

Early Development of the Sacramento Perch

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Abstract.—Populations of the Sacramento perch *Archoplites interruptus*, California's only native centrarchid, are declining and early development of this imperiled species is poorly known. Therefore, the main objective of this study was to describe the early development of Sacramento perch from fertilization to initiation of exogenous feeding. Embryos were obtained in the laboratory by spawning induction via photoperiod–temperature control or gonadotropin-releasing hormone analog treatment. Gross morphology and histological analyses were integrated to describe the early development. Cleavage was synchronous until the 64-cell stage, which allowed us to calculate the duration of one mitotic cycle during early synchronous cleavage (τ_0) as 10–16 min at 25.4–25.6°C. A description of histological events from time to morulation, gastrulation, hatch, and exogenous feeding larvae is provided. Yolk sac larvae tended to adhere to the spawning substrate or sediment during the critical organogenesis stages. Larvae swam up and began exogenous feeding within 4 d postfertilization. Primordial germ cells were observed in 10-d-old larvae, but sex differentiation occurred after 43 d. External sexual dimorphism was not observed in early life stages.

Populations of the Sacramento perch *Archoplites interruptus* have declined significantly in their native range (Moyle 2002). The Sacramento perch is a relict centrarchid and the only centrarchid that is native to areas west of the Rocky Mountains (McCarragher and Gregory 1970; Moyle et al. 1974; Aceituno and Nicola 1976; Wang 1986). The native range of the Sacramento perch includes the waters of the Central Valley, the Clear Lake basin, Sacramento–San Joaquín River drainage, and the Pájaro and Salinas River systems of California (McCarragher and Gregory 1970; Aceituno and Nicola 1976; Gobalet 1990a, 1990b). According to archeological evidence, the Sacramento perch was once the most abundant species found at freshwater sites in Native American fisheries of the central California coast and inland sites (Gobalet and Jones 1995; Gobalet 2001). Unfortunately, the Sacramento perch has reportedly been declining since 1960 and has been classified as a depleted species (McCarragher and Gregory 1970; Miller 1972; Vanicek 1980). Some authors noted a decline in Sacramento perch popula-

tions before the turn of the 19th century (Jordan and Evermann 1896, cited in Imler et al. 1975), and subsequent studies indicated a major reduction in the distribution and abundance of this species (Aceituno and Nicola 1976; Aceituno and Vanicek 1976; Scopettone and Smith 1978) except at some transplant sites outside the native range (Saiki and Martin 2001). Currently, the Sacramento perch is depleted in its native range and efforts have been directed toward its recovery (Schwartz and May 2004).

The decline of Sacramento perch populations has been attributed to several causes that include interspecies competition for food and space, predation, habitat degradation, and parasite infestation (Murphy 1948; Aceituno and Nicola 1976; Goude and Vanicek 1985). Population decline in the Sacramento–San Joaquín River systems of California has been directly attributed to the introduction and establishment of nonindigenous species (McCarragher and Gregory 1970; Aceituno and Nicola 1976). Competition with other exotic centrarchid species (e.g., bluegill *Lepomis macrochirus*) might have played a major role in reducing the distribution and abundance of Sacramento perch (Aceituno and Vanicek 1976; Vanicek 1980; Marchetti 1997).

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Nevertheless, rapid anthropogenic changes have altered the species' distribution (Gobalet 1990b).

Life history traits of the Sacramento perch could render it more susceptible to environmental stressors than other fish species within its native range. First, the Sacramento perch is an inshore-oriented fish that is found in littoral areas during all seasons and prefers warm, shallow, slow-moving waters (Vigg 1980; Gobalet et al. 2004). The nearshore distribution makes the Sacramento perch more vulnerable to anthropogenic disturbances, such as effluents carrying environmental toxicants. Second, the sedentary nature of Sacramento perch (LaRivers 1962) can contribute to extended exposures to environmental stressors. Not surprisingly, relative abundance decreases, reduced growth, and reproductive failure of Sacramento perch have been documented in waters with a history of habitat or chemical degradation (e.g., agricultural fertilizer; Vanicek 1980). Moreover, the water quality in river systems previously inhabited by Sacramento perch has been affected by the release of contaminants from multiple sources. Therefore, exposure to environmental stressors can potentially induce abnormalities that alter patterns of early development or impair the reproductive capability of Sacramento perch.

Unfortunately, detailed records of early development in Sacramento perch are lacking. Therefore, our goal was to document early life stages to better assess the contribution of environmental stressors to the imperiled status of this species. Our main objective was to describe and identify the embryonic and postlarval developmental stages of the Sacramento perch from fertilization to initiation of exogenous feeding. Our study integrates gross morphology and histological data to describe and identify the early life stages and organogenesis of Sacramento perch.

Methods

Captive rearing.—The original source of Sacramento perch broodfish was maintained at the Contra Costa Mosquito and Vector Control District (CCMVCD) aquaculture facilities in Concord, California. Additional broodfish were maintained at the Center for Health and the Environment and the Center for Aquatic Biology and Aquaculture at the University of California–Davis since November 2002. Broodfish were reared in a partially closed, recirculating, Plexiglas aquarium system equipped with water pump, flowmeter, biological sand filters, particle filter, activated charcoal filter, and ultraviolet light sterilizer. Each aquarium system included spawning stations covered with Spawn Tex commercial spawning substrate (Bolnick and Miller 2006). Reconstituted water for rearing broodfish was prepared according to the

guidelines of the U.S. Environmental Protection Agency (Horning and Weber 1985). Water was maintained at a hardness of 80–100 mg CaCO₃/L, a pH of 8.0 ± 0.1, a dissolved oxygen level of 7.0 ± 1 mg/L, and a temperature of 25 ± 2.0°C. Ammonia, nitrite, and nitrate were kept below detectable limits. Broodfish were stocked in 75.7- or 151.4-L tanks at a density of 2.6–5.6 mm total length (TL) per liter and at a sex ratio of two males per female; these conditions have been found to favor captive breeding (Miller 2002). Broodfish were fed floating trout pellets (Silver Cup) and were routinely monitored for indications of courtship behavior. The protein and fat levels in the diet were 42% and 12%, respectively.

Spawning induction and artificial fertilization.—We induced spawning in broodfish in the laboratory by photoperiod control or hormonal treatment. Six spawning events were observed between January and September 2003. Five of those events were induced by photoperiod and temperature control in captivity. Photoperiod was 16 h light : 8 h dark, and spawning temperature was 25.0 ± 1.0°C. Alternatively, broodfish were induced to spawn by means of a neurohormone, the gonadotropin-releasing hormone analog (GnRHa). We induced mature males and females with a single intramuscular (IM) injection of 20 µg GnRHa/kg ([des-glycine¹⁰, D-Alanine⁶, proline⁹-NH₂-ethylamide]-luteinizing hormone releasing hormone; Bachem). Embryos were obtained from one female that received GnRHa treatment. The GnRHa treatment induced egg maturation within 24 h after a single injection of one female. Before induction, responsiveness of adult females to hormonal treatment was evaluated by examining eggs extracted by catheterization to confirm egg maturation. Prior to ovulation, catheterized eggs were adhesive and demersal and were characterized by an opaque yolk. A responsive female was identified by examining catheterized ovarian follicles placed in a clearing solution (60 mL of 95% ethanol, 30 mL of 37% formalin, and 10 mL of glacial acetic acid) to facilitate observation (Stoekel and Neves 1992; Stoekel 2000). Specifically, female responsiveness to hormone injection was indicated by observing the stage of germinal vesicle migration and determining whether a single oil globule surfaced in the developing egg; both characteristics were observed in catheterized eggs. Before hormone injections, adult fish were lightly sedated by brief immersion in a solution of tricaine methanesulfonate (MS-222; ~91.7 mg/L) and sodium bicarbonate. Subsequently, two females and three males that received a single IM injection of 20 µg GnRHa/kg were placed in separate tanks before spawning. Females were catheterized every 24 h after hormone injection to monitor changes in egg maturation.

tion. After egg maturation, ovulation time was estimated by recording the time between injection and ovulation of induced females. A second female spawned within 32 h after induction with GnRH α . One of the females died within 1 week after spawning. In males, GnRH α treatment induced courtship behavior and the darkening of body coloration. All eggs examined after spawning induction by GnRH α treatment had been fertilized and showed normal development. Among eggs that were left in the spawning station, the majority were dead and decomposed by pathogens unless otherwise treated with embryo rearing solution. However, negligible mortality was observed in eggs removed from the spawning station and incubated in rearing solution.

Mature eggs and milt of five induced fish were obtained for artificial fertilization. Viable eggs were collected by strip-spawning a female for artificial fertilization. Before artificial fertilization, milt collected from one of the induced males was examined under a light microscope to observe sperm motility. Duration of sperm motility was approximately 1 min. A dry method was used to artificially fertilize Sacramento perch eggs. First, strip-spawning was conducted if the fish did not ovulate or spermiate within a reasonable period (i.e., 3–5 d). Strip-spawning was done by gentle compression of the abdomen to release gametes. Second, male gametes were removed and sperm motility was evaluated by microscopic examination of extracted milt diluted in water. The duration of sperm motility was recorded to determine the maximum amount of time in which eggs and milt should be mixed for optimal fertilization. Third, a drop of milt was placed over stripped eggs in a glass Petri dish and 20–30 mL of water was then added. Finally, gametes were swirled gently until there was a single layer of eggs on the Petri dish. Subsets of eggs were sampled at intervals between 6 and 26 min based on the occurrence of cleavage activity in continuously monitored live eggs and fixed in formalin for later analysis of gross morphology and histology. Live eggs were maintained at a temperature of 25.4–25.6°C and a pH range of 7.72–7.80. Live eggs were monitored to document the timing of cleavage stages, embryonic, and larval development. Finally, fecundity of Sacramento perch was estimated as 4,006–16,260 larvae/brood ($n = 6$).

Early development and organogenesis.—Cleavage stages, embryonic and larval development, and organogenesis were documented by coupling gross morphological and histological analyses. The duration of one mitotic cycle during the period of synchronous cleavage divisions (τ_0) was estimated according to Dettlaff (1995). Gross morphology of live embryos was documented by observing embryonic development

under a dissecting scope (Olympus; Model SZX12). Timing of embryonic development was determined by periodic observation and digital photography of live embryos. Live embryos were collected from the Spawn Tex spawning substrate or bottom of the fish tank by using a wide-mouthed pipette or a siphon egg collector. Collected embryos were either treated overnight with a solution of methylene blue (10% NaCl, 0.3% KCl, 0.4% CaCl $_2 \cdot 2H_2O$, 0.797% MgSO $_4$ anhydrous, 0.01% methylene blue in distilled H $_2O$) or preserved in 10% formalin for later examination. Methylene blue solution was previously used in our laboratory to inhibit the growth of aquatic mold and bacteria that affected fish egg development. Egg samples were preserved in 5–10% buffered formalin at regular intervals. Preserved eggs were processed for glycomethacrylate embedding, serially sectioned at 4–5- μ m thickness, and stained in Mayer's hematoxylin and eosin (Burns and Bretschneider 1981). The histological sections provided a detailed account of the timing and the characteristics of events during early development and organogenesis. Timing and characteristics of normal development from fertilization to the postlarval stage were documented with digital images.

Larval development.—Larval development was described from a sample of larvae reared at CCMVCD. Larvae were fed live saltwater rotifers *Brachionus plicatilis* upon hatching. Saltwater rotifers were offered twice daily to achieve an estimated density of 5 rotifers/mL in suspension. Brine shrimp *Artemia* spp. were introduced as a second food item at 11 d posthatch. Brine shrimp were offered ad libitum to larvae twice daily. Larvae were sampled at 5-d intervals from hatch to 30 d posthatch. Larval samples were fixed in 10% formalin and examined under a dissecting microscope coupled with a digital camera (Nikon; COOLPIX 950). Digital images were archived from each individual and were used to estimate larval TL to the nearest 0.001 mm by use of National Institutes of Health Image software.

Results

Early Development and Organogenesis

Zygote period.—The blastodisc, a single large cell that forms at the animal pole, formed within 3–30 min after artificial fertilization (Figure 1A). The hemispherical structure of the blastodisc occupied one-sixth to one-third of the zygote volume. Most of the zygote volume was occupied by yolk platelets that were in contact with the blastodisc and surrounded by outer membrane layers. Water hardening of the embryo was indicated by elevation of the outer membrane. As the outer membrane was elevated, the vitelline membrane separated from the egg surface, thus creating a

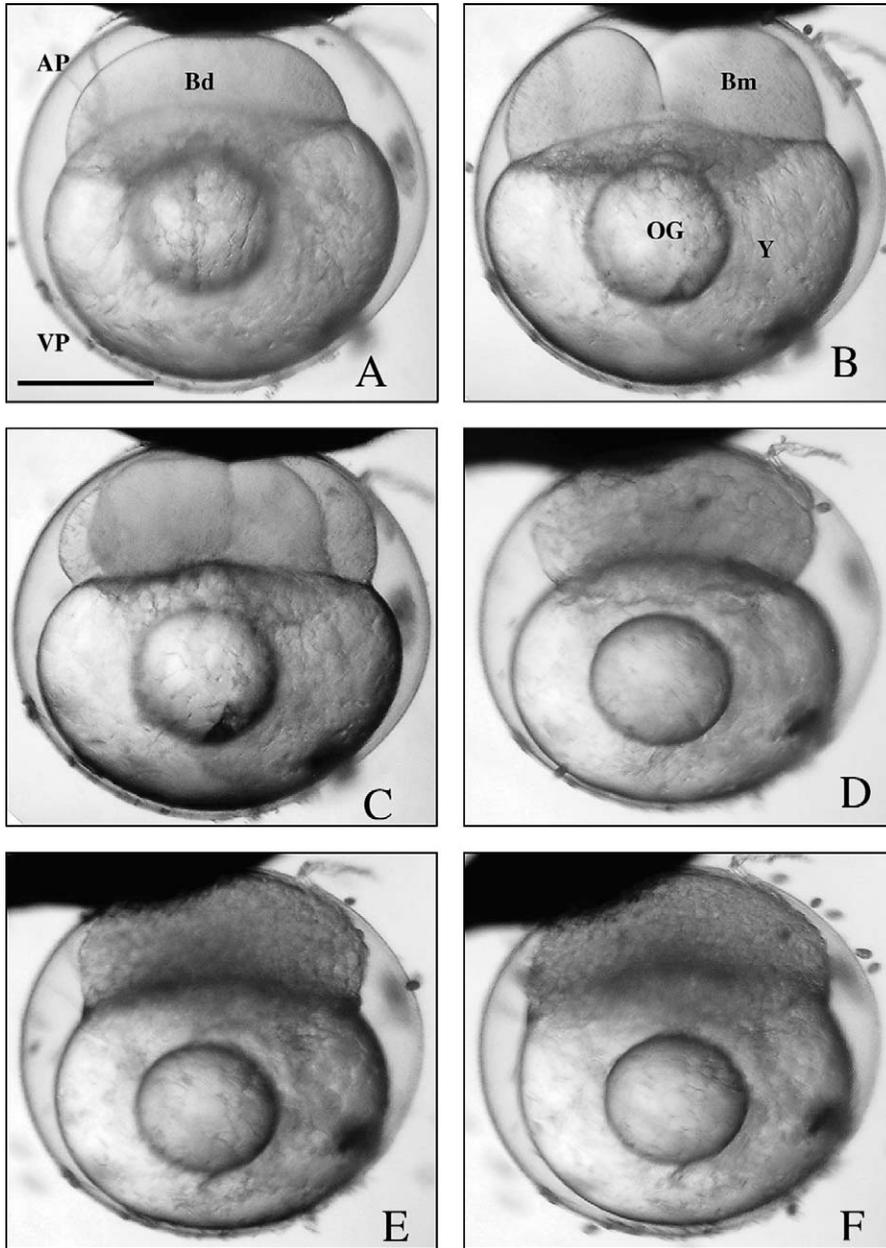


FIGURE 1.—Images of cleavage stages in Sacramento perch: (A) perivitelline space and blastodisc (Bd) formed within 3 min after fertilization (MAF; AP = animal pole; VP = vegetal pole; scale bar = 0.1 mm), (B) two-cell stage (48–56 MAF; Bm = blastomeres; OG = oil globule; Y = yolk), (C) four-cell stage (75–84 MAF), (D) morula stage (153–156 MAF), (E) blastula stage (170–206 MAF), and (F) gastrulation (215–720 MAF). Mean \pm SD egg diameter (mm) was 0.33 ± 0.04 ($n = 9$), temperature range was 25.4–25.6°C, and pH range was 7.72–7.8.

perivitelline space. The elevation of the outer membrane occurred on the animal pole of the zygote, above the blastodisc. Below the blastodisc, a single oil globule was often evident at the surface near the vegetal pole of the zygote. As the zygote developed,

the blastodisc increased in size and occupied approximately one-fourth of the volume. Moreover, the single oil globule migrated to just below the blastodisc, while the perivitelline space increased within 40 min after fertilization (MAF).

Cleavage period and embryonic stages.—The onset of the cleavage period was indicated by a series of mitotic divisions of the blastodisc that produced a mass of blastomeres in the animal pole. These mitotic divisions were synchronous from the first cleavage until the 64-cell stage, which allowed us to calculate a τ_0 range of 10–16 min for a temperature range of 25.4–25.6°C. The first cleavage furrow appeared at the midline of the blastodisc and formed two blastomeres of equal size (48–56 MAF; Figure 1B). A second cleavage furrow began to form perpendicular to the first cleavage furrow (65 MAF). The second cleavage furrow was completed within 10–19 min and formed four blastomeres of equal size (Figure 1C). Within 40 min after the onset of the cleavage period, a third cleavage furrow appeared and formed eight blastomeres. The orientation of the eight blastomeres was no longer symmetrical. As cleavage progressed, the blastomeres rearranged position until they stood at equal heights above the yolk mass (97 MAF). The blastomeres spread over the embryonic surface and became constricted in the region of contact with the yolk, thus forming a dome-shaped structure. Subsequently, a fourth cleavage furrow was formed and resulted in 16 blastomeres (102 MAF). Five of these blastomeres were distinguished on a face view of the cleaving egg. A new mitotic cycle formed a fifth cleavage furrow that resulted in 32 blastomeres (112 MAF).

Once the embryo reached the 32-cell stage, the cells began to arrange in discrete tiers or rows. Some of these cells were closer to the interface with the yolk mass, while others were closer to the tip of the animal pole. The cells rearranged into a discrete mound with two tiers within 2 h after fertilization (HAF). As cleavage proceeded and the embryo reached the 64-cell stage, the boundaries between cells in the mound became less discrete. Cell divisions became asynchronous, and a high mound of compacted small cells stood above the animal pole (2.5 HAF). At this point, the embryo reached the morula stage, also known as the “mulberry stage” (2.6 HAF; Figure 1D). During the morula stage, blastomeres continued to rearrange and began to migrate actively toward the vegetal pole (2.8 HAF). As migration ensued, the interface between the blastomeres and yolk mass became less pronounced and the cells appeared to be loosely attached, thus forming a blastoderm.

Formation of the blastoderm indicated the onset of the early blastula stage (Figure 1E). During the late blastula stage, the blastoderm became flattened over the yolk mass due to active cell migration toward the vegetal pole. The blastoderm began to move over the embryo sphere, indicating the onset of gastrulation (3.6

HAF). As gastrulation proceeded, there was a slight change at the center of the migrating cell mass. Specifically, the interface between the blastoderm and the yolk mass increased due to the formation of a subgerminal cavity known as the blastocoel (4.4 HAF).

All of the examined embryos showed varying degrees of cell migration at 6 HAF. Cell migration indicated cleavage stages ranging from early morula to early gastrula (Figure 1D–F). The latter stage was indicated by a flattened mass of spreading cells identifiable as mesenchyme. As gastrulation advanced, the round mesenchyme cells showed marked changes in shape (9 HAF). Cells near the surface acquired an elongated shape, while other groups assumed the cuboidal and columnar shapes that are characteristic of more advanced tissues (e.g., secretory tissues).

Neurulation and anterior–posterior differentiation were observed in live embryos when the development of blastomeres was at less than 50% epiboly (9.3 HAF). During the epiboly stage, the yolk mass was overgrown by blastomeres that presumably comprised the ectoderm and mesoderm. Epiboly proceeded at a steady pace, and about one-half the yolk mass was covered within 5 HAF. Anterior–posterior differentiation of the embryo became evident as the early embryo elongated and the yolk mass appeared medially constricted by the embryonic tissue (9–22 HAF; Figure 2A). At this stage, the embryo and the yolk mass were separated by a syncytial layer known as the preblast.

As anterior–posterior differentiation proceeded, the embryo advanced to the segmentation period and began to develop the supporting axis of the body. Various embryonic structures were formed, the head broadened, and the tail became tapered during the segmentation period (15 HAF). The broadened head stretched the outer membrane and forced the egg to assume an oval shape. In addition, an axial strand coiled around the yolk mass and several somites were distinguished on histological sections of the embryonic axis. The embryonic axis was formed by two types of cells. One type formed the somites and appeared slightly stretched, while the other type was tightly packed and formed a rudimentary notochord.

During the late segmentation period, brain tissue and blood tissue appeared in the embryo. As segmentation proceeded, further broadening of the head occurred and the tip of the tail touched the head as the body extended across the yolk mass. Development of the brain in the embryo was indicated by two dense lobes in the head. Moreover, formation of the optic cups and otoliths became evident as segmentation ensued (12–18 HAF; Figure 2B). Development of blood during the segmentation period was indicated by the appearance of red blood cells inside the pericardial cavity. The pericardial

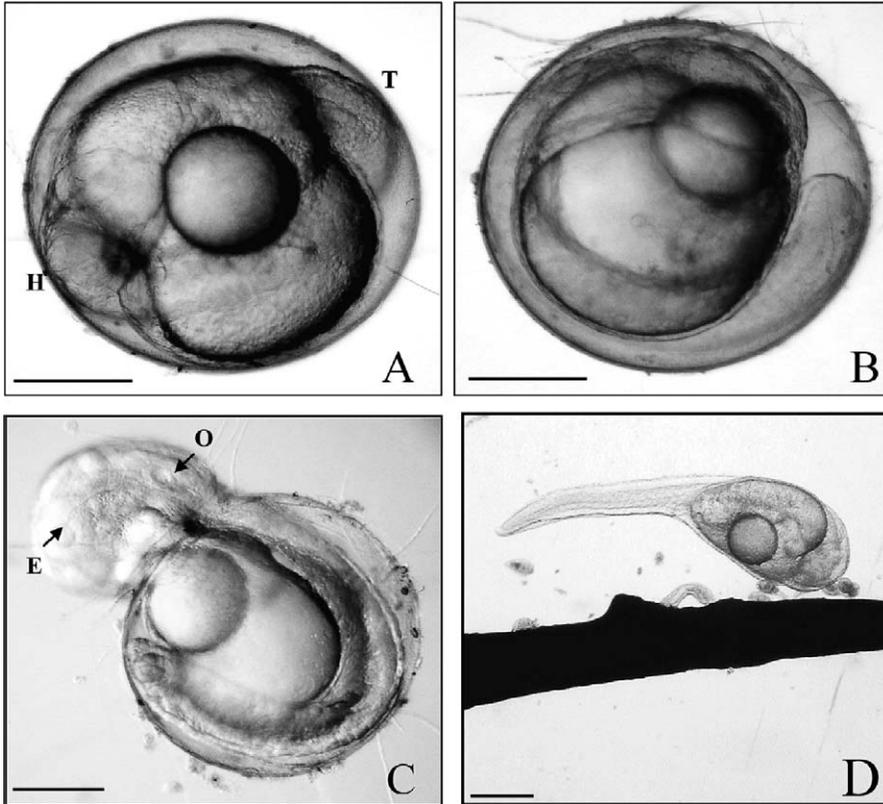


FIGURE 2.—Images of Sacramento perch embryonic stages and hatching (scale bars = 0.1 mm): (A) anterior–posterior differentiation of embryo (12.8 h after fertilization [HAF]; H = head; T = tail), (B) eye cup and otolith development (12.8 HAF), (C) prelarval emergence, head first (19.8 HAF; E = eye cup; O = otolith), and (D) hatching (0.580 mm TL) attachment to spawning substrate. Mean \pm SD egg diameter (mm) was 0.33 ± 0.04 ($n = 9$), and temperature range was 23.0–25.0°C.

cavity became increasingly differentiated during the prehatch period.

Before hatch, formation and evagination of the optic cup and optic placode were evident. Terminal notochord cells then formed as a result of the vacuolation of original notochord cells. Subsequently, clear spaces developed along the notochord and the developing neural tube became evident as a clear rod along the body axis.

Hatching and Larval Development

Sacramento perch hatching occurred within 19–36 HAF. Hatching appeared to be facilitated by the presence of a hatching gland and muscular contractions on the body axis of the embryo. Up to 33 myomeres appeared as the somites elongated and assumed the tapered shape of these muscle fibers on the body axis (28–44 HAF). As the body axis became longer than the circumference of the yolk sac and as the tail was freed, vigorous movements of the trunk and tail prompted the newly hatched larvae to emerge. Larvae emerged head

first in all observed hatching events (Figure 2C). The hatching gland was formed in the anterior region of the head. Subsequently, newly hatched larvae attached to the spawning substrate (i.e., Spawn Tex) with a thin filament that extended from their head region (Figure 2D). At this stage, the head rested on the yolk mass; these larvae are referred to as yolk sac larvae until the onset of exogenous feeding.

In general, yolk sac larvae are poorly developed at the moment of hatch. For instance, there was no eye pigmentation or fin development. Eye pigmentation was observed for the first time once the eyes differentiated into multiple layers of pigmented cells and a lens became evident (34–44 HAF). On the other hand, early fin development was evident once the surface area of the fin fold increased and accumulated a high density of mucous cells. Fin fold development was followed by the appearance of pectoral fin buds (53–66 HAF). Pectoral fin buds appeared as a rounded mass of mesodermal cells oriented upwards between the yolk sac and the body axis. Pectoral fin movement began as

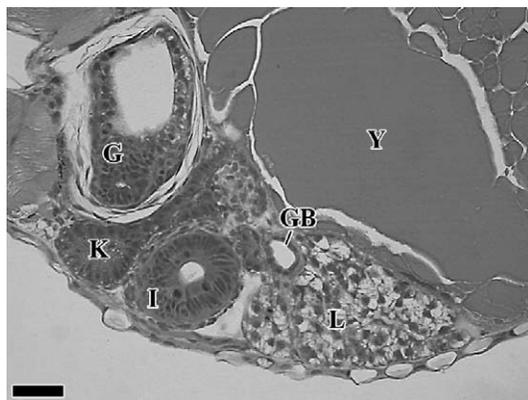


FIGURE 3.—Organogenesis of Sacramento perch, as shown by a longitudinal histological section of a larva (66 h postfertilization; Y = yolk; scale bar = 0.05 mm; stained with hematoxylin and eosin). The gallbladder (GB) forms by dilation of the gall tube and is adjacent to the liver (L). The intestine (I) is characterized by the presence of tall columnar epithelial cells with a brush border facing the lumen. The pronephric duct (K) in its early developmental stage appears as a dense cellular cord lining with cuboidal epithelial cells. The gas bladder (G) is composed of cuboidal epithelial cells and is surrounded by fibrous tissue.

gentle twitching (73 HAF). As the pectoral fins became larger, they extended sideways (126 HAF). In addition, further specialization of the fin fold formed a preanal fin fold that appeared as a slight swelling on the preanal segment of the fin fold (65 HAF). This structure was presumably the primordium of the anal fin. Finally, the contour of the yolk sac larval fin fold acquired curves that indicated further fin development (81 HAF).

Yolk sac larvae exhibited limited organogenesis. For instance, they had kidney tubules (i.e., primordial kidney ducts), a liver bud, spleen, and nervous system structures. The kidney tubules were developed above the stomach and intestines (Figure 3). The spleen appeared as a reddish brown spot over the yolk (73 HAF). Moreover, nervous system structures became apparent in yolk sac larvae. The brain was differentiated into forebrain, midbrain, and hindbrain. The cerebellum and the brain ventricle that houses spinal fluid became evident at 38–44 HAF.

The earliest indications of cardiovascular system development were the presence of a heartbeat (18.8 HAF) and blood circulation around the yolk sac within the periblast (i.e., yolk syncytial layer; [28 HAF; Figure 4). Circulation of nucleated red blood cells became more conspicuous with the increase in blood pigmentation (38 HAF). As the blood acquired a bright red color, the Cuvier duct was revealed as a thick blood vessel located on the anterior section of the yolk sac above the tubular heart. Both the tubular heart and aortic arches were ventral to the neck. The tubular heart was more conspicuous as yolk resorption progressed and the pericardial cavity enlarged. Moreover, the tubular heart became highly differentiated before initiation of active movement in larvae. The volume of the pericardial cavity was mostly occupied by a well-developed two-chambered heart. The two-chambered heart was observed for the first time when the anterior portion of the yolk sac was resorbed in yolk sac larvae (49 HAF). The heart chambers acquired the shape of an inverted “L” (65 HAF), and a clear network of blood

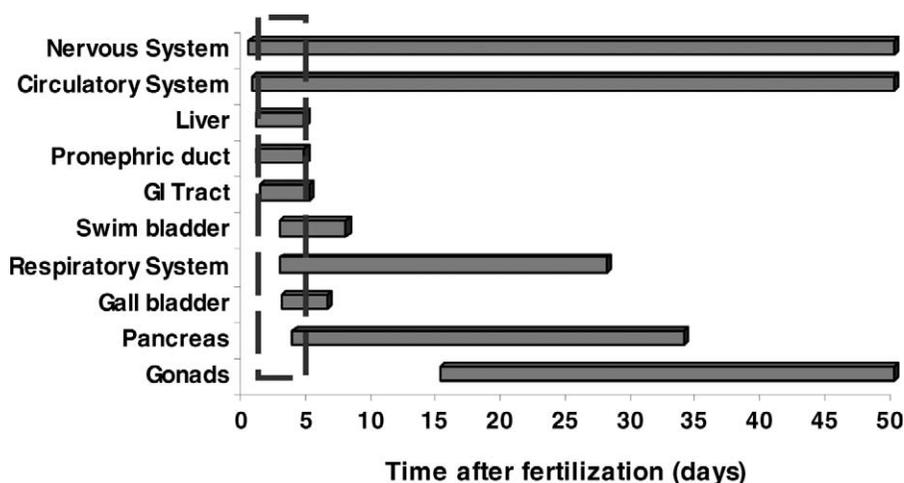


FIGURE 4.—Timing of organogenesis in the Sacramento perch (d postfertilization). Gross morphology and histological examination data were combined to determine the timing of development for each organ or system (GI = gastrointestinal). Dashed rectangle indicates the duration of the yolk sac larval stage.

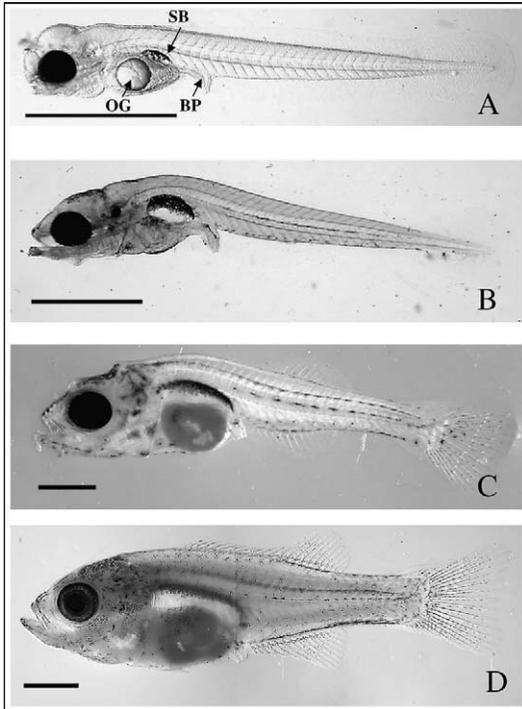


FIGURE 5.—Images of Sacramento perch larval development (scale bars = 1 mm): (A) pigment plug present in the gastrointestinal tract (0 d post-swim-up; BP = bile plug, OG = oil globule, SB = swim bladder), (B) initiation of exogenous feeding, as indicated by the presence of rotifers in the gut and absence of an oil globule (10 d post-swim-up); (C) larva feeds exogenously on *Artemia* (20 d post-swim-up), and (D) larva displays advanced fin development (30 d post-swim-up).

vessels distributed over the yolk sac became evident a few hours later (73 HAF).

In yolk sac larvae, the gas bladder first became evident above the oil globule and yolk remnants, just below the notochord of yolk sac larvae. In addition, gill filaments were first evident in yolk sac larvae (69 HAF) and later became highly differentiated in larvae (124 HAF). The gas bladder was lined by thick gas cells (Figure 3), which appeared to aid the gas bladder in its progressive expansion before the onset of larval swim-up (122 HAF). The operculum was first evident within 5 d post-swim-up.

The gastrointestinal (GI) tract appeared shortly after hatch. The first sign of GI tract development was the presence of an anal opening posterior to the lemon-shaped (i.e., ovoid) yolk sac (26–27 HAF; Figure 4). Subsequently, an oral groove was observed and cartilaginous jaws appeared on the yolk sac larvae (28–66 HAF). The mid-intestine was distinguished before stomach differentiation (57 and 65 HAF, respectively). The gallbladder formed between the GI

tract and the liver (66–77 HAF; Figure 3). The posterior portion of the intestine continued to elongate before the lumen of the esophagus and the mouth formed (69 HAF). Mouth formation completed the GI tract development at 89–90 HAF. Before the mouth was completely formed, accumulation of bile fluid in the GI tract produced a golden-yellow pigment plug (i.e., “bile plug”; 85 HAF; Figure 5A). At 93 HAF, the larvae displayed frequent jaw movements and water uptake prompted the elimination of the bile plug by some yolk sac larvae (110 HAF).

Yolk sac resorption was almost complete after elimination of the bile plug. The yolk sac became progressively elongated and granular during resorption. As the yolk was resorbed, the head straightened (65 HAF) and the large intestine was exposed in the yolk sac larvae (Figure 5A). The remaining yolk was limited to the periphery of the oil globule before swim-up and the onset of exogenous feeding.

Exogenous feeding began as the larvae initiated swim-up into the water column (123 HAF). Larvae developed an olfactory bulb, olfactory lobes, and nostrils at 53–66 HAF. In addition, other GI changes associated with exogenous feeding larvae included coiling and thickening of the intestine and development of accessory glands. The pancreas appeared as indicated by the presence of zymogen cells at 90 HAF. Nevertheless, these larvae had little yolk left, and the remaining yolk mainly surrounded the GI tract (Figure 5A, B). There was no significant mixed feeding period (i.e., concurrent use of exogenous and endogenous food sources). Feed was administered before or immediately after the swim-up period to ensure adequate food intake.

At 0 d post-swim-up, Sacramento perch larvae attained a mean TL of 3.1 mm (range = 2.9–3.2 mm, SD = 0.1, $n = 11$; Figure 5A). At this time, the posterior region of the body was straightened, while the anterior region was slightly bent downwards. In addition, myomere mass was distinctively smaller in the posterior region; thus, muscle mass was less conspicuous toward the tail. Moreover, the oil globule was anterior and slightly smaller than the gas bladder until it was finally resorbed within 5 d post-swim-up.

At 5 d post-swim-up, larvae attained a mean TL of 3.6 mm (range = 3.3–3.8 mm, SD = 0.1, $n = 11$). At this stage, the larvae experienced changes in oral morphology and pigmentation. A change in oral morphology was evident as the snout protruded and the position of the mouth was rearranged from slightly subterminal to distinctively terminal. Mouth rearrangement coincided with the initiation of exogenous feeding, as confirmed by the presence of rotifers in the digestive tract of preserved specimens. Pigmenta-

tion of the gas bladder spread ventrally, and stellate melanophores spread over the dorsal region of the body. Sparsely concentrated stellate melanophores occurred along the ventral surface of the body and head. For instance, melanophores formed a line along the middle part of the notochord. In latter stages, melanophores were observed along the tail (81 HAF) and proliferated on the dorsal region of the gas bladder (85–97 HAF). Melanophores continued to migrate on the body axis as they became aligned on the ventral side of the tail and abdomen (155 HAF). Moreover, melanophore abundance increased as melanophores spread over the brain, cerebellum, and otic region (250 HAF).

At 10 d post-swim-up (Figure 5B), the larvae attained a mean TL of 4.2 mm (range = 3.7–4.8 mm, SD = 0.3, $n = 10$). At this stage, the lateral line and the reproductive system were observed for the first time. The lateral line was distinguished from the notochord. The notochord had two lines of pigment cells in some specimens. Primordial germ cells were observed in histological sections. Moreover, pigment cells became more concentrated above the head region and the gas bladder during this stage.

At 15 d post-swim-up, Sacramento perch larvae attained a mean TL of 4.8 mm (range = 4.2–5.8 mm, SD = 0.6, $n = 13$). Larvae presented significant fin ray development and swim bladder differentiation at this stage. Up to 14 caudal fin rays were present in most of the preserved specimens. As the caudal fin rays developed, the distal portion of the notochord bent upwards and the swim bladder became longer. Both fin ray development and swim bladder elongation were more advanced in larger specimens.

At 20 d post-swim-up (Figure 5C), larvae attained a mean TL of 5.9 mm (range = 5.1–6.5 mm, SD = 0.5, $n = 11$). At this stage, larvae had already begun to ingest brine shrimp and showed further development of previous characters. Brine shrimp nauplii were observed in the GI contents of most larvae. The mass of ingested brine shrimp nauplii and cysts was large enough to occupy most of the gut. Primordial dorsal and anal fin development and up to 18 caudal fin rays were evident. Caudal fin ray development from the ventral region toward the dorsal region displaced the notochord upwards. Once most of the fin rays developed, the homocercal tail of larval specimens had a greater definition (Figure 5C). The regions of homocercal tail and dorsal and ventral fin development were also the only areas in which portions of the fin fold blade remained. Pigmentation became more abundant on the head and opercular regions, and the lateral line was darkened by a dense pigment stripe.

At 25 d post-swim-up, larvae attained a mean TL of

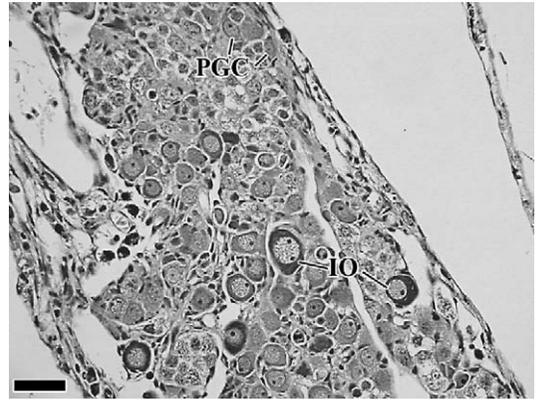


FIGURE 6.—Histological section of an immature ovary from a female Sacramento perch at 43 d post-swim-up (IO = immature oocytes; PGC = primordial germ cells; scale bar = 0.05 mm; stained with hematoxylin and eosin).

7.4 mm (range = 6.3–8.5 mm, SD = 0.8, $n = 10$). The larvae at this stage showed further fin and swim bladder development and more extensive pigmentation than observed 5 d earlier. Up to 14 anal and 18 dorsal fin rays were evident. Dorsal fin rays had differing lengths. Caudal fins were highly pigmented, and their development appeared to have displaced the notochord from the homocercal tail region in most of the specimens examined. Larvae showed further elongation of the swim bladder, almost to the length of the coiled digestive tract.

At 30 d post-swim-up (Figure 5D), larvae attained a mean TL of 9.4 mm (range = 8.2–10.8 mm, SD = 0.8, $n = 14$). Fin development and other morphological changes related to swimming and exogenous feeding appeared to be complete. For instance, the caudal fin was fully developed with up to 24 rays. In addition, there were up to 15 ventral and 22 dorsal fin rays present. Melanophores were sparsely spread along the homocercal tail.

At 43 d post-swim-up, Sacramento perch showed the first sign of sexual differentiation. Specifically, a histological section of one fish revealed immature oocytes and primordial germ cells (Figure 6).

Discussion

Early Development and Organogenesis

The Sacramento perch has a meroblastic pattern of cleavage similar to other modern teleosts and elasmobranchs. The patterns of cleavage are determined primarily by the amount and distribution of yolk (Witschi 1956). Meroblastic cleavage has three distinctive characteristics: incomplete (discoidal), even, and liquefied extra-embryonic yolk. Sacramento perch

cleavage is incomplete, since it is restricted to a disk-shaped area of the animal pole, the blastodisc. In addition, meroblastic cleavage is even and mostly synchronous, thus producing blastomeres of similar size. Cleavage proceeds in meridional direction up to 32 cells and an equatorial direction that elevates the blastodisc over the yolk. Furthermore, the yolk plates become fused, hydrated, and surrounded by a periblast, the syncytial layer that mediates the interaction between the embryo and yolk sac through early development (i.e., cleavage and gastrulation) and organogenesis.

Early development timing can be used as an endpoint in comparative studies of Sacramento perch. Specifically, our results provide baseline information to determine alterations that result from alternative rearing conditions. For instance, developmental studies can address aspects such as in vitro effects of gonadotropic pituitary hormones on Sacramento perch oocytes, duration of mitotic phases, and variations in the patterns of cleavage as compared with other teleosts.

The τ_0 could be used to compare the early development of a larger number of animals (Dettlaff 1988, 1995). Nevertheless, we found that cleavage divisions became asynchronous at the 64-cell stage; therefore, further comparisons of early development timing and organogenesis between the Sacramento perch and other fishes beyond this stage would not be meaningful. Instead, such comparisons could be made for period from fertilization to the onset of the 64-cell stage.

Larval Development

Larval growth and survival can be determined in part by the adequate development of structures that will facilitate exogenous feeding. Development of the eyes and GI tract structures is advanced in larvae. This observation suggests that Sacramento perch larvae are ready for sight feeding once they reach swim-up and initiate exogenous feeding. For instance, saltwater rotifers were selected as larval feed, since Sacramento perch reportedly forage by capturing zooplankton and other small animals in midwater (Moyle et al. 1974; Aceituno and Vanicek 1976; Vinyard 1982). Brine shrimp are an effective food source for Sacramento perch cultured in the laboratory (Wang 1986). Sacramento perch have been categorized as stenophagous, since they can subsist on a limited variety of foods (Vigg and Kucera 1981). Therefore, adequate and timely intake of exogenous foods is crucial for the growth and survival of larvae.

The onset of particular developmental events in Sacramento perch may be dependent more on larval size than age. Our observation of more advanced fin

ray development and swim bladder elongation in larger specimens of the same age-group suggests that these changes are size dependent. Based on histological evidence, sexual differentiation was evident at 43 d post-swim-up. Gross morphology evidence of sexual differentiation was absent at early life stages and was minimal in adults. For instance, adult males are much darker than females, and their dark vertical bands are almost indistinguishable from the background (Aceituno and Vanicek 1976). In this study, pigmentation patterns in larvae were similar to previous observations (Wang 1986). In addition, females tend to be longer than males, most noticeably at older ages, and females are known to be heavier and grow faster than males throughout life (Aceituno and Vanicek 1976). Nevertheless, no genetic sexual dimorphism was found in karyotype analysis for this species (Busack and Thorgaard 1979). Therefore, further studies might be required to determine whether the onset of sexual differentiation in Sacramento perch is size or age dependent.

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