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# Embryonic and Larval Development of Sacramento Splittail, *Pogonichthys macrolepidotus*

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## ABSTRACT

Embryonic and larval development of Sacramento splittail (*Pogonichthys macrolepidotus*) was characterized from zygote to metamorphosis in laboratory conditions. Fertilized eggs were obtained from induced and natural tank spawning of adults caught in the Yolo Bypass of the Sacramento River. Splittail produced transparent adhesive eggs with a moderate perivitelline space. Duration of embryonic development from fertilization to hatching was 100 h at 18 ± 0.5 °C. Newly hatched larvae were 5.2 to 6.0 mm total length with no mouth opening. Yolk-sac larvae were demersal and absorbed the yolk within 10 days post-hatch. Exogenous feeding started at 6 days post-hatch, concomitant with swim bladder inflation and swim-up movement. Fin differentiation began at approximately 10 d post-hatch (ca. 8.3 to 8.85 mm total length) and was completed at 50 d post-hatch (ca. 19.6 to 20.85 mm total length) when larval finfold was fully resorbed and the adult complement of fin rays was present in all fins, but scales were still lacking.

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## KEY WORDS

splittail, embryo, larva, development

## INTRODUCTION

Sacramento splittail is a cyprinid endemic to California's Central Valley and the San Francisco Estuary (Meng and Moyle 1995; Moyle 2002). It is the only extant species in genus *Pogonichthys,* after the extinction of the congeneric Clear Lake splittail *P. ciscoides* in the 1970s (Moyle 2002). Splittail has been listed as a species of special concern by the California Department of Fish and Game since 1989, then as threatened by the U.S. Fish and Wildlife Service in 1999, primarily due to low population abundance during drought years in 1987 to 1992 (Meng and Moyle 1994, 1995; Sommer and others 1997, 2007, 2008; Moyle and others 2004). The species was removed from the federal list of threatened species in 2003, after revision of population abundance and distribution (USFWS 2003). In April 2010, the U.S. Fish and Wildlife Service initiated a new status review to determine whether listing of splittail was warranted (USFWS 2010a). The review concluded in October 2010 that splittail does not warrant protection under the Endangered Species Act because no recent declines in abundance and distribution sig-

nificantly threaten the species at the population level (USFWS 2010b).

Sacramento splittail is one of the relatively abundant native species which has a significant ecological role in the San Francisco Estuary (Meng and others 1994; Matern and others 2002; Feyrer and Healey 2003). There is an ample literature on the splittail life history and reproductive ecology (Daniels and Moyle 1983; Wang 1986; Meng and Moyle 1995; Sommer and others 1997, 2008; Moyle 2002; Moyle and others 2004). Adult splittail consume benthic invertebrates in the Sacramento–San Joaquin Estuary and usually reach sexual maturity at the age of two years. In December–March, they migrate upstream to spawn on seasonally inundated floodplains. Spawning was observed in March and April. By June, most juveniles migrate downstream to tidal freshwater and brackish waters, where they spend 1 to 2 years before migrating upstream to spawn. Reproductive success of splittail depends on spring flooding and the availability of zooplankton for early life stages (Sommer and others 1997, 2008; Moyle and others 2004). Laboratory experiments revealed that splittail tolerates a wide range of temperatures, low dissolved oxygen, and moderate salinity (Young and Cech 1996). In spite of its environmental tolerance, splittail is clearly affected by ecological changes in the San Francisco Estuary (Moyle 2002), and by modifications of spawning and nursery habitats (Feyrer and others 2005).

Some features of splittail embryos and larvae were described in previous reports (Wang 1986, 1995; Bailey 1994). Splittail eggs have adhesive and transparent chorion and yellowish and granular yolk. Field observations indicate that the eggs attach to submerged vegetation in flooded areas where they undergo development (Moyle 2002). Wang (1986, 1995) described the morphology of several larval stages collected in the field. However, developmental time-frames with stage-by-stage descriptions of embryonic and larval development of splittail have not been reported. Developmental time-frames and behavioral observations can provide basic information to support management strategies that maintain how long floodplains are inundated, which is critical to sustain recruitments of splittail population.

The purpose of this paper is to more completely characterize early development of splittail, from fertilization to the juvenile period. This knowledge of splittail development will be useful in research on reproductive ecology, toxicology, and environmental requirements for spawning and nursery habitats. Developmental time-frames are also important for fisheries modelers and managers to predict population dynamics.

## MATERIALS AND METHODS

Six females and four males (30 to 42 cm in total length, TL; 204 to 608 g in wet weight; ca. 3 to 4 years old) were collected in January 2003 by the California Department of Water Resources using a fyke trap in the Yolo Bypass of the Sacramento River. Fish were immediately transported to the Center for Aquatic Biology and Aquaculture, University of California, Davis, and placed in a 1.85-m diameter circular tank supplied with well water at a constant temperature of 18°C. The fish were fed Silver Cup trout feed (Nelson & Sons, Inc., UT) at 2% body weight per day, using an automatic belt feeder. In mid-February, artificial spawning substrates (Spawntex, Aquatic Eco-Systems, Inc., FL) were lined along the tank wall, with two plastic boxes placed on the tank bottom for egg collections. Spawning occurred during the early morning hours of February 21, five days after the placement of the spawning substrates. Approximately 20,000 adhesive eggs were collected from the spawning substrate. Several developmental stages of the naturally spawned eggs were undocumented due to the early-morning spawn, so additional batches of eggs with a known fertilization time were obtained by inducing gamete maturation with injection of 10 to 20 μg $kg^{-1}$ of gonadotropin-releasing hormone analog [D–$Ala^6$, $Des–Gly^{10}$]–LH–RH Ethylamide (Peninsula Laboratories, CA), followed by stripping and artificial fertilization.

Eggs were incubated in beakers or glass trays in water bath at 18 ± 0.5 °C under a natural photoperiod. Containers were aerated with 75% water renewal twice a day. Unfertilized and dead eggs were manually removed to prevent fungal growth. Hatched larvae were reared at the same temperature in flow-

through rectangular plastic containers. They were fed a pelleted diet (Deng and others 2002) supplemented with cultured rotifers (*Brachionus plicatilis*) and brine shrimp nauplii.

The description of the embryonic stages of splittail follows that of Kimmel and others (1995) for zebrafish, *Danio rerio*, a cyprinid species commonly used as a laboratory model. A similar description of stages is frequently applied to other species, sometimes in a different order of teleosts (e.g., the perciforme Nile tilapia, *Oreochromis niloticus*; Morrison and others 2001). Timing of each stage was recorded when more than 50% of individuals in a sample showed similar characteristics of the stage. Larvae were sampled at 0, 1, 3, 6, 10 days post-hatch (dph) and at a 10-day interval until 50 dph. The developmental periods for larvae are divided into pre-flexion, flexion, and post-flexion phases based on caudal notochord flexion (Kendall and others 1984; Blaxter 1988; Snyder and others 2004).

Embryos and larvae were anesthetized in a bath of 100 mg $L^{-1}$ solution of tricaine methanesulfonate (MS222, Argent Laboratories, WA) and photographed using a digital camera (Nikon Coolpix 950) mounted on an ocular lens on an Olympus SZX12 dissecting microscope. An additional ten larvae of each stage were euthanized with an overdose of anesthetic, and fixed in 10% phosphate-buffered formalin for measurements and histology. Two to three subsamples of selected stages were dehydrated in a graded ethanol, embedded in JB–4 glycol methacrylate (Polysciences, PA), serially sectioned at 5 µm, stained with toluidine blue, and photographed under a BH–2 Olympus microscope. Egg diameter and larval total length (TL) were measured under a dissecting microscope using a digital image-analyzing tablet (Nikon Microplan II, ± 0.01 mm), or a micrometer caliper for larger animals. Spawning, handling, and rearing of animals followed the Animal Care and Use Protocol, approved by the University of California, Davis. Terminology of embryonic and larval development follows ZFIN (*http://zfin.org/zf_info/zfbook/stages/gloss.html*) and Synder and others (2004).

## RESULTS

### Embryonic Development

The timing and main characteristics of embryonic stages are given in Table 1. The duration of the embryonic period from fertilization to 50% hatch was 100 h at 18 ± 0.5 °C (Table 1). Ovulated eggs of splittail had a mean diameter of 1.37 ± 0.1 mm (mean ± SD, n = 50), were translucent, and had yellowish yolks. Eggs became adhesive immediately after fertilization. The yolk retained a globular structure throughout development, and oil droplets were not discernable under a dissecting microscope.

During the zygote phase (2-h duration), cytoplasm streamed toward the animal hemisphere to form a prominent blastodisc. The perivitelline space formed before cleavage by imbibition of water and separation of the chorion from the yolk cell and blastodic (Figure 1A). At the start of cleavage, the diameters of hardened eggs and yolk cell with blastodisc were 2.1 ± 0.1 and 1.4 ± 0.1 mm (n=20), respectively. Two layers with different optical densities were distinguishable in the perivitelline space. The inner, denser layer gradually expanded during development, and occupied the entire perivitelline space at 25-somite stage.

Early cleavage (4-h duration) included six synchronous cell division cycles, resulting in 64-cell embryos. The cleavage furrows of the first four cycles passed vertically at approximately 30- to 40-minute intervals (Figure 1A, 1B). Cell division continued during the blastula phase (approximately 10-h duration) resulting in elevation of the blastodisc. In the high blastula stage, the blastodisc was hemispherical, and blastomeres were still visible under a dissecting microscope (Figure 1C). Histological examination of the blastula did not reveal a blastocoel but showed the formation of the yolk syncytial layer, YSL (Figure 2A). Epiboly started after the sphere stage, manifested by thinning and spreading of the blastoderm over the yolk cell, with the blastoderm margin above the egg equator (Figure 1D). Blastoderm cells were no longer detectable under a dissecting microscope.

The gastrulation phase (14- to 16-h duration) was manifested by the formation of a thickened germ ring

Table 1 Stages of embryonic development in Sacramento splittail (constant 18 ± 0.5 ºC)

| Phase/Stage | Time (hrs) post-fertilization | Characters of stage |
|---|---|---|
| **Zygote** | 0:00 + | Formation of perivitelline space and blastodisc |
| **Cleavage** | | |
| *2-cell* | 2:45 | 1st cleavage (two cells, Figure 1A). |
| *4-cell* | 3:30 | 2nd cleavage (four cells, 2 × 2). |
| *8-cell* | 4:20 | 3rd cleavage (eight cells, 2 × 4, Figure 1B). |
| *16-cell* | 4:45 | 4th cleavage (16 cells, 4 × 4) |
| **Blastula** | | |
| *High* | 6:15 | High blastodisc (Figure 1C). |
| *Sphere* | 11:25 | Flattened blastodisc. (Figure 1D). |
| *Start of epiboly* | 16:45 | Dome-shaped blastoderm. |
| **Gastrula** | | |
| *Germ-ring* | 19:40 | Formation of germ ring at 50% epiboly |
| *80% epiboly* | 23:10 | Yolk plug (80% epiboly). Thickened dorsal side (Figure 1E). |
| *Tail Bud* | 25:25 | Tail bud stage, yolk plug closed (Figure 1F). |
| **Segmentation** | | |
| *5-somite* | 32:10 | Brain primordium and optic vesicles. |
| *8-somite* | 34:20 | Eye cups with discernible creases. |
| *16-somite* | 40:10 | Auditory vesicles, Kupffer's vesicle. |
| *22-somite* | 43:30 | Chevron-shaped somites (Figure 1G). |
| *25-somite* | 47:30 | Tail elongated and partly separated from yolk. Eye lens placodes. Muscle movements (Figure 1H). |
| *38-somite* | 57:30 | Myotomes W-shaped. |
| *44- to 46-somite* | 68:30 | Heart beating. Blood circulating. |
| **Pharyngula** | | |
| *Hatching gland* | 80:00 | Yellowish blood cells. Visible ducts of Cuvier. |
| *Pectoral fin bud* | 93:30 | Pectoral fin buds and pigmentation of eyes. |
| **Hatching** | 94–105:30 | Transparent embryos with pigmented eyes. Bent Head (Figure 2A, 3A). |

and embryonic shield at approximately 50% epiboly. As epiboly advanced, the embryonic shield elongated and thickened. At yolk plug closure (100%-epiboly), the embryo had a prominent tail bud and a thickened brain rudiment (Figure 1F).

Various organ rudiments appeared during the segmentation phase (approximately 38-h duration), when the central nervous system, sensory organs, notochord, somites, and tail developed. As somitogenesis proceeded in an anteroposterior direction, organ rudiments appeared in the following order: optic vesicles at the 5-somite stage, auditory placodes and Kupffer's vesicle at the 16-somite stage, and pear-shaped yolk at the 22-somite stage (Figure 1G). The eye lens placodes differentiated and the tail elongated at the 25-somite stage, when the first muscular twitch was observed (Figure 1H). The Kupffer's vesicle disappeared at 28-somite stage, when the

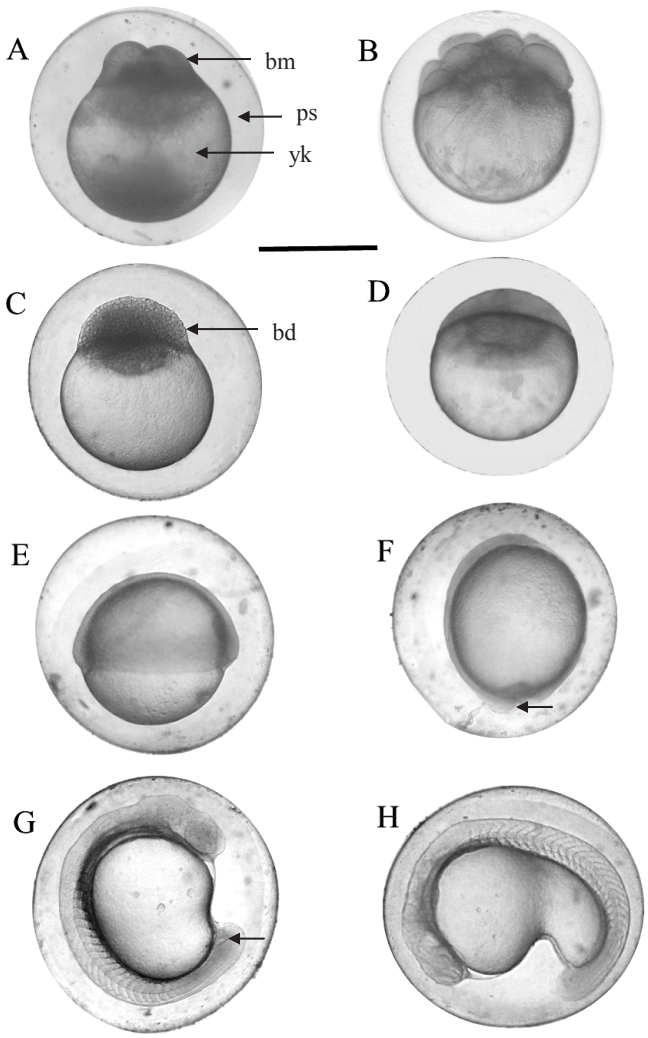


**Figure 1** Embryonic development in splittail. **A.** 2-cell stage: bm, blastomere; ps, perivitelline space; yk, yolk. **B.** 8-cell stage. **C.** High blastula: bd, blastodisc. **D.** Sphere blastula. **E.** Germ ring stage. **F.** Tail bud (arrow) stage. **G.** 22-somite stage, Kupffer's vesicle (arrow). **H.** 25-somite stage. Bar = 1 mm, egg diameter, 1.8 to 2.2 mm.

forebrain, midbrain, otoliths, and eye lenses became distinct. At the 46-somite stage, the hindbrain and olfactory organs developed, the tail elongated and the heart started beating. Blood, pale in color, circulated in a single loop: heart → paired mandibular aortic arches → paired lateral dorsal aortae → medial dorsal aorta → caudal vein → ducts of Cuvier → heart.

The pharyngula phase (25-h duration), in reference to differentiation of pharyngeal pouches (Ballard 1981), was characterized by formation of pectoral fin buds on the anterior dorsal part of the yolk sac, a change in blood color from pale to red, and development of a cranial loop of circulatory system and Curvier's ducts. Hatching glands appeared at 14 h before hatching as the small granules on the dorsal surface of the head. Inner ears had otoliths and eyes were pigmented, but the body remained unpigmented. Branchial arches started to differentiate, but jaws remained unformed.

Hatching (12-h duration) started at 94 h, peaked at 100 h, and ended at 105 h post-fertilization. At hatching, the embryo twisted vigorously to break through chorion with the tail, and continued twisting to remove chorion from the head.

## Larval Development

The major characteristics for larvae of selected ages are summarized in Table 2. Detailed developmental events of the corresponding ages are described below.

Newly hatched yolk-sac larvae (length 5.6 ± 0.36 mm, n = 10) had transparent bodies devoid of pigment, darkly pigmented eyes, and the head bent down over the yolk-sac at approximately a 45° angle, with no mouth opening (Figure 2B, 3A, 4A). Hatching glands densely covered the head and were sparsely scattered on the anterior yolk-sac surface (Figure 4A). The anterior yolk-sac was bulbous, and its posterior part was elongated and cylindrical. A wide median finfold enabled larvae to move with a jerky motion in the water column, however yolk-sac larvae mostly lay on the bottom of the tank. When a piece of artificial spawning substrate was placed in the tanks, the yolk-sac larvae hid under the substrate.

By 2 dph, approximately 50% of the yolk was absorbed and external body pigments became evident. Melanophores appeared on the head and along the dorsal and lateral midlines of the body. By 3 dph, the head straightened, melanophores increased in numbers and size in the dorsal region (Figure 4B), and a mouth opening formed on the ventral side of the head (Figure 4C). The swimbladder was not inflated but had a narrow lumen lined with columnar epithelium (Figure 2C). Mandibular and hyoid arches elongated, and branchial arches were distinct

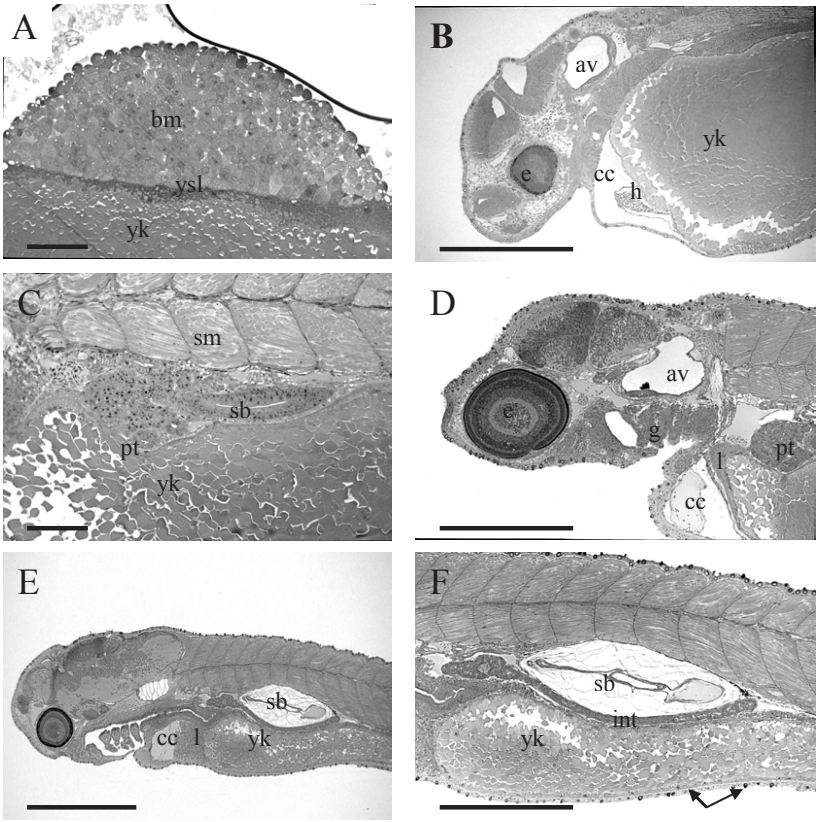


**Figure 2** Histology of splittail embryos and larvae. **A.** High blastula. **B.** Newly hatched embryo. **C.** Larva 3 dph (myotomes and swimbladder anlage). **D.** Larva 3 dph, head region and gill arches. **E.** Larva 6 dph. **F.** Larva 6 dph, enlarged area of dilated and partially inflated swimbladder. Arrows pointed to mucous cells. Abbreviations: av, auditory vesicle; cc, cardiac cavity; bm, blastomere; e, eye; g, gills; h, heart; int, intestine; l, liver; pt, proneohric tubules; sm, somites; sb, swimbladder; ysl, yolk syncytial layer; yk, yolk-sac. Scale bars: A, B, C, E = 0.1 mm; D, F = 0.5 mm.

**Table 2** Larval periods of Sacramento splittail development (constant 18 ± 0.5 °C)

| Phase/Days (posthatch) | Characteristics |
|---|---|
| **Pre-flexion** | |
| Day 1 | Lifted head from yolk-sac. No body pigments. |
| Day 2 | Melanophores on the head and along the dorsal and lateral medlines. |
| Day 3 | Straightened head. Heart at anterior position. Mouth orifice, jaw cartilages, and gill arches visible. |
| Days 4–5 | Large melanophores on dorsal head and on swim bladder rudiment, and along notochord. Gill filaments and opercula differentiated. Jaws moving. |
| Day 6 | Swim bladder inflation. Wide preanal finfold. Elongated pectoral fins. Onset of exogenous feeding (Figure 3B). |
| **Flexion** | |
| Day 10 | One-chamber swim bladder. Caudal notochord flexed, and 4 to 5 fin rays in the lower caudal fin (Figure 3C). |
| Day 20 | Dorsal and post-anal finfolds reduced. Appearance of fin rays in dorsal and anal fins (Figure 3D). |
| **Post-flexion** | |
| Day 30 | Two-chamber swim bladder. Dorsal and anal fins with 10 and 9 rays (Figure 3E). |
| Day 40 | 5 fin rays in pelvic fin and reduced ventral finfold. |
| **Juvenile** | |
| Day 50 | Resorbed pre-anal finfold. No scales formed (Figure 3F). |

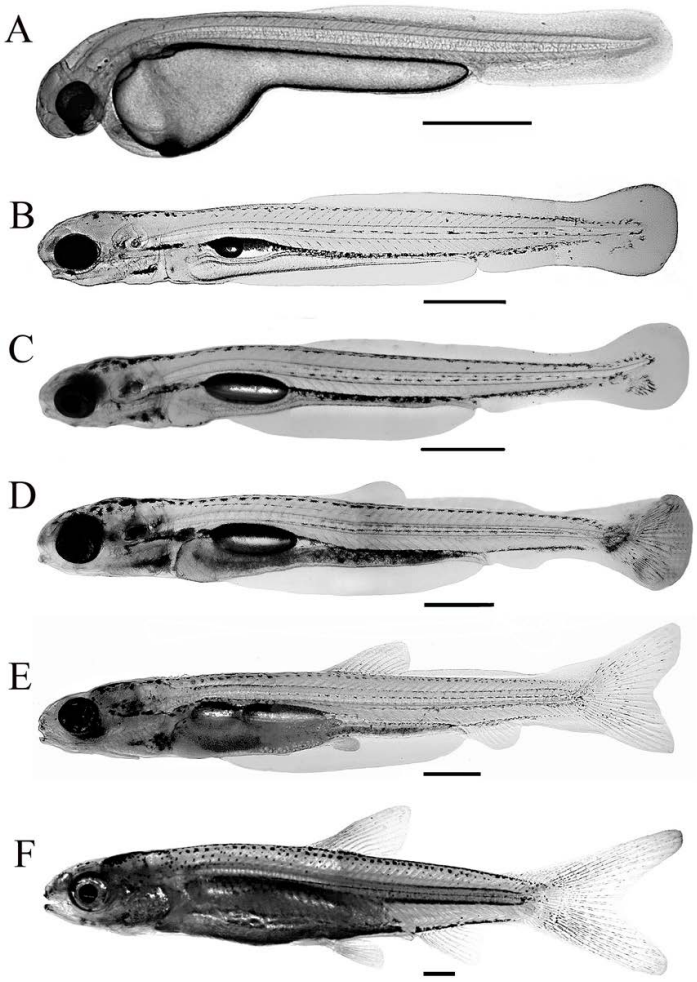


**Figure 3** Posthatch development of splittail yolk-sac larvae and larvae. **A.** Newly hatched embryo (5.9 mm) with large yolk-sac. **B.** Preflelxion larva (6 dph, 8.0 mm). **C.** Flexion larva (10 dph, 8.5 mm). **D.** Flexion larva (20 dph, 9.7 mm). **E.** Postflexion larva (30 dph, 11.5 mm). **F.** Postflexion larva (50 dph, 22 mm). Bars = 1 mm.

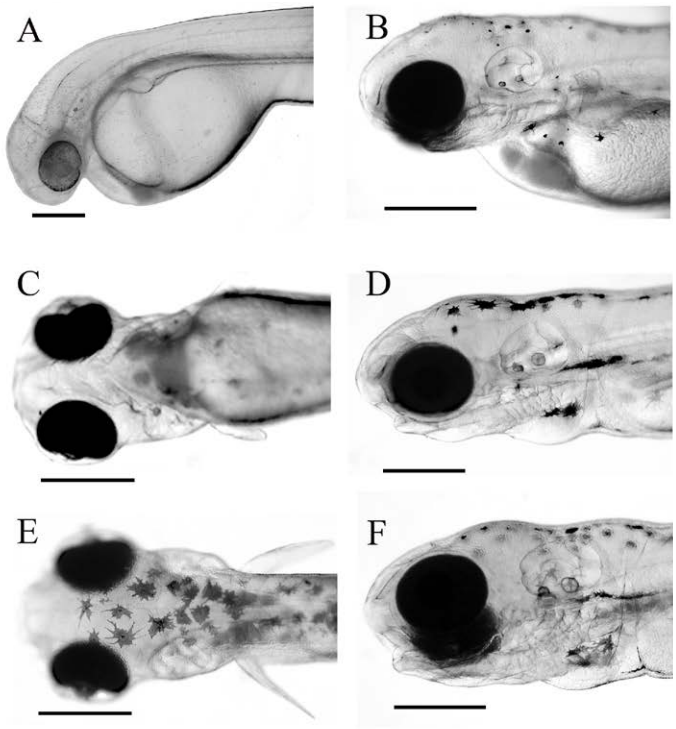


**Figure 4** Head development of splittail preflexion lavae. **A.** Newly hatched embryo, lacking pigment cells; hatching glands are the small dots on head and anterior yolk-sac surface. **B.** Larva 3 dph, pigment cells appear on the dorsal region; **C.** Larva 3 dph (ventral view), mouth opens and lower jaw develops; **D** and **E.** Larva 4 dph, side and top views, showing developing pigmentation, lower jaw, gills, and elongated pectoral fins; **F.** Larva 5 dph, with well developed jaws, gills, and opercula. Bars = 1 mm.

but devoid of filaments. A liver rudiment formed at the anterior part of the yolk-sac (Figure 2D). The pericardial cavity enlarged, and the heart had two distinct chambers. Blood flow reached the end of the tail via dorsal aorta and returned to the atrium via the posterior cardinal vein and the ducts of Cuvier. At 4 to 5 dph, pigmentation increased, especially on the dorsal part of the head (Figures 4D, 4E), and jaw movement began.

At 6 dph (TL 8.1 ± 0.1 mm, n =10; Figures 3B, 2E, 2F), pre-flexion larvae inflated their swimbladders, swam up in the water column, and started feeding, with approximately 10% to 20% of the yolk remaining. Rotifers were observed in the tube-like intestine which was lined with simple columnar epithelium (Figure 2F). Gill filaments developed on four branchial arches, and the opercules completely covered the gills.

By 10 dph, the swimbladder was enlarged, yolk was completely absorbed, and the urostyle had begun to flex upward with 4 to 5 fin rays in the lower portion of the caudal fin, marking the beginning of the flexion phase (Figure 3C). At 20 dph, dorsal and postanal regions of the median finfold were partly lost, differentiation of principal fin rays in the caudal fin was almost completed, and differentiation of the fin rays of the dorsal and anal fins began (Figure 3D). Differentiation and inflation of the anterior lobe of the swimbladder occurred at 30 dph in post-flexion larva, when dorsal and ventral lobes of the caudal fin developed. The dorsal and anal fins had 10 and 9 principal rays, respectively (two rays based on the last pterygiophore were counted as one). At this age, rudiments of pelvic fins appeared on both sides of the preanal finfold (Figure 3E). At 40 dph, pelvic fins had 5 fin rays, and the preanal finfold was reduced. The complete resorption of the preanal finfold occurred by 50 dph (TL 20.2 ± 0.5 mm, n=10), marking the transition to the juvenile period (Figure 3F). The body of the juvenile was less transparent and the skin was silvery, but no scales were apparent under the dissecting microscope.

The greatest increase of total length happened within 3 dph, primarily from the straightening of head and the enlargement of the caudal finfold (Table 3). Yolk remnants remained as larvae left the substrates, became more pelagic, and began feeding (6 to 10 dph). The rate of growth in length slowed between 6 and 20 dph, but increased again thereafter (Table 3).

**Table 3** Total length and growth increments in TL of Sacramento splittail larvae (n = 10, 18 ± 0.5 °C)

| Post-hatch (days) | Mean ± S.D. (mm) | Minimum (mm) | Maximum (mm) | Mean increment (mm $d^{-1}$) |
|---|---|---|---|---|
| 0 | 5.60 ± 0.36 | 5.20 | 6.00 | |
| 1 | 6.06 ± 0.45 | 5.40 | 6.49 | 0.46 |
| 3 | 7.33 ± 0.40 | 6.80 | 7.73 | 0.64 |
| 6 | 8.08 ± 0.13 | 7.90 | 8.21 | 0.25 |
| 10 | 8.50 ± 0.14 | 8.30 | 8.85 | 0.11 |
| 20 | 9.94 ± 0.66 | 9.00 | 10.70 | 0.14 |
| 30 | 13.84 ± 0.79 | 12.40 | 15.40 | 0.39 |
| 40 | 17.38 ± 0.74 | 15.63 | 18.00 | 0.35 |
| 50 | 20.24 ± 0.54 | 19.60 | 20.85 | 0.29 |

## DISCUSSION

Comparison of the embryonic development of Sacramento splittail to the developmental staging system for the model species zebrafish (Kimmel and others 1995) shows similarities in the developmental pattern and corresponding developmental stages of the two species. As in zebrafish and other teleosts, splittail embryos had similar meroblastic cleavage, did not form blastocoel and retained granular yolk matter throughout their entire embryonic development (Collazo and others 1994; Kimmel and others 1995). Both species exhibited similar sequence of organogenesis, as well as similar formation of the blood circulatory system as described in the pharyngula phase (Isogai and others 2001). However, splittail and zebrafish differ in the timing of the appearance of organ rudiments relative to a number of somites in the embryo. For instance, Kupffer's vesicle, a transient feature observed only in teleost embryos, appeared at the 16-somite stage in splittail whereas in zebrafish it occurred at 5- to 9-somite stage. The trunk somites in splittail became chevron-shaped at the 22-somite stage but at the 14-somite stage in zebrafish (Kimmel and others 1995). Larger egg and greater somite numbers in splittail embryos may explain these discrepancies. The mean diameter of splittail ovarian eggs was 1.37 mm, approximately two times the diameter and eight times the volume of zebrafish ovulated eggs (0.7 mm diameter; Kimmel and others 1995). A fully developed splittail embryo had 44 to 46 somites compared to 30 to 34 somites in zebrafish embryos (Kimmel and others 1995). In addition, differences were also observed in the appearance of hatching glands and pigmentation. Hatching glands were prominent in the pharyngula phase in both splittail and zebrafish. They developed mainly on the head region in splittail; but in zebrafish, they grew on the anterior yolk-sac (Kimmel and others 1995). External body pigmentation in splittail formed much later at about 2 dph, while in zebrafish

body pigmentation presented during the pharyngula phase of an embryo.

The length of splittail pre-flexion and flexion larvae recorded in this study is in general agreement with that at similar stages previously reported in field and laboratory studies. Wang (1986, 1995) reported that wild splittail larvae of 5.5 to 6.5 mm TL had no mouth opening and were poorly pigmented, which was observed in the newly hatched larvae in our study. Bailey (1994) noted that larvae reared under laboratory conditions completed yolk absorption and started feeding at 7 to 8 mm TL (5 to 7 dph), which is similar to 10-dph larvae in this current study. Moyle and others (2004) observed that wild splittail can be easily identified at 20 to 25 mm TL. Our observation confirmed that individuals at this size range were late post-flexion larvae that had transformed into juvenile stages (17 to 21 mm TL, 50 dph) with the adult complement of morphological features, except the scale cover.

The laboratory observations in this study support the notion that shallow waters play an important role in the splittail reproduction and recruitment. Previous field studies indicated that splittail larvae used shallow-water wetlands, including floodplains, as nursery areas (Sommer and others 2002; Crain and others 2004). A long-term analysis of the distribution and habitat of age–0 splittail (<50 mm fork length) revealed that the young fish also favored similar types of habitat with low-flow velocity (Feyrer and others 2005). A relatively long developmental presence of large finfold in splittail yolk-sac larvae may support this apparent reliance upon shallow and slow moving waters. With large finfolds, the larvae exhibit poor swimming ability that may not withstand strong currents in the river channel. The observed poor pigmentation and hiding behavior of yolk-sac larvae suggest that yolk-sac larvae are photonegative and hide in the bottom substrates to avoid predation. In addition, we observed splittail larvae attached to the substrate or the wall of glass beakers with their ventral region, presumably by the secretion of mucins from numerous epithelial mucous cells (Figure 2F). Such behavior could help larvae hold their positions in shelters to avoid predators, as well as preventing them from drifting in strong currents or from sinking into muddy bottoms with poor oxygenation.

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