# Characterization of Development in Columbia River Prolarval Eulachon, *Thaleichthys pacificus*, Using Selected Morphometric Characters.

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## CONTENTS

## INTRODUCTION

Active spawning of eulachon, *Thaleichthys pacificus*, has been observed in various tributaries of the Lower Columbia River, including the Cowlitz River, the Kalama River, the Lewis River, and the Sandy River (Smith and Saalfeld 1955). However, little is known about the spawning distribution of eulachon in the Lower Columbia River mainstem itself.

Catches of very recently hatched eulachon larvae in plankton net hauls might indicate proximal spawning grounds and hence provide a useful tool for mapping and defining spawning habitat distribution in the mainstem Columbia River. To achieve this, developmental observations from larvae of known ages are required to provide a baseline against which larval assemblages collected in the field can be compared.

Upon hatching, larvae are incorporated into the drift and, depending on local current velocities, are presumably transported substantial distances from their spawning grounds in relatively short periods of time. For an aging methodology to be an effective tool in pinpointing spawning areas, short-term developmental changes - measured on an hourly time scale - must be identified.

Eulachon larvae have been successfully propagated by several workers for various studies that include: spawning substrate preference (Wendler 1937); assessment of possible population heterogeneity in the Lower Columbia River and its tributaries

(Delacy 1963); and effects of temperature on incubation periods (Smith and Saalfeld 1955). Parente (1970) provides a pictorial record and discussion of egg and (to a limited extent) larval development. However, a systematic, quantitative assessment of eulachon larval development has not been previously described as it has for other, related species such as the Rainbow smelt, *Osmerus mordax*, (Cooper 1978). Given the lack of information regarding eulachon larval development our goal was to artificially propagate larvae, establish a time/temperature dependent developmental baseline and subsequently evaluate the efficacy of our proposed methodology.

## METHODS

### Artificial Propagation

Adult brood fish were collected with dip-nets from the Cowlitz River (March 6, 2001) and Sandy River (March 14, 2001). Broodstock collected from the Cowlitz River (39 males, 42 females) were transported to facilities of the Oregon Department of Fish and Wildlife (ODFW) in Clackamas and held in large circulating tanks (males and females separated) until ripe. The fish matured rapidly in the holding tanks - probably a result of increased water temperatures observed in the Clackamas spring water (57 $^{o}$F) relative to the Cowlitz River (43 $^{o}$F). Despite the separation of the sexes, all of the females extruded their eggs and subsequently died during the night of March 11, 2001. However, one female from this batch had been sacrificed for initial fertilization experiments March 8. Broodstock collected from the Sandy River (15 males, 12 females) were ripe upon collection and were artificially spawned March 14 and 15, 2001.

Eggs were manually stripped from females into glass Petrie dishes and covered with milt from ripe males. Water was added to activate the spermatozoa and thus initiate fertilization. The eggs/milt were gently stirred with the caudal fin of a eulachon (Wendler 1937) to ensure adequate mixing of sex products.

After a short period (minutes) the eggs were gently washed with fresh water and then transferred to two incubation environments consisting of 1) McDonalds jars supplied with water from a closed system cooled by a portable chiller unit to 48$^{o}$F, and 2) water filled petrie dishes placed into a walk-in chiller to incubate at a constant temperature of 48 $^{o}$F. During the incubation period, water in the petrie dishes was changed daily and dead/fungused eggs were removed. All water used in the propagation experiments came from the local spring at the ODFW Clackamas complex.

At the peak of hatching, larvae were transferred to water filled Petrie dishes so that each dish contained larvae emergent within a 30-minute time period. Larvae were then transferred to the chiller and allowed to develop. Chiller temperature was adjusted to 53 $^{o}$F at this time to reflect that observed contemporaneously in the Columbia River.

Larvae were preserved in 10% buffered Formalin at post-hatch ages of 0, 6, 12, 24, 36 and 48 hours and then subsequently at intervals of 24 hours until total yolk sac absorption. Preservation of the 24-hour age class failed and so no results from this time period are included.

## Morphometric Characters

Basic morphometric observations were chosen to characterize larval development. Morphometric measurements included total length, snout to vent length, and yolk sac length (Figure 1). Measurements were made with an ocular micrometer read to the nearest 0.1mm. Ten larvae per age class were evaluated.

Measurements were also obtained from larvae taken in plankton net tows from the Cowlitz River and the Lower Columbia River shipping channel in the vicinity of Price Island (River kilometer 55). Fifty individuals from each sample were randomly selected for characterization. These measurements were taken to compare development in larvae taken from a known spawning area (Cowlitz River) against those from a location in the study area assumed to be substantially downstream from major spawning areas.

## Data Analysis

Analysis of Variance (ANOVA) procedures were used to test for significant differences in yolk sac length between age classes of propagated larvae. Tukey's multiple comparison procedure was used to isolate differences among groups. Linear regression analyses were conducted to define at-hatching values for each of the morphometric characters. T-tests were used to test for significant differences in morphometric characters between larvae taken from the Cowlitz River and the Columbia River. All statistical procedures were carried out using SigmaStat 2.0 software. Tests were conducted at $\alpha = 0.05$ level of significance.

## RESULTS

The majority of the fertilized eggs (approximately 60 thousand eggs) were placed in the McDonalds jar system to incubate. No eggs survived after a day in this environment due to the failure of the chiller unit and stresses induced by turbulence on the eggs. Eggs incubated in the petrie dishes were initially subject to high mortality and fungusing as a result of overcrowding. We reduced egg densities and mortality was reduced.

Eggs fertilized March 8 began hatching April 24 and continued through April 29. Eggs fertilized March 14 began hatching May 01 and continued through May 10. For each batch first hatch occurred approximately 47 days after fertilization. Water temperature throughout this period was a constant 48 $^{o}$F. Using 32 $^{o}$F as the assumed biological zero for eulachon (Delacy and Batts, 1963) the eggs accumulated 752 Thermal Units (TU's) before hatching.

Most larvae were observed to emerge tail first from their egg casing. Initial observations showed that the time between rupture of the egg membrane and full emergence of the larvae varied widely from a few minutes to several hours (see Parente, 1970 for a pictorial record of a eulachon larva hatch sequence). Many individuals at this stage showed a marked curvature in the anterior portion of their bodies (probably a remnant of coiling in the egg) and gradually assumed a straighter form after approximately 72 hours (Figure 2). Coiling proved problematic for obtaining true measurements of total and snout to vent lengths. Data for these characters appears to

show a relatively rapid rate of growth from hatching to approximately 72 hours after which length increases are less pronounced (Figure 3). This is attributable to coiled larvae straightening out over time (Figure 2). Yolk sac measurements were unaffected by coiling and showed a more linear trend over time (Figure 3).

Larvae were strongly attracted to, and stimulated by, light sources. When placed in water filled beakers most individuals exhibited pelagic swim-up behavior, remaining at the water surface for extended periods. Yolk absorption was complete in larvae 21 days post hatch and few individuals survived beyond this age.

Mean total length of larvae at hatching (0 hours) was 4.3 mm (+/- 0.51 SD). Mean snout-to-vent length at hatching was 3.0 mm (+/- 0.82 SD). Mean yolk sac length at hatching was 0.86 mm (+/- 0.14 SD). However, at hatching total length and snout-to-vent length means were assumed invalid due to imprecise measurements taken from coiled individuals. Linear regression analysis of total length and snout-to-vent length with 0, 6, 12 and 36-hour age class data removed gave a total length at-hatching value of 5.7mm ($R^2$=0.372, SE = 0.39) and snout to vent length at-hatching value of 4.1 mm ($R^2$=0.333, SE=0.38). Linear regression analysis of all yolk sac data gave an at-hatching length of 0.8 mm ($R^2$ = 0.746, SE = 0.118). ANOVA showed no statistically significant differences in mean yolk sac length between age classes 0, 6, 12, 36, 72 and 96 hours post-hatch (Tukey multiple comparison, $P > 0.05$).

Significant differences between the mean values of each morphometric character were observed between larval assemblages collected in the Cowlitz River and in the Columbia River in the vicinity of Price Island (t-tests, $P < 0.001$ in all cases; Table 1 and Figure 4).

## DISCUSSION

Our results show that changes in larval morphology are not identifiable over a time scale of even a few days. Since it is likely that currents in the Columbia River would carry larvae substantial distances (kilometers) in the matter of only a few hours it appears unlikely that spawning areas could be located using our proposed methodology. However, morphometric data from larvae collected from the Columbia River and Cowlitz River suggest that identifiable development does occur as larvae out-migrate to the estuary and ocean (Figure 4, Table 1).

In this study eggs accumulated 752 TU's before hatching – a figure markedly greater than that observed in previous investigations. Smith and Saalfeld (1955) reported TU's of 378 & 369.6 from hatchery experiments performed in 1946 (Kalama River Hatchery) and 1949 (University of Washington School of Fisheries) while Delacy and Batts (1962) found a range of 349.7 to 387.9 TU's in their investigations. Wendler (1937) reported larvae hatched 24 days after fertilization at a mean water temperature of 40.7 $^{o}$F during incubation - translating to approximately 209 TU's. Adult eulachon first entered the Cowlitz River in the first week of March this year (personal observation) and plankton net sampling by staff of Washington Department of Fish and Wildlife showed

larvae were present in substantial numbers during the last week of March – approximately three weeks after the arrival of adults. Water temperature in the Cowlitz during this time was around 48°F. This leads to a very rough estimate of 336 TU's for egg incubation in the Cowlitz this year (21 days at 48°F), a figure close to those reported in previous experiments.

It is unclear why the incubation period was so protracted in our study although, in our experiment, eggs were incubated in the absence of light, continuous water exchange, and temperature fluctuations - relatively static environmental conditions compared to those in all previous studies and under natural conditions. Environmental conditions in our experiment may also have protracted the development of larvae. In Parente (1970) a photograph of a six-day-old eulachon larva shows almost complete utilization of yolk sac contents – a stage only reached after almost 21 days for larvae in our experiment.

The remnant coiling observed in many hatchlings in our experiment could be considered a useful qualitative descriptor of larval age. However it was not seen in any larvae collected from the Cowlitz River despite the close proximity of the sampling location to spawning areas. This suggests the characteristic was also an artifact of our experimental conditions.

Repetition of this experiment with propagation conducted under more natural conditions might lead to increased developmental rates allowing changes to be identified over short time periods. However, the high morphological variability in individuals

observed in each age class in our study (Figure 3) as well as those larvae taken from the Cowlitz River (a site where all individuals are presumably of very similar ages; Figure 4) might still preclude this.

## ACKNOWLEDGMENTS

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Table 1. Morphometric measurements (mm) of larval eulachon taken from the Cowlitz River and Columbia River during April, 2001. Each couplet gives the mean and one standard deviation (in parentheses).

| | N | Total Length | Snout to Vent Length | Yolk sac Length |
|---|---|---|---|---|
| Cowlitz River | 50 | 5.5 (0.5) | 4.0 (0.4) | 0.9 (0.1) |
| Columbia River | 50 | 6.1 (0.4) | 4.5 (0.3) | 0.5 (0.1) |

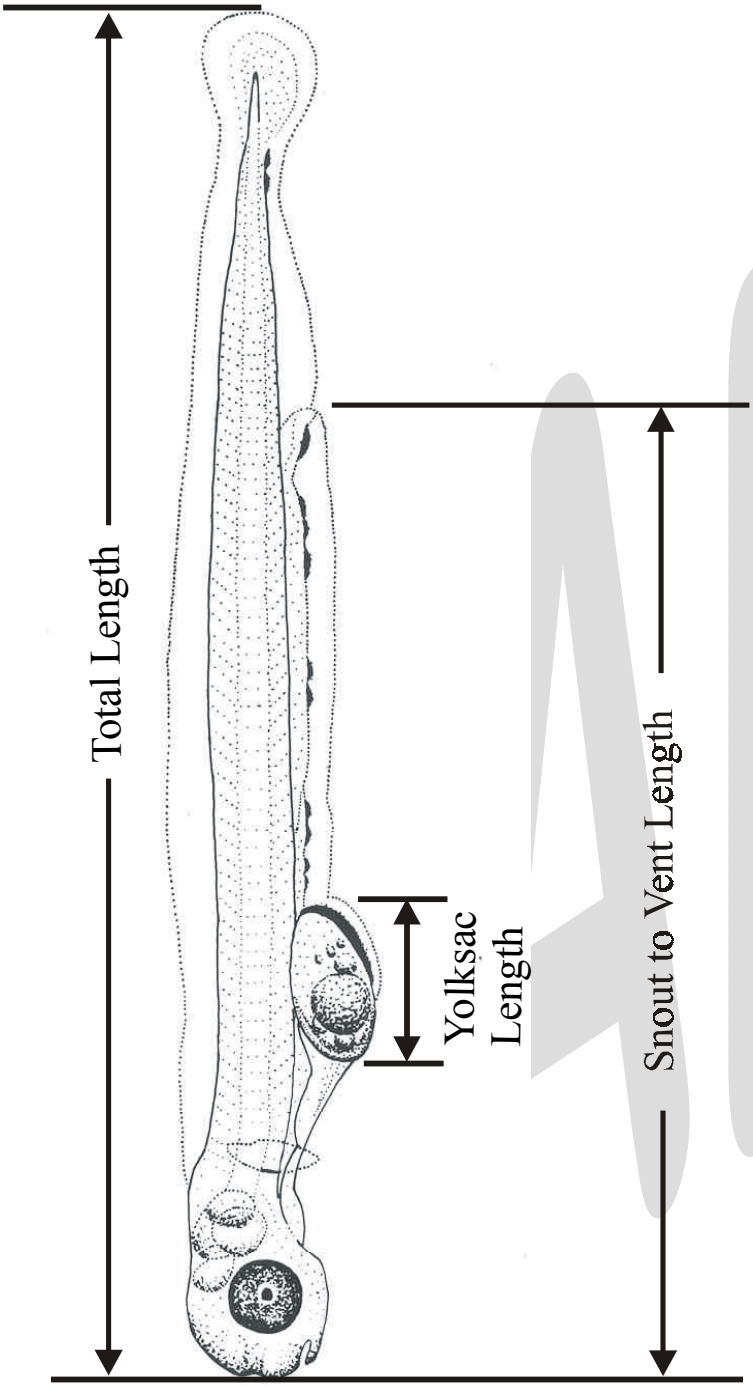
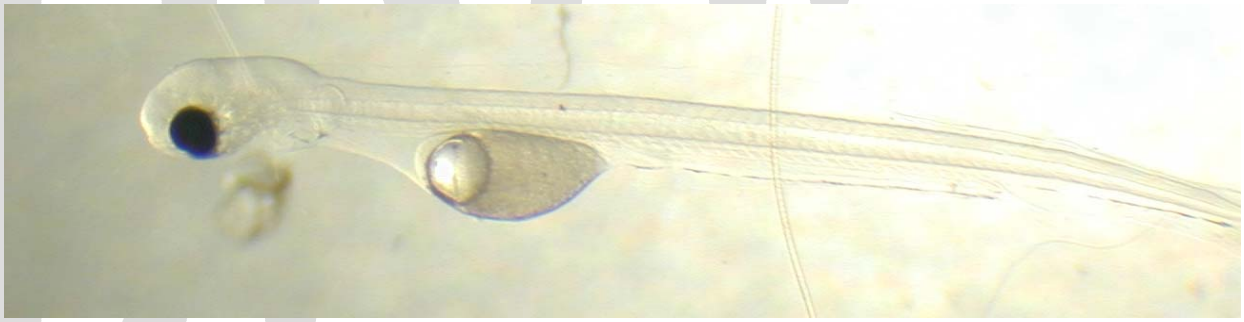



Figure 1. Morphometric characters used in eulachon larval development study.

0 Hours post hatch.

36 hours post hatch.



72 hours post hatch.

Figure 2. Diminishment of remnant egg coiling over time in artificially propagated larval eulachon.

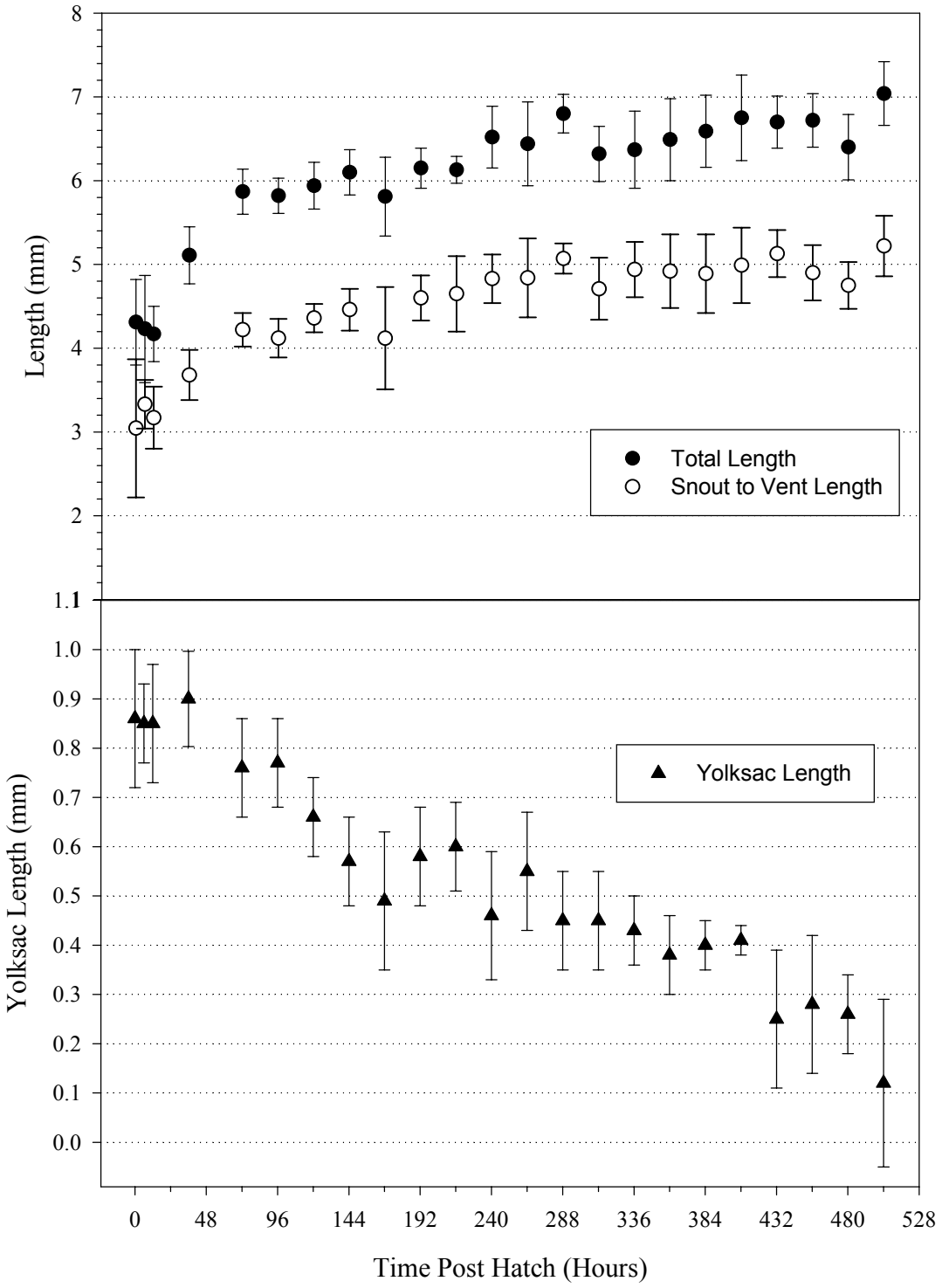



Figure 3. Morphological changes in artificially propagated eulachon larvae over time. Individual plots are means with error bars representing 1 standard deviation.

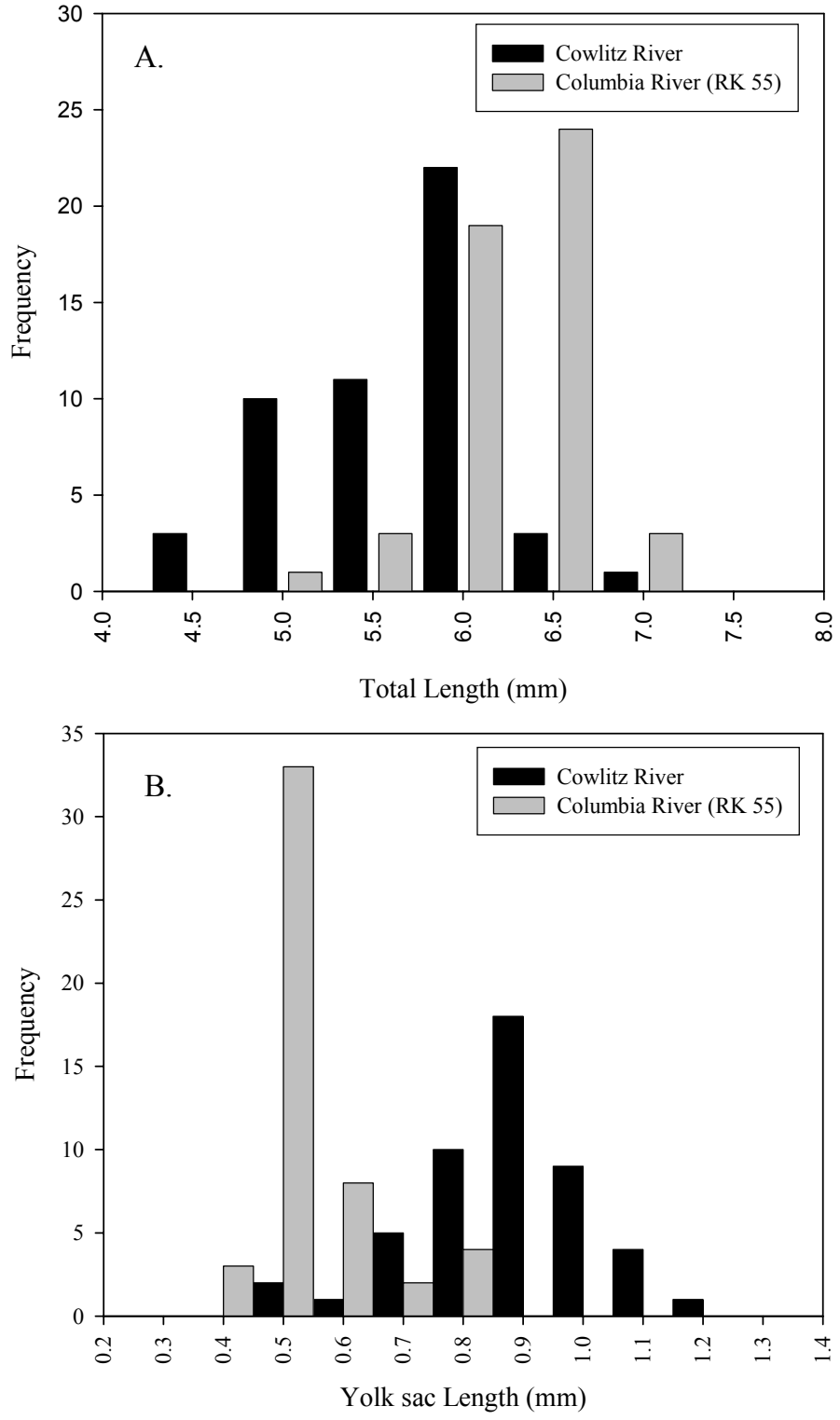



Figure 4. Length frequency distributions for A) total length and B) yolksac length in prolarval eulachon taken in plankton net tows in the Cowlitz and Columbia Rivers during April, 2001. N = 50 larvae for each location.