DEVELOPMENTAL STUDY OF BROOK, BROWN, RAINBOW, AND CUTTHROAT TROUT LARVAE

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ABSTRACT

A detailed comparative study of the larvae of brook, brown, rainbow, and cutthroat trouts is being conducted to determine and describe the obvious morphological characters of diagnostic value in the identification of collected specimens. Cultured series for each species contained approximately 50 specimens with developmental stages from just-hatched mesolarvae to recently transformed juveniles. These series are being subjected to rigorous morphometric and meristic analyses as well as detailed examinations of specific structures, melanophore pigmentation patterns and the chronology of selected ontogenetic events. The methodology for this study is briefly discussed herein. Although the project is still underway, preliminary observations of pigmentation and oil droplets in the yolk indicate that these characters are of diagnostic value for segregating the larvae of brook and brown trout from the others and each other.

In the management of specific species of fish or bodies of water, as well as the monitoring of the impacts of man's modifications of these waters it is necessary and useful to study the distribution, abundance, and general biology

of larval fish. Unfortunately such investigations, even for game species, can be precluded or limited by an inability to identify fish larvae. Such is often the case with even the more widely distributed salmonids such as the brook, brown, rainbow, and cutthroat trouts (Salvelinus fontinalis, Salmo trutta, Salmo gairdneri, and Salmo clarki respectively).

Although the larvae of all but the cutthroat trout have been described to some degree (Bacon 1954, Crawford 1925, Knight 1963, Lister 1980, Wales 1941), these descriptions are largely incomplete and inadequate for identification purposes. Perhaps the most useful literature to date for distinguishing the brook, brown and rainbow trouts is provided by Marcinko (1978) and Weisel (1966). Larval cutthroat trout are especially similar to the larvae of closely related rainbow trout and are reputed to be especially difficult to separate.

The objectives of this investigation are to provide detailed and comparable descriptions of the forementioned trout, verify the diagnostic characters suggested by previous investigators, and determine additional and perhaps more obvious and consistant differences for segregation of the larvae and early juveniles of these fishes. The purpose of this paper is to briefly report on the approaches and methods used in conducting this study. Although the project is still underway, the results of some pigment pattern and oil droplet observations are presented herein with a few general comments on trout development.

SPECIMENS EXAMINATED

Approximately 50 specimens from just hatched to early juvenile stages were examined for each of the four species. All were obtained from cultured sources between 1976 and 1982 and were preserved in 3% buffered formalin. All but the rainbow trout series were reared at approximately 12 C from fertilization or

an eyed stage at the Colorado Division of Wildlife Hatchery at Bellvue. The brown and cutthroat (greenback subspecies, S. c. stomias) trout originated from eggs taken from Colorado brood stock in Delaney Butte Lake and Island Lake, respectively. The brook trout came from California's Mount Whitney Hatchery where they were incubated at 6-9 C to an eyed stage prior to shipment to the Bellvue hatchery. The rainbow trout (Tasmanian strain) were originally fertilized and incubated at 6 C at Colorado's Rifle Falls Hatchery, then transferred as eyed eggs to Colorado State University where they were subsequently reared at 15-17 C. Two juvenile rainbow trout (Arlee strain) were also studied. These originated from brood fish at Colorado's Crystal River Hatchery and were subsequently reared at the Bellvue Hatchery.

METHODS

Equipment

Specimens were examined and measured under a variable power dissecting microscope with a 10 mm eyepiece reticle, polarizing filters and both transmitted and reflected lighting as needed. Due to the large size of trout larvae it was necessary to use a supplemental 0.5X objective lens to halve the power of magnification. For measuring, the scope was first focused on the specimen at approximately 5X or 10X, depending on whether the eyepiece reticle was to be calibrated as a 10 mm or 20 mm scale, respectively. The scale in the reticle was then calibrated against a stage micrometer positioned in the plane of focus by adjusting the variable power control. The polarizing filters were of limited value in counting the myomeres of these relatively large and thick bodied larvae, but they were useful in illuminating the fin rays and pterygiophores.

Morphometrics

Morphometric analysis consisted of the 32 specific measurements illustrated in Figures one through three. Lengths were measured from the anterior margin of the snout to a specific structure or point along imaginary lines parallel to the horizontal axis of the body (Fig. 1). The distance between any two points of reference is simply determined by subtraction (e.g. length of the base of a fin is the measurement to the fin's insertion minus the measurement to its origin). Fin lengths are measured as the maximum distance between the origin of the fin (anterior most point of attachment) and its most distal margin (Fig. 3). Depths and widths were measured perpendicular to the horizontal axis of the body (Fig. 2). With one exception (AMPM), the location of depth and width measurements correspond to specific points of reference for specific length measurements. Typically recorded to the nearest tenth of a millimeter, the measurements are later converted to percent standard length to facilitate comparison between specimens of different sizes.

Meristics

The meristics considered in this study included fin ray and myomere counts. Fin ray counts included both principal and secondary elements (Fig. 3) and were recorded in Arabic and lower case Roman numerals respectively. Myomere counts were made from the most anterior unit, often somewhat deltoid shaped and located immediately behind the occiput, to a specific point or structure of reference. All myomeres transected in any part by an imaginary verticle line from that point of reference were included in the count (Siefert 1969). To make myomeres more visible, it was sometimes necessary to gently scrape away heavily pigmented surface tissues. Several specimens were cleared with trypsin

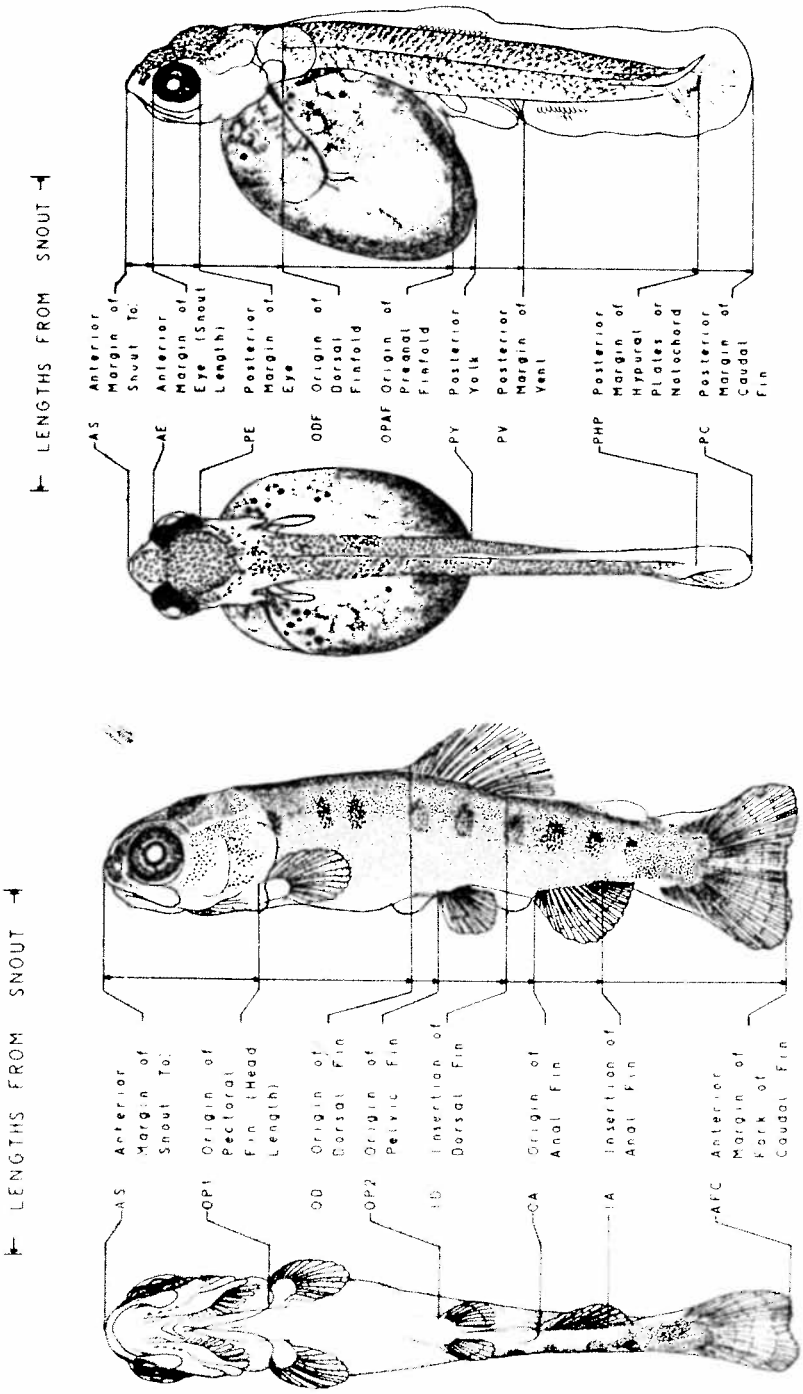

FIGURE 1. Length measurements for morphometric analysis of salmonid larvae and early juveniles. All are measured from the anterior margin of the snout to a specific point of reference and made on all developmental stages in which the referenced structure exists.

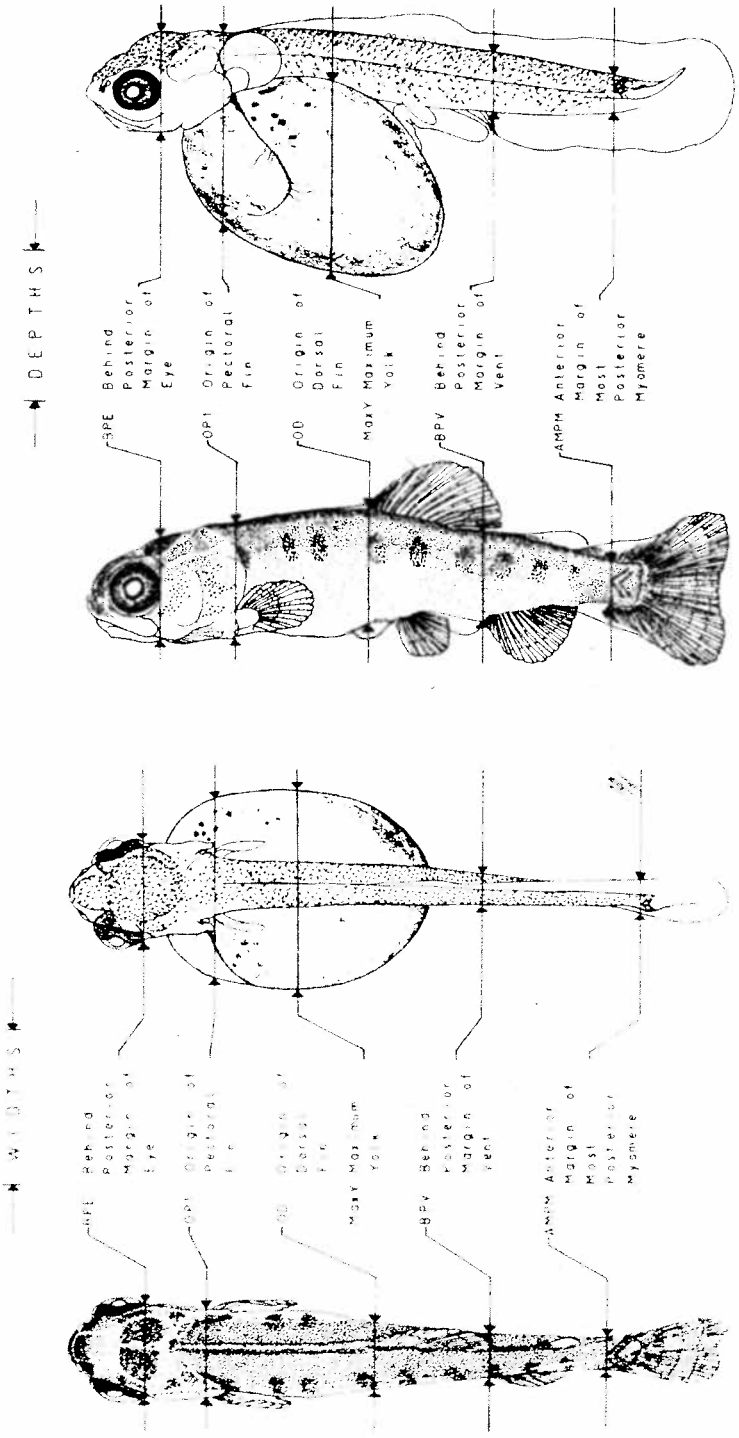

FIGURE 2. Depth and width measurements for morphometric analysis of salmonid larvae and early juveniles.

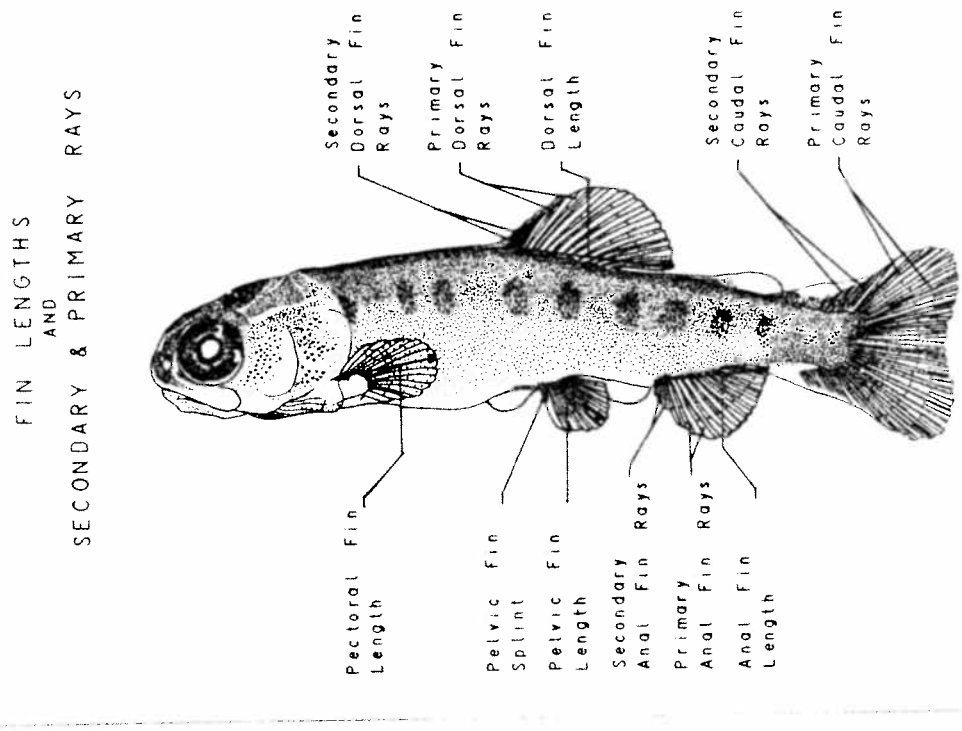

FIGURE 3. Fin length measurements for morphometric analysis and primary (principal) and secondary rays as differentiated for meristic analysis of salmonid larvae and early juveniles.

and glycerin and stained with alizarin red as described by Taylor (1967) to verify fin ray and total myomere counts.

Total myomere counts correspond almost one to one with counts of total vertebrae (Fish 1932, Snyder 1981) and were verified accordingly. Vertebral counts included the first unit which is fused to the cranium but excluded the last three centra which comprise the urostyle (Vladykov 1954). Occasionally observed compound or fused vertebrae were easily distinguished by the presence of two hemal or neural spines, and were counted as two units.

Analysis of Morphometric and Meristic Data

Following the developmental terminology recommended by Snyder (1976 and 1981) most of the specimens were designated as mesolarvae, metalarvae or early

juveniles (a few prematurely hatched specimens lacked median fin rays and thereby qualified as protolarvae). Most of the morphometric and meristic characters were summarized according to developmental phase. Selected length measurements were also graphed with standard length of the specimens on the y-axis and percent standard length for specific characters on the x-axis, thereby providing a visual representation of relationships between the various measures as the fish increase in size. These graphs were done on transparent mylar sheets to allow direct comparison between species by overlay. Selected data is also being subjected to discriminant function analysis.

Other Characters

Other characters specifically considered in this investigation included the shape or form of the yolk-sac and oil globules, melanophore pigmentation patterns and the size at which specific developmental events occur. Emphasis was placed on the more potentially diagnostic structures, pigmentation, and events.

SOME PRELIMINARY RESULTS

The trout studied typically hatched at approximately 10-14 mm TL (total length) with fin rays already evident in the developing caudal fin thereby skipping the protolarval phase and beginning the larval period as mesolarvae. The mesolarval phase was short in duration and distinguished from metalarval phase by the lack of a full adult complement of principle rays in the dorsal, anal and caudal fins. The pelvic fin buds, also required prior to transition to the metalarval phase, developed before or shortly after hatching. The absorption of the preanal finfold was the last of the criteria required

for transition to the juvenile period and occurred, depending on species, between 30 and 50 mm TL.

The preliminary results of observations on oil globules and pigmentation are summarized in Tables 1 and 2. Among the mesolarvae and earlier metalarvae, which possessed a substantial amount of yolk, differences were observed in the size and abundance of various size oil droplets near the surface of the yolk. Differences in pigmentation were particularly obvious on the dorsal fin, adipose fin, caudal fin, the pterygiophore ridge of the anal fin, and the lower jaw. Both sets of characters have diagnostic value for distinguishing the larvae of brook and brown trout from each other and from the rainbow and cutthroat trout. However differences in these characters between the rainbow and cutthroat trout were inadequate for identification purposes.

DISCUSSION

Much of this investigation is patterned after that presented by Snyder (1981) for cypriniform fish larvae but with modifications and additions to accommodate the unique characters of larval Salmoninae. Snyder provides a detailed discussion of characters useful in the identification of cyprinid and catostomid larvae; some of these are expected to be useful for salmonid larvae as well.

Upon completion of this investigation, the results are expected to reveal other and in some instances more obvious and definitive diagnostic characters than has been presented by previous researchers (see references in introduction) or in the preliminary results herein. The results will be initially prepared as a graduate thesis and subsequently prepared for publication in a technical journal, and as part of a series of Larval Fish Identification Circulars planned by the Colorado State University (CSU) Larval Fish Laboratory (Snyder 1980).

TABLE 1. Summary of melanophore pigmentation patterns on selected structures for separating brook, brown, rainbow, and cutthroat trout larvae. All length measurements refer to total lengths

Size Range Examined	Brook 12-32 mm	Brown 12-30 mm	Rainbow 12-44 mm	Cutthroat 10-44 mm
Dorsal	-Light pigmentation on the anterior margin, ≥ 16 mm	-Light pigmentation on the anterior margin, ≥ 22 mm	-Bold pigmentation on the anterior margin, > 22 mm	-Bold pigmentation on the anterior margin, ≥ 19 mm
Adipose Fin	-Bold pigmentation on the posterior margin, ≥20 mm	-Scattered pigmentation over entire fin with no areas of concentration, > 29 mm	-Light pigmentation on the posterior margin, ≥ 23 mm	-Light, inconspicuous pigmentation on the posterior margin, ≤ 37 mm -Distinct pigment on the posterior margin > 37 mm
Anal Pterygiophores	-Dense pigment on the anterior anal pterygiophores, > 21 mm	-Pigment forming shallow "V" which envelopes the anus, pointing posteriorly ≥ 23 mm	-Scattered pigment with no areas of concentration, > 23 mm	-Scattered pigment with no areas of concentration, > 20 mm
Caudal Fin	-Heavy pigment on the area of the horizontal midline, 12-32 mm	-Scattered pigment tended to line the distal portions of the principle rays ≥ 19 mm	-Pigment scattered distally with no pattern or areas of concentration, > 22 mm	-Pigment scattered distally with no pattern or areas of concentration, ≥ 31 mm
Chin and Anterior Margin of the Mandible	-Very few to no pigment on the chin, ≥ 15 mm -Dense pigment on the anterior margin of the mandible, ≥15 mm	-Scattered pigment on the chin, > 17 mm -Dense pigment on the anterior margin of the mandible, ≥ 22 mm	-Scattered pigment over entire mandible with no areas of concentration, > 23 mm	-Scattered pigment over entire mandible with no areas of concentration, ≥ 21 mm

TABLE 2. Summary of the abundance and size distribution of oil droplets observed on the surface of the yolk of mesolarvae and earlier metalarvae of the brook, brown, rainbow, and cutthroat trout. All measurements are of oil globule diameters.

	Brook	Brown	Rainbow	Cutthroat
OIL GLOBULES:				
< 0.5 mm	very numerous none larger than about 0.4 mm	many	many	many
0.5-1.0 mm	none	several	several, seldom over 0.8 mm	rarely larger than ~0.5 mm
> 1.0 mm	none	0-4	none	very rare, only in one specimen

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