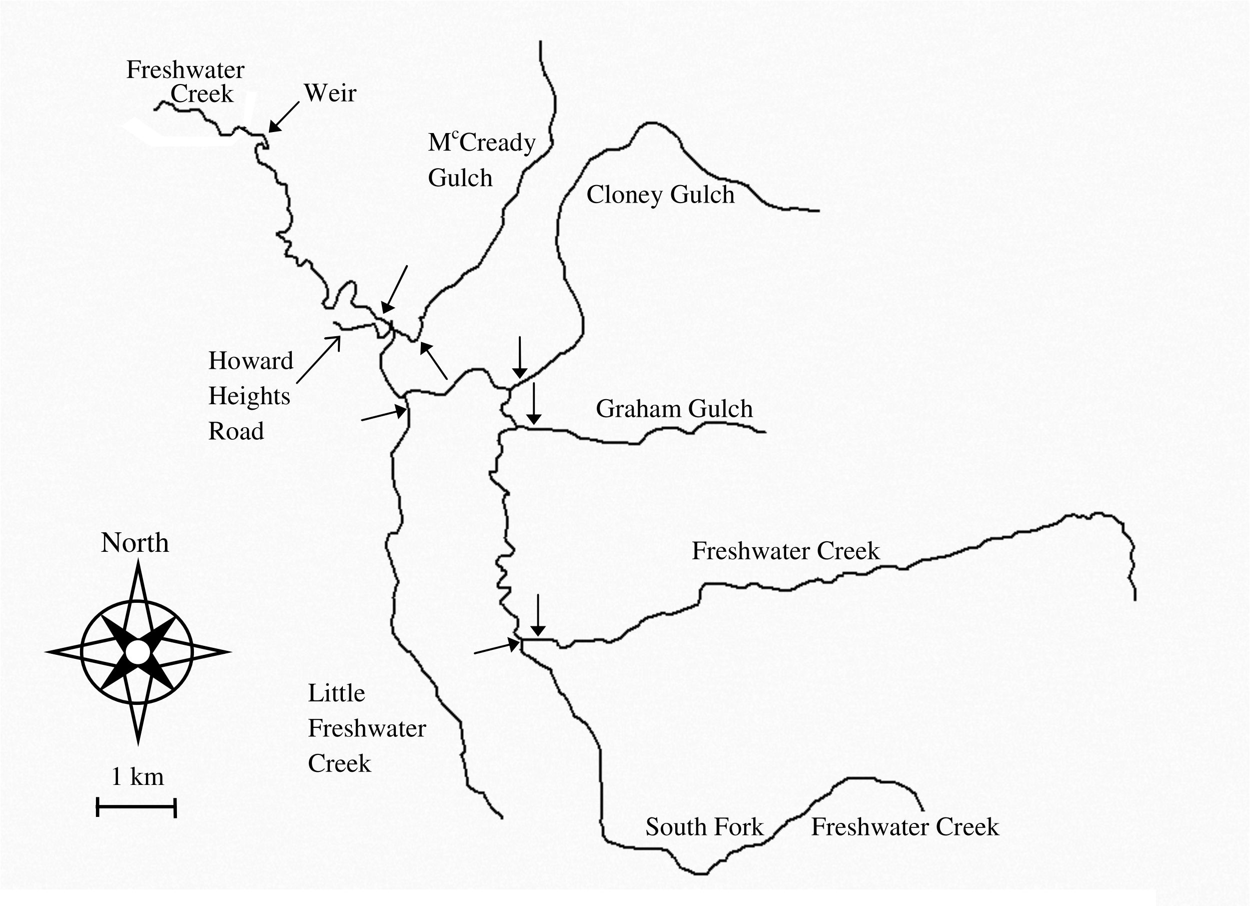

Figure 2. Map of Freshwater Creek, Humboldt County, California and the major tributaries. Closed arrows indicate locations of downstream migrant traps.

column in the lower reach has not been observed during summer low flows, and water temperatures were within the non-lethal range for coho salmon (i.e., did not exceed 18 ^{o}C) upstream of the weir (Wallace 2006).

The upper reach of Freshwater Creek is associated with coniferous forest and is managed for timber production, while the lower reach, associated with pastures, has limited riparian development. The riparian community for Freshwater Creek consists of: redwood (*Sequoia sempervirens*), Douglas-fir (*Psuedotsuga menziesii*), white fir (*Abies concolor*), Sitka spruce (*Picea sitchensis*), willow (*Salix spp*.), red alder (*Alnus rubra*), black cottonwood (*Populus trichocarpa*), blackberry (*Rubus ursinus*), salmonberry (*Rubus spectasbilis*), and other herbaceous plants. The salmonid assemblage includes coho salmon, Chinook salmon (*O. tshawytscha*) and occasionally chum salmon (*O. keta*), as well as steelhead and cutthroat trout (Ricker 2006a).

MATERIALS AND METHODS

Estimates of freshwater growth for coho salmon (*Oncorhynchus kisutch*) were based on analysis of scales from juvenile and adult fish that were organized into four datasets from 2004 through 2007: 1) paired scales from the same fish, taken at juvenile and adult stages, for which rearing location was known (n = 22 fish from the upper reach; n = 7 fish from the lower reach); 2) scales from juvenile fish that were marked and recaptured from the lower reach during sampling events from summer through fall (n = 31); 3) scales from juvenile fish that were captured for marking using downstream migrant traps at or upstream from the Howard Heights Bridge (n = 39); and 4) scales from adult fish of unknown rearing origin (n = 50). All salmonids encountered in this study were handled following accepted standards as approved by the Institutional Animal Care and Use Committee (IACUC No. 07/08.F.43.A).

Juvenile fish marked and recaptured from the lower reach were assumed to have reared in the lower reach of Freshwater Creek, and juveniles captured in downstream migrant traps at or upstream from the Howard Heights Bridge were assumed to have reared in the upper reach of the creek. These assumptions are supported by qualitative observations made by CDFG employees, as marked juveniles from the upper reach have not been observed to pass through the inclined-plane trap more than once (Ricker 2008, personal communication). During a mark and recapture study, Wallace (2006) observed that young-of-the-year coho salmon migrated down into the lower reach shortly after emergence and utilized the lower reach from April to mid-September.

Juvenile coho salmon were captured from seven locations in the upper reach (Figure 2) from 2004 through 2007. Emigrating smolts were sampled from mid-March through mid-June using a floating inclined-plane trap located downstream of Howard Heights Bridge and six pipe traps located at the mouths of each of the five major tributaries (Cloney Gulch, Graham Gulch, Little Freshwater Creek, McCready Gulch, and South Fork Freshwater Creek) and the upper mainstem. During the sampling season, fish were removed from trap boxes on a daily basis. Salmonids were identified to species, and their ages were estimated. Salmonids over 70 mm were anaesthetized with tricaine methane sulfonate and marked with passive integrated transponder (PIT) tags. Salmonids less than 100 mm were marked with an 11 mm PIT tag, whereas salmonids over 100 mm were marked with a 23 mm PIT tag (Ricker 2006b). Scale samples were collected from the left side of each individual coho salmon in an area between the dorsal fin, lateral line, and caudal fin (Ricker 2008, personal communication). After recovery from the anesthetic, fish were returned to Freshwater Creek in the immediate vicinity of the trap (Ricker 2006b). The CDFG provided scales that represented a randomized set of samples collected during the 2004, 2005 and 2006 field seasons from juveniles that reared in the upper reach (not including those in the paired juvenile and adult data set).

Juvenile coho salmon were captured from six locations in the lower reach (Figure 1) from 2004 through 2006. Fish were sampled in mid-March through October using a beach seine and minnow traps (Wallace 2006), while a pipe trap was affixed to the weir and operated from mid-March through mid-June (Anderson 2008, personal communication). Juveniles captured in the pipe trap were processed following the same procedure as mentioned above for the upper reach (Ricker 2006b). Juveniles captured using the beach seine (9.1 m X 1.8 m, 6.4 mm mesh) and minnow traps were processed as follows: 1) at each sample site, fish were anaesthetized with Alka Seltzer^{TM}, observed for marks and (or) tags, measured to fork length and weighed; 2) fish were sorted by species and life history stages (young-of-the-year, pre-smolts, smolts, and adults); and 3) scale samples were collected from the first 10 coho salmon (Wallace 2006) in a process similar to that described above (Wallace 2008, personal communication). Yearling and older juvenile coho salmon were marked with PIT tags. After recovery from the anesthetic, fish were released into Freshwater Creek in the immediate vicinity of the sample site (Wallace 2006). Scale samples were collected from recaptured individuals from the right side of each tagged fish in an area opposite to that described above (Wallace 2008, personal communication). Some fish were recaptured up to five times.

Scales from juveniles that reared in the lower reach (not including those in the paired juvenile and adult data set) were selected from samples provided by CDFG in which a minimum duration between initial marking and recapturing was three weeks, and a maximum was three months. Initial mark dates ranged from mid-June to mid-August, while final recapture dates ranged from mid-August to mid-November. These dates were chosen to encompass any growth that occurred during the low flow season for Freshwater Creek in order to ascertain whether a growth check was recorded in the scale circulus pattern during this season. The CDFG provided scales from ten to 11 juveniles from each of the three field seasons (2004, 2005, and 2006).

Returning adult coho salmon were detained at the permanent weir on Freshwater Creek from late October or early November through early June in 2005 through 2007. The weir consisted of a concrete base that lined the creek bed providing attachment for a series of modular metal panels. The weir trap, located on the north side of the stream, consists of concrete walls and metal panels. During the sampling season, the weir was monitored daily for the presence of fish. Captured fish were removed from the weir with nets and submerged in a tagging cradle without the use of anesthetic. Species were identified, measured to fork length, and examined. Coho salmon were scanned for the presence of individual identifying PIT tags that might have been inserted during downstream migration (Ricker 2006a). Scale samples were collected from tagged fish from the right side in an area opposite to that described above (Ricker 2008, personal communication). All fish were then returned to Freshwater Creek immediately upstream of the weir (Ricker 2006a).

Paired scales taken from the same individual at juvenile and adult stages were limited by the number of marked adults that had returned to the weir. Scales from adults of unknown rearing origin were obtained from a random sample of scales collected from adults that were captured at the weir (25 of 263 unmarked adults for the 2006 field season and 25 of 215 unmarked adults for the 2007 field season).

Scale analysis was conducted using digital analysis software following CDFG Freshwater Salmonid Monitoring Project protocols (see Appendix A). Adult scales were washed, dried, and mounted between two glass slides for analysis, whereas juvenile scales were not cleaned prior to mounting because of their small size. Digital images of scales were captured at 6.3x magnification using Spot 4.6 ™ software and optimized using the best-fit enhancement filter in Image-Pro Plus 4.1 ™ software. Measurements were taken from the scale focus at 20^o on either side of the longest axis. Circuli were identified and the distance between adjacent circuli was measured in millimeters to the sixth decimal place using Image-Pro Plus ™. Software analysis of circuli presence and placement was confirmed through visual inspection done by one reader.

The first intercircular spaces on scales from juvenile coho salmon that reared in the upper and lower reaches of Freshwater Creek were compared to determine if growth differences between the two reaches could be inferred (Figure 3). Scales collected from juvenile fish of both reaches were also examined for the occurrence and location of a growth check. A growth check was identified by alternating groups of widely and narrowly spaced circuli, with the last narrowly spaced circulus within the narrow group being interpreted as a growth check (Bilton and Robins 1971). Scales from marked and recaptured juveniles that reared in the lower reach were examined to ascertain the timing of a summer growth check.

The paired juvenile and adult data set was analyzed to determine if the end of juvenile growth, which was represented by the total number of circuli on scales of juveniles, coincided with “ocean entry” on scales of adults (Figure 4). On scales of adults, the juvenile growth pattern (i.e., total number of circuli) up to the “ocean entry” check was determined. An “ocean entry” check was identified by a pair of widely spaced circuli, being wider and more robust than the preceding narrowly spaced circuli (Beamish et al. 2004). Scales from adult fish were visually inspected during the measuring process

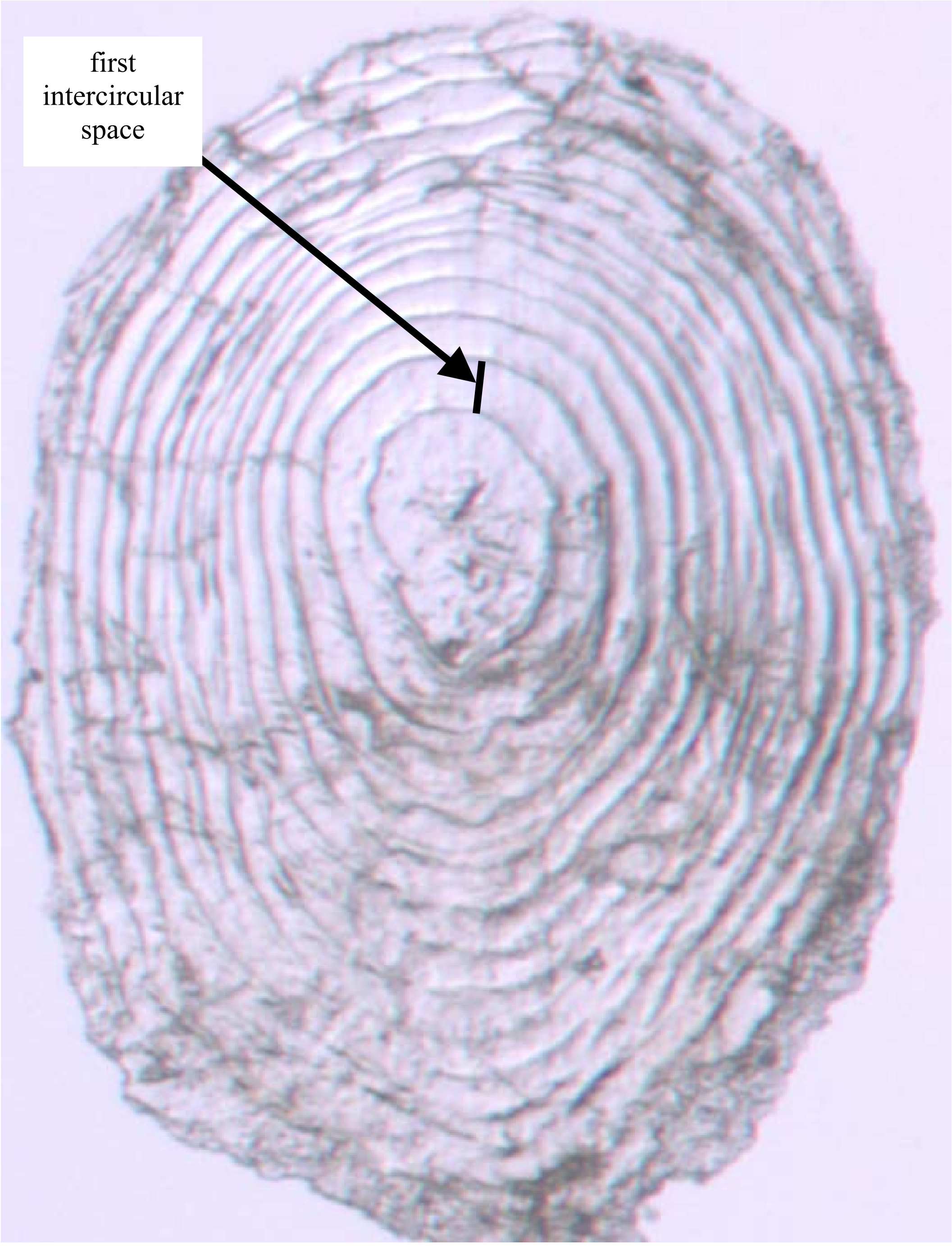

Figure 3. Scale from juvenile coho salmon showing the location of the first intercircular space (image taken at 6.3x magnification).

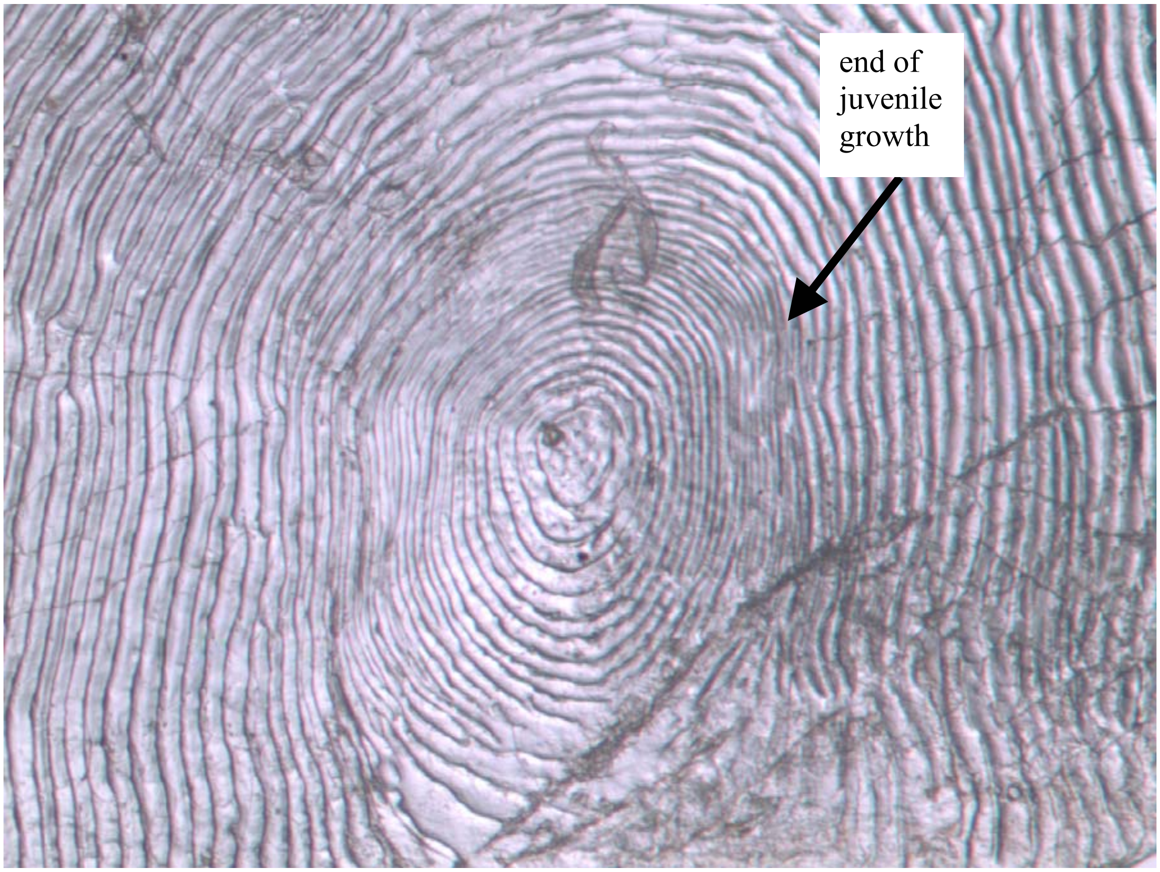

Figure 4. Scale from adult coho salmon showing the end of juvenile growth (image taken at 6.3x magnification).

to ensure that the “ocean entry” checks were present in the data. The first 17 intercircular spaces on scales from adults that reared in the upper and lower reaches of the creek were compared.

Scales of adult fish from unknown rearing grounds were examined in order to assign each individual back to its rearing grounds (upper watershed or lower stream-estuary ecotone) so that the relative contribution of each life history to adult escapement could be determined.

Accuracy of scale analysis was assessed by comparing measurements of all circulus spacing representing juvenile growth from 10 sets of paired juvenile and adult scales to measurements made by a second scale reader. The mean coefficient of variation of the readings was 9.33 percent. Variance in circulus spacing between juvenile coho salmon reared in upper and lower reaches was compared with an *F*-test. Differences in mean circulus spacing on scales between the two groups were analyzed with a two-sample *t*’-test. Minimum means were estimated to define the summer growth check on scales from juvenile and adult fish that were associated with known rearing grounds. Minimum means represent the smallest average distance between adjacent circuli for all individuals in a data set. If an individual was recaptured multiple times, then all measurements were averaged in calculating the minimum means for a data set. The relationship between number of circuli in scales of juveniles and number of circuli to the “ocean entry” check on the scales of their adult counterparts was evaluated with linear regression. Levene’s test for equal variances was used to verify the homogeneity of the variances in circulus spacing of scales from adults that reared upstream versus adults that reared in the lower reach. Once confirmed, data were analyzed using a general linear model to determine: 1) if mean circulus spacing differed between individuals from the upper and lower reaches; and 2) if mean circulus spacing differed for all gaps between circuli. Sample sizes for the Levene and general linear model tests were limited by the number of lower reach samples (n = 7). Statistical analyses were run in Minitab 15.0 ™ software.

RESULTS

Mean circulus spacing of scales from juvenile coho salmon was greater in fish that reared in the upper reach than in fish that reared in the lower reach (two-sample *t*' = 2.41; df = 60; *p* = 0.02) at the first intercircular space. Having demonstrated this difference, no further analyses were performed. For this analysis, the sample size was limited by the number of lower reach samples (n = 38). Spacing was 33.67 µm in scales from upper reach fish and 31.49 µm in scales from lower reach fish.

The smallest distance between adjacent circuli on scales of juveniles that reared in the upper reach averaged 12.67 µm (SD = ±3.74; n = 61) (Figure 5). Among scales examined, the smallest distance between adjacent circuli occurred with the greatest frequency at gap number 10 (Table 1). The smallest distance between adjacent circuli on scales of juveniles that reared in the lower reach averaged 12.53 µm (SD = ±3.96; n = 38) (Figure 6). The smallest distance between adjacent circuli occurred with the greatest frequency at gap number 15. The total number of gaps found on scales of lower reach individuals was 22, while the number on the scales of upper reach individuals was 26.

Examination of mark and recapture data for juveniles from the lower reach revealed that the growth check occurred between late July and early September. Since juveniles from the upper reach were marked during migration out of the system, recapture data are not available to allow determination of timing of the growth check in individuals from this reach. However, perusal of the relationship between circulus spacing and gap number on scales from individuals that reared in the upper and lower reaches (Figures 5, 6) suggests that the growth check occurred sooner during juvenile growth in the upper

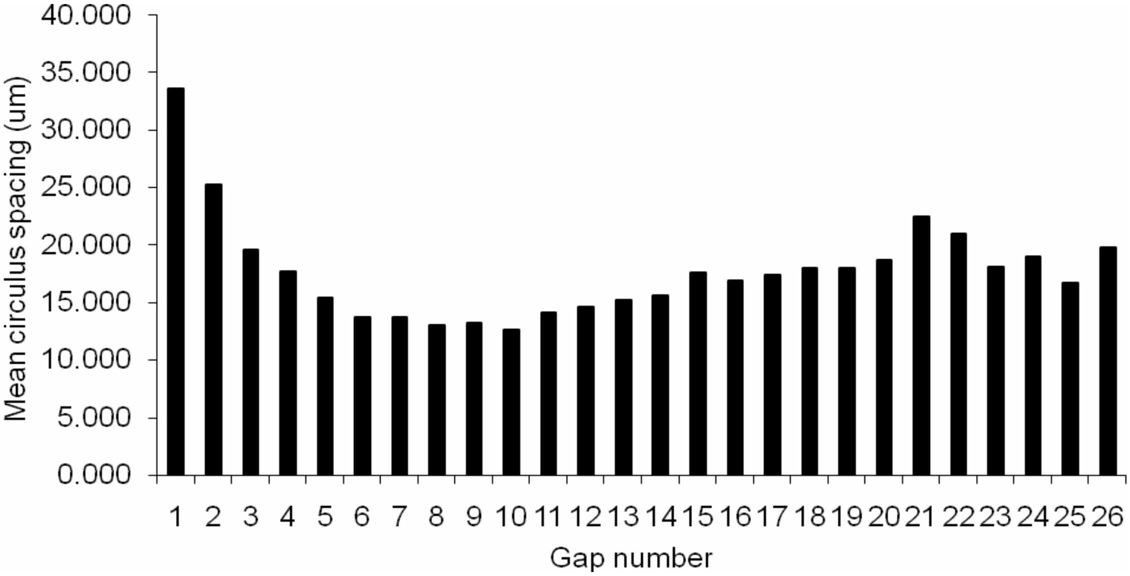

Figure 5. Mean circulus spacing (μm) on scales of juveniles that reared in the upper reach (n = 61) of Freshwater Creek, Humboldt County, California.

Table 1. Frequency with which the smallest distance between adjacent circuli occurred at differing gap numbers on scales of juvenile coho salmon that reared in the lower and upper reaches of Freshwater Creek, Humboldt County, California.

	Frequency of Occurrence	
Gap Number	lower reach	upper reach
3	0	2
4	0	1
5	0	1
6	0	5
7	1	6
8	1	3.5
9	4	2.5
10	2	14
11	3.5	6
12	4.5	4
13	5	4
14	6	7
15	7	3
16	1	0
17	1	1
18	1	1
…		
24	1	0

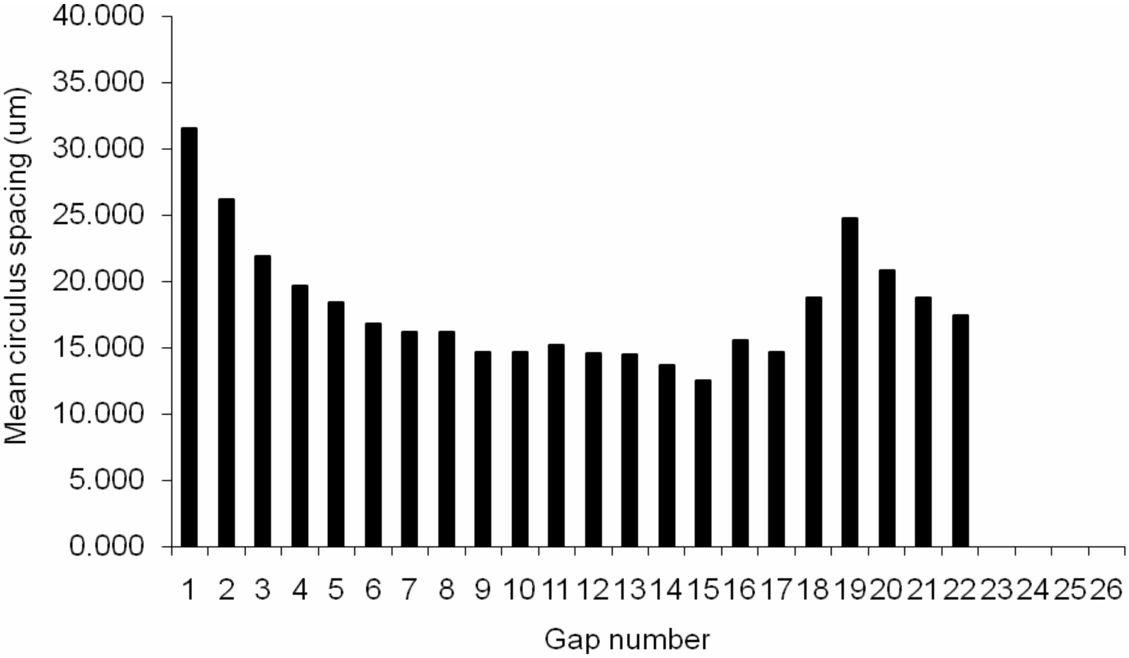

Figure 6. Mean circulus spacing (µm) on scales of juveniles that reared in the lower reach (n = 38) of Freshwater Creek, Humboldt County, California.

reach than in the lower reach, assuming that emergence times of juveniles were equal between locations.

The number of circuli to the "ocean entry" check on scales of adults did not correspond with the number of circuli on their juvenile counterparts ($R^2 = 0.09$; $p = 0.13$; slope = 0.37) (Figure 7). However, when the analysis was restricted to scales of fish from the upper reach of Freshwater Creek, the number of circuli to the "ocean entry" check on scales from adults was related to the number of circuli on their juvenile counterparts ($R^2 = 0.42$; $p = 0.002$; slope = 0.77) (Figure 8).

Scales from paired juvenile and adult data set were also analyzed to determine the existence and location of a summer growth check on adults that reared as juveniles in the upper and lower reaches of the creek (Figure 9). The smallest distance between adjacent circuli on scales of adults from the upper reach averaged 16.40 µm (SD = ±8.53; n = 22) at gap number 6, and the smallest distance between adjacent circuli occurred at spaces somewhat evenly distributed across circuli 4-11, 14, 16, and 18 (Table 2). For adults that reared in the lower reach, the smallest distance between adjacent circuli averaged 13.79 µm (SD = ±3.52; n = 7) at gap number 7, while the smallest distance between adjacent circuli was dispersed evenly across spaces 6, 7, 9, 11, 12 and 15.

Mean circulus spacing differed among gap numbers ($F = 5.73$; $p < 0.001$). However, circulus spacing did not differ between individuals from the upper and lower reaches ($F = 2.33$; $p = 0.13$) or with the interaction between gap number and location ($F = 0.86$; $p = 0.62$).

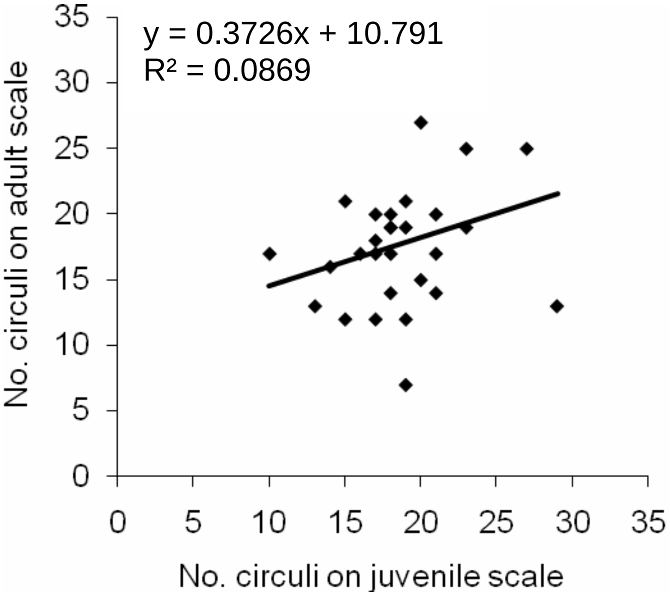

Figure 7. Relationship between the number of circuli on scales from adult coho salmon and the number of circuli on their juvenile counterparts from Freshwater Creek, Humboldt County, California. Number of circuli on scales from adults includes all circuli from the focus to the "ocean entry" check. Data are from all individuals tagged as juveniles that were recaptured as returning adults (n = 29).

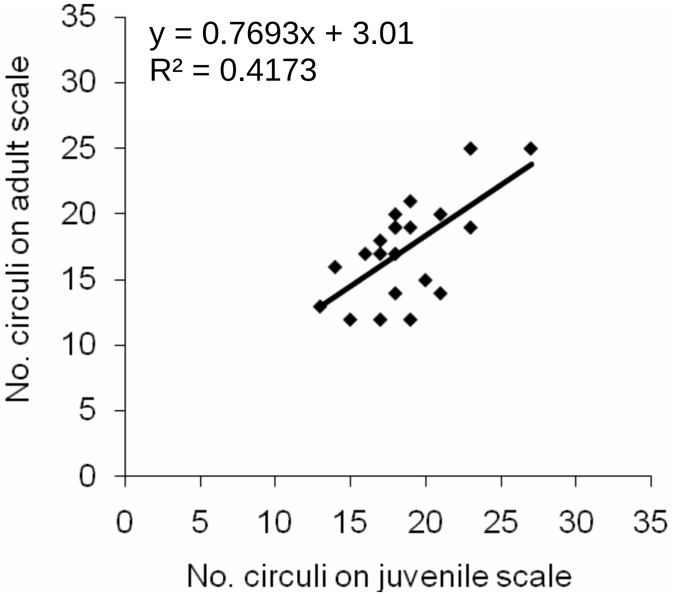

Figure 8. Relationship between the number of circuli on scales from adult coho salmon and the number of circuli on their juvenile counterparts from Freshwater Creek, Humboldt County, California. Number of circuli on scales from adults includes all circuli from the focus to the “ocean entry” check. Data are from individuals reared in the upper reach (n = 22) that were tagged as juveniles and recaptured as returning adults.

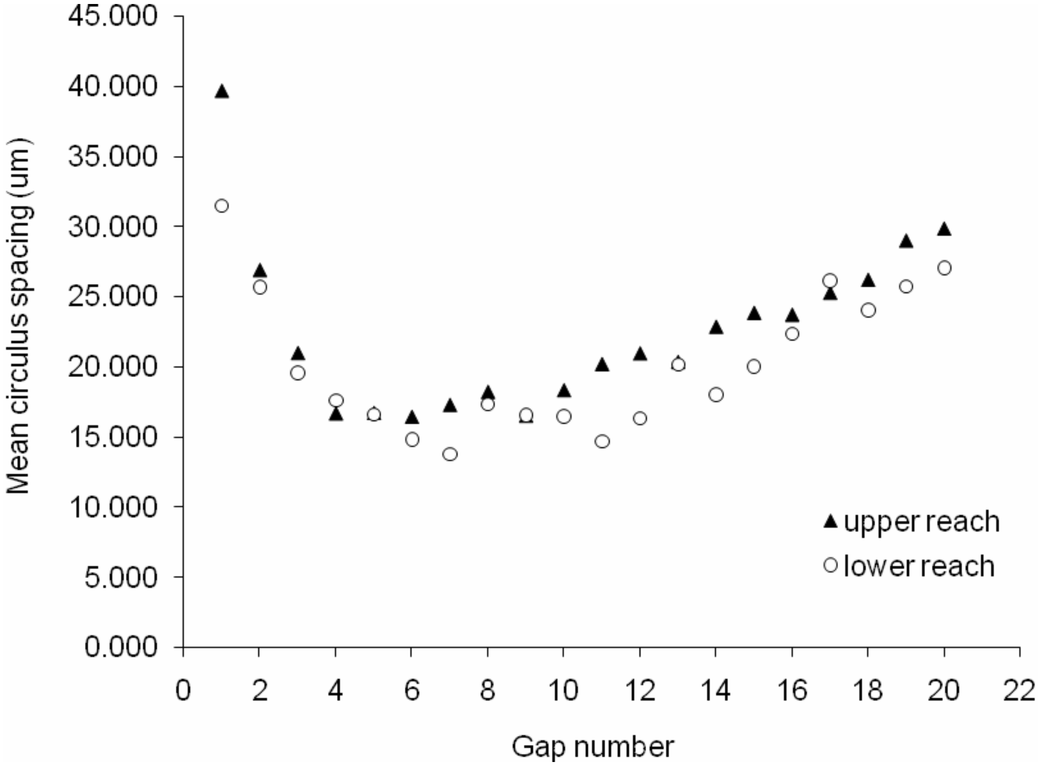

Figure 9. Mean circulus spacing (µm) on scales from adults that reared in the upper (n = 22) and lower (n = 7) reaches of Freshwater Creek, Humboldt County, California.

Table 2. Frequency with which the smallest distance between adjacent circuli occurred at differing gap numbers on scales of adult coho salmon that reared in the upper and lower reaches of Freshwater Creek, Humboldt County, California.

	Frequency of Occurrence	
Gap Number	upper reach	lower reach
4	3	0
5	1	0
6	2	2
7	4	1
8	2	0
9	3	1
10	1	0
11	2	1
12	0	1
13	0	0
14	1	0
15	0	1
16	1	0
17	0	0
18	2	0

DISCUSSION

Salmonids display dominance based on size (Newman 1956, Jenkins 1969, Wankowski and Thorpe 1979), with size differences in juvenile salmonids resulting from egg sizes or emergence time. Individuals that emerge sooner (Chandler and Bjornn 1988) and (or) hatch from larger eggs (Fowler 1972, Beacham and Murray 1985, Hutchings 1991) tend to be larger than individuals that emerge later and (or) hatch from smaller eggs. Dominant juveniles often establish territories for foraging, while some subordinate juveniles may become non-territorial foragers or may be displaced downstream (Nakano 1995). Martel (1996) found that non-territorial juveniles grew slower than territorial individuals, and Chapman (1966) established that non-territorial juveniles will form territories when presented with the opportunity to do so. Analysis of scales from juvenile coho salmon that reared in Freshwater Creek suggested that initial growth was slower for coho salmon that reared in the lower stream-estuary ecotone than for coho salmon that reared in the upper watershed. This may suggest that individuals rearing in the lower reach originate from smaller subordinate foragers that, after migrating downstream, are able to establish territories in the lower reach.

The difference in the number of circuli spaces on scales of juveniles between lower and upper reaches (22 versus 26, respectively) was most likely an artifact of collection times for the two locations. Lower reach samples were collected in summer, whereas upper reach samples were collected the following spring, allowing for the deposition of more circuli on the scales of upper reach juveniles.

Although growth differences could not be detected in scales of adult coho salmon, the analysis was most likely limited by the small sample size of adults from known rearing locations. Thus, the proportion of adult escapement representing the upper and lower reaches could not be determined. As mentioned previously, larger salmonid smolts have an increased chance of surviving size-selective predation upon entering the marine environment (Mathews and Buckley 1976, Bilton 1978, Olson 1978, Bilton et al. 1982, Holtby et al. 1990, Koenings et al. 1993). This would lead one to believe that larger juveniles rearing in the lower reach of Freshwater Creek should have an advantage in marine survival over the smaller upstream juveniles. However, the juveniles that reared upstream are heavier than their longer downstream counterparts (Ricker 2008, personal communication). Beamish et al. (2004) hypothesized that similarly sized juvenile coho salmon having greater or lesser proportions of lipid stores will survive their first winter at sea to a greater or lesser degree, respectively. Therefore, the greater weight of the smaller juveniles may put them on equal footing with the thinner, larger juveniles in terms of adult survival.

In the process of this study, evidence was obtained that supported observations that: 1) a summer growth check occurs prior to the “ocean entry” check on scales from coho salmon in this system; and 2) juveniles that rear in the upper watershed do not spend substantial amounts of time rearing in the lower reach before migrating to sea (Figures 5, 6, 8). Wallace (2006) reported yearling coho salmon marked at the inclined-plane trap had a mean residence time in the lower reach of 1.4 weeks (range between one to three weeks). The relationship between the number of circuli on scales of upper reach juveniles and on the juvenile growth portion of their adult counterparts suggests that little growth is occurring between the downstream migrant traps where the scales from juveniles were collected and “ocean entry”. Since the “ocean entry” check has not been defined for this system, this could be occurring farther downstream in the brackish portion of the watershed, in Humboldt Bay, or in the Pacific Ocean. Further investigation is necessary to clarify this. The smaller number of circuli in juvenile growth pattern of scales from adults may have resulted from resorption and (or) erosion leading to loss of peripheral rings (Jearld 1983) and (or) from the collection of scales at a less than “preferred location” on returning adults.

Identifying the occurrence of a growth check prior to the end of the first year of growth eliminates confusion of this feature with an annulus, thereby ensuring greater accuracy of aging estimates for coho salmon in this system. While the summer growth check appears to occur at two different points in time between the upper and lower reaches, this may simply be the result of faster growth in the lower reach, leading to the development of more circuli over a given period of time relative to the upper reach. It has been estimated that the rate of marine circuli deposition in salmon is four per month in spring and summer but only two per month in autumn and winter (Friedland et al. 1993). However, this estimate is confounded by the fact that no deposition or even resorption may occur during poor growth conditions (Bilton 1975). Also, early emergence and (or) greater size at emergence may explain differences in growth between individuals rearing in the upper and lower portions of the watershed (Rosenau 1991, Ramstad et al. 2003). A mark and recapture study of upper reach juveniles could help to differentiate between these possibilities. The variation in measurement and location of summer growth checks between juvenile and adult counterparts is likely attributed to individual variation between scales from a given individual.

One juvenile sampled in the tidally influenced portion of the creek had 28 circuli, substantially more circuli than the other individuals sampled, with a summer growth check at gap number 24. If this individual laid down circuli at the same rate as other juvenile coho salmon in the system, then this may lend support to observations of a two-year juvenile life history in the creek.

If lower reach juveniles do originate from non-territorial foragers that are able to find suitable habitat in which to establish territories in the lower reach, then restoration to improve habitat conditions within this reach may provide more opportunities for displaced juveniles to shift from slower growing non-territorial foragers to faster growing territorial foragers. Increased growth would allow these individuals to obtain larger sizes before migrating to sea, thereby, increasing their ability to avoid size-selective predation in the marine environment. Marking of juvenile coho salmon and subsequent recapture of adults in Freshwater Creek should, over time, provide a larger sample set for analysis that may be able to establish differences in growth potential between the two rearing locations, and the effect of rearing location on adult escapement.

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Appendix A. California Department of Fish and Game Freshwater Salmonid Monitoring Project scale analysis protocols.

I. Cleaning Scales:

1. Supplies: forceps; probes; pencil; finger bowl; dish soap; tepid water; Rite in the Rain paper; slides; adhesive tape
2. Cover surface of working area with black paper.
3. Tape a quarter sheet of Rite in the Rain paper to the desktop.
4. Fill a finger bowl with tepid tap water, add a drop of dish soap, and mix slowly to avoid causing bubbles.
5. Do not clean juvenile scales! Juvenile scales are too small to clean. They may get lost.
6. Scales have been dried in dehydrator for five hours. Therefore, in order to clean adult scales, soak them in the finger bowl.
7. For adult scales, wipe both sides of scales on Rite in the Rain paper to remove debris.
8. Clean finger bowl after each use to avoid mixing samples.
9. Clean slides before mounting scales.
10. Place 10 adult scales on a slide in two rows, orienting them consistently.
11. Use a dissecting scope to mount juvenile scales; place 20 juvenile scales on a slide in two rows.
12. Place a second slide on top of the first slide, and wrap adhesive tape around both ends of slides.

13. Place slides on white paper, and label the adhesive tape using a pencil.
14. Wipe off black paper to avoid mixing samples.

II. Photographing Scales:

1. Open Spot Basic ^{TM} using the icon on the desktop.
2. After the program opens, turn on the camera and wait for the toolbar icons to appear (about 10 seconds).
3. Turn on the light source.
4. Position slide-mounted scales on microscope so that the best scale is centered.
5. Make certain that the lever on the left of the microscope in set to photo/bino.
6. Click the Live button on the toolbar.
7. Adjust the focus of the image and the background lighting as needed. Click the Match Color button to make the image black and white. Click the dropper on the background of the image. Click the Set White Balance… button and okay. Click the Make White/Gray button and click okay.
    a. Suggested settings: lowest setting on the light source; mirror turned slightly; highest magnification level (6.3).
8. Note what magnification level is being used on the microscope.
9. Make certain the image fits within the Live window.
10. Click the camera icon on the toolbar to capture the image.
11. Save the image using a unique nomenclature that is descriptive of that sample.
    a. Include in the file name: species code; sample number (Fish no. or PIT tag no.); photograph number; magnification level (NOTE: “63” = 6.3)

12. Note: files were stored in the X: drive on the CDFG wetlab computer.

III. Making Measurements:

1. Open Image-Pro Plus ™ using the icon on the desktop.
2. Then open a new Excel ™ spreadsheet.
3. In Image-Pro Plus ™, open the saved image. Adjust the magnification by clicking the right mouse button and selecting the appropriate zoom level.
4. Click the Edit menu to access the Rotate option. Use this to align the scale image so that the longest axis is vertical.
5. Calibrate the image by using the Measure menu to select the Calibration option, then the Select Spatial… option.
    a. Select the appropriate magnification level in the dialogue box.
    b. Click okay.
6. Click the Best Fit Equalization button on the toolbar.
7. Click the Measure menu, and select the Caliper option.
    a. Set up Edge Detectors by clicking New.
        i. For type, select Derivative.
        ii. Click Select.
        iii. Click Detect Valleys.
        iv. Click OK.
    b. On the Measurements tab:
        i. Click the Measurements button.
            1. Click Add.

        2. Select Distance from sampler's origin.

           [Valley (A)]

        3. Click OK.

    ii. Click the Measurements button.

        1. Click Add.

        2. Select Distance between markers of an edge detector.

           [Valley (A)   Valley (A)]

        3. Click OK.

c. On the Input/Output tab:

    i. For data to output, select Measurements.

    ii. For destination, select DDE to Excel.

    iii. Click Options… button.

        1. Select Active Sheet.

        2. Input the appropriate row and column into which the data set will be exported in the Excel ™ spreadsheet.

        3. Select Append next data set to bottom.

        4. Click OK.

d. On the Options tab, input or select the following:

    i. Smoothing: 11

    ii. Thickness: 1

    iii. Calibration: Use spatial calibration

    iv. Marker: Show label

        - v. Precision: 6 decimal places
    - e. Use the line tool and the transparency overlay to make a reference line at a 20 degree angle to the vertical axis.

8. Zoom in on the line by right clicking and selecting zoom in.
9. Adjust the end points if necessary.
10. Select the red minus icon to add or remove tick marks.
    - a. To remove, click on a red tick mark.
    - b. To add, click on the yellow line.
11. Export data to Excel ^{TM} by clicking the Send Data button on the Input/Output tab.
12. In the Excel ^{TM} spreadsheet:
    - a. Type Fish no., PIT tag no., file names, and/or other identifying information.
    - b. Delete excess information.
    - c. Save the Excel ^{TM} file using a unique nomenclature that is descriptive of that sample. It is also useful if the file name of the data set reflects the file name of the image it was acquired from.
    - d. SAVE OFTEN!
13. Note: files were stored in the X: drive on the CDFG wetlab computer.