DEVELOPMENT OF THE EGGS AND LARVAE OF THE YELLOWCHIN SCULPIN, *ICELINUS QUADRISERIATUS* (PISCES: COTTIDAE)

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ABSTRACT

The development of the eggs and larvae of *Icelinus quadriseriatus* is described from laboratory-reared and field-collected specimens.

The eggs have diameters from 1.08 to 1.17 mm, an adhesive chorion, and multiple oil globules. Before hatching the oil globules coalesce into one 0.14-0.19 mm in diameter. The embryo develops a patch of tubercles on the dorsal surface of the head that are lost immediately after hatching.

The larvae hatch at 2.6-3.4 mm. Distinguishing characters are 1-6 rows of ventral gut melanophores, 25-63 postanal ventral melanophores, and lower jaw angle pigment. Larvae over 3.9 mm may develop chin and pectoral insertion melanophores. Nasal and parietal spines appear at 9.3 mm. Postflexion larvae develop three patches of pigment dorsolaterally on the body by 10.5 mm and transform to juveniles by 16.3 mm.

Scorpaeniform fishes are represented in the North Pacific Ocean by a large group of endemic taxa whose early life histories are poorly known. The early life histories of many sculpins (Cottidae), the second largest family in the order, were recently described (Richardson and Washington 1980). Larvae of several species of Artedius, Clinocottus, and Oligocottus have been described by Washington (1986). Synchirus gilli larvae were described by Marliave et al. (1985). Reared and field-collected larvae of Chitonotus pugetensis have been described (Misitano 1980; Richardson and Washington 1980); however, the larval stages of the closely related Icelinus, including nine described species and one undescribed species (Yabe et al. 1980; R. Rosenblatt²), are unknown. The purpose of this paper is to describe the eggs and larvae of the yellowchin sculpin, Icelinus quadriseriatus, using both laboratory-reared and field-collected material.

Icelinus quadriseriatus occurs along the coast of California north to Sonoma County (lat. 38°23.5'N; long. 123°08'W) and south to Cabo San Lucas, Baja California (Miller and Lea 1972; Eschmeyer et al. 1983). Adults are usually collected at depths from 18 to 90 m (based on Natural History Museum of Los Angeles County (LACM) and California Academy of Sciences (CAS) adult collection data); occasionally they range beyond these limits, being found in the intertidal zone and as deep as 201 m (Love and Lee 1974). The period of peak occurrence of prespawning females for *I. quadriseriatus* ranges from January to April, but mature oocytes have been found in females in every month except October (in one year of a 2-yr study) indicating an almost year-round spawning capability (Goldberg 1980).

MATERIALS AND METHODS

Adult *I. quadriseriatus* were collected by otter trawl off Santa Monica, CA, on 8 February 1981, 3 July 1981, and 11 March 1982 and off of Huntington Beach, CA, on 19 March 1981. The females were separated from the males (easily recognized by their darkly pigmented anal fin and gill membranes) and their eggs stripped into petri dishes filled with seawater. Sperm stripped from the males was then added to selected clutches of eggs while other clutches were left unfertilized. One clutch from the spawn of July 1981 and two clutches from the spawn of March 1981 were split in half and only onehalf was fertilized.

The eggs were incubated in natural seawater in a refrigerated 227 L tank with undergravel filter and within a temperature range of $13^{\circ}-16^{\circ}$ C (the March 1981 spawn was kept at $14^{\circ}-16^{\circ}$ C). The egg clutches were kept separate in plastic containers each with its own airstone. For the last spawning, March 1982, the undergravel filter was not used and the gravel removed completely in an attempt to cut

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down on bacterial and nematode infestation. The seawater was initially UV sterilized and filtered before use in the tank. Ten percent of the water was replaced every week.

The developing eggs of the first three spawnings were sampled every 2 hours for the first 12 hours and then only once a day. The eggs of the last spawning, March 1982, were sampled at 16, 38, 63, 89, and 110 hours and 6, 8, and 10 days. Living eggs were illustrated using a Wild M5⁸ dissecting microscope with camera lucida. Measurements of egg diameter, perivitelline space, and oil globule diameter were taken with an ocular micrometer.

After hatching the larvae were put into 10 L plastic buckets and later transferred to the main culture tank. Ten percent of the water was changed every day. The larvae were fed the rotifer, *Brachionus plicatilis* (Hunter 1976; Misitano 1978). For the March 1982 spawn the culture tank bloomed initially with algae, including species of *Tetraselmas* and *Isochrysus*. Fresh rotifers and algae were added daily. After 20 days, *Artemia salina* nauplii were added in addition to the rotifers.

The reared larvae were sampled and viewed at hatching and every day thereafter for 12 days, and then less frequently thereafter up to 35 days. A total of 40 larvae were preserved in 4% formalin after being tranquilized with dilute quinaldine. Twentyfive larvae were preserved for analysis of pigment characteristics and morphometric comparison. Length was recorded as notochordal (NL), flexion (FL), or standard (SL) depending on the stage of caudal fin development. Selected sizes of preserved specimens were drawn using the camera lucida.

Field-collected larvae were obtained from the King Harbor Ichthyoplankton collection and the Bightwide Ichthyoplankton Program collection (LACM). Two juveniles (LACM 21639, 43579-1) were obtained from the LACM adult fish collection. Additional specimens were obtained from Marine Ecological Consultants (MEC) of Encinitas, CA. The larva from the King Harbor Ichthyoplankton collection was collected in King Harbor using a single conical 1 m diameter plankton net with 335 μ m mesh towed just below the surface (McGowen 1978). A total of 420 larvae was collected by the Bightwide Ichthyoplankton Program along the California coast between Point Conception and the Mexican border using either an Auriga net (benthic sampler) or a 70 cm diameter bongo net for oblique and middepth tows (R. J. Lavenberg pers. commun.⁴ and G. E. McGowen pers. commun.⁵). A 16.3 mm juvenile was collected by the Bightwide Ichthyoplankton Program with an Auriga net set with 2 mm diameter mesh. Five specimens from MEC were collected off San Onofre, CA, and the Santa Margarita River, CA, using an Auriga net (W. Watson pers. commun.⁶). Transforming larvae >10.5 mm and <16.3 mm were absent from the above collections.

Sixteen specimens were double-stained for bone and cartilage using alizarin red and alcian blue stains (Dingerkus and Uhler 1977) including one juvenile (LACM 43579-1). Eleven specimens including the juvenile were used for meristic counts.

Field-collected larvae were identified by comparing the pigmentation and myomere counts of smaller size larvae (2.7-5.8 mm) with reared larvae and by comparing larger size larvae (<9.3 mm) with cleared and stained specimens identified using vertebral, dorsal fin, anal fin, and pelvic-fin ray counts. A 10.5 mm larva was identified by meristics, including radiograph vertebral counts, and consistent spine and pigment development. A total of 425 fieldcollected larvae was examined; 55 larvae and 2 juveniles were observed for detailed pigment characteristics, morphometrics, and meristic counts.

Definition of terms

- Preanal length = distance from the snout to a vertical line through the anus.
- Body depth = depth of body at the pectoral fin base.
- Pectoral fin length = horizontal distance from upper fin base to posterior edge of fin or end of longest ray.
- Head length = distance from snout to posterior edge of opercle.
- Flexion length (FL) = distance from the snout to the posterior tip of notochord during the stage when the posterior notochord starts to bend upward until the stage when the hypural plates are formed and in their permanent orientation, their posterior edges almost vertical.

Eye diameter = horizontal diameter of eye.

Pectoral insertion = ventral attachment of pectoral fin.

⁸Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

⁴R. J. Lavenberg, Natural History Museum of Los Angeles County Section of Fishes, 900 Exposition Boulevard, Los Angeles, CA 90007, pers. commun. winter 1982.

⁶G. E. McGowen, Natural History Museum of Los Angeles County, Section of Fishes, 900 Exposition Boulevard, Los Angeles, CA 90007, pers. commun. winter 1982.

⁶William Watson, Marine Cological Consultants, 531 Encinitas Boulevard, Suite 110, Encinitas, CA 90024, pers. commun. summer 1981.

Trunk = muscular section of body not including the head, abdominal cavity or caudal fin.

RESULTS

Fertilization

Eight whole egg clutches were externally fertilized with freshly stripped sperm, and five contained eggs that developed successfully into the embryo stage. Three whole egg clutches were left unfertilized, and none contained eggs that developed successfully into the embryo stage. Three whole egg clutches were split in half; one half was fertilized with sperm and the other was left unfertilized. Eggs in all three of the fertilized halves developed successfully into the embryo stage; eggs in the unfertilized halves did not. Therefore, there was no evidence of internal fertilization.

Egg Description

Icelinus quadriseriatus eggs are adhesive and negatively buoyant after being stripped from the female and formed a single clutch of 200-250 eggs (excluding eggs that may have been left in the abdomen; Goldberg [1980] reported an average clutch size of 284 eggs in 28 gravid fish). The eggs are 1.08-1.17 mm in diameter and are initially transparent, but the chorion becomes more opaque and textured during development. A pale-green yolk fills most of the egg except for a small perivitelline space (0.024-0.096 mm).

At spawning there are about 15 yellow oil globules, the largest of which is 0.14 mm in diameter, which coalesce to one oil globule of 0.14-0.19 mm diameter by the eighth day of development. An opaque, flocculant mass is suspended in the yolk next to the oil globules.

Embryonic Development

The eggs develop for 12-13 days in 13° -16°C seawater before hatching. Sixteen hours after artificial fertilization the blastodisc is well formed (Fig. 1A). By 38 h the eggs are in the crescent stage of gastrulation (Fig. 1B). The germ ring can be seen making its way around the yolk. At 63 hours somites begin to form along the embryo and the eye capsule is present (Fig. 1C). At 89 hours the heart (Fig. 1D), brain, and otic capsules are visible, and the body is lined with 30 or more myomeres. The heart begins beating after 110 hours, and the vitelline veins can be seen coursing across the surface of the yolk (Fig. 1E). At 6-7 days the anus forms, the eyes become darkly pigmented (Fig. 1F), and the tail begins flexing back and forth. A patch of tubercles forms on the interorbital section of the head and persists until immediately after hatching.

At 7-8 days the embryos develop pectoral buds (Fig. 1G) and melanophores begin forming on the anus and surrounding yolk sac. The eyes turn a metallic green and blood begins circulating through the ventral veins and arteries of the body.

Numerous melanophores cover the yolk sac, anus, and postanal ventral midline (Fig. 1H) in late-stage embryos. The head flattens against the chorion and the tubercles spread from the snout to just dorsal to the otic capsule.

After 10-12 days eggs reared at 13°-16°C begin to hatch.

Description of Larvae

The larvae hatch with the oil globules positioned at the anterior of the yolk sac (Fig. 2A). The yolk is absorbed after 6-7 days. The oil globules disappear along with the yolk in reared larvae; whereas, the oil globule(s) in field-collected larvae move about the abdominal cavity, are fragmented, possibly increase in diameter, or are absent completely (Fig. 2B-C). Evidence of the globule's increase in diameter in the field-collected larvae is found in some specimens (6.1-8.0 mm SL) with oil globules from 0.26 to 0.36 mm diameter, about twice the diameters of oil globules in the eggs and yolk-sac larvae.

The percentage of field larvae with oil globules (based on a subsample of 129 larvae) drops from 93% in larvae with yolk sacs (N = 15) to 70% in larvae <3.5 mm (N = 79) to 45% in larvae 3.6-6.0 mm (N = 20) to 27% in larvae 6.1-8.0 mm (N = 15). Larvae over 8.0 mm possess minute or no oil globules.

Reared larvae hatch at 2.7-3.4 mm NL (after preservation); field-collected larvae are found as small as 2.6 mm. The larvae shorten to a varying degree, depending probably on the reaction to quinaldine and Formalin. Reared larvae shrink 5-17% after anesthesia and fixation.

Myomere counts range from 31-37. Double-stained specimens have vertebral counts of 33-35.

Morphometrics are given in Table 1. From 6.0 to 9.4 mm the larvae become more deep-bodied (27-30% SL) and the head length increases to 34% of the standard length.

A pigmented preanal finfold does not preserve in the field larvae as it does in the laboratory-reared larvae. There is a skin connection between the anus and the rest of the gut, usually with one or more



FIGURE 1-Eggs of *Icelinus quadriseriatus*: A) 16 hours (LACM 44159-1); B) 38 hours (LACM 44159-2); C) 63 hours (LACM 44159-3); D) 89 hours (LACM 44159-4), arrow indicates heart; E) 110 hours (LACM 44159-5); F) 6 days (LACM 44159-6), arrow indicates anus; G) 8 days (LACM 44159-7), arrow indicates pectoral bud; H) 10 days (LACM 44159-8).

melanophores, but it is not formed into a finfold as in the reared larvae.

Pigmentation

Pigment in the larvae at hatching is restricted to the postanal ventral midline, the ventral abdominal surface, the dorsoposterior peritoneum, the anus, and the lower jaw angle (Fig. 2A). The postanal ventral midline melanophores are positioned in a single row, number from 25 to 37 in yolk-sac larvae and increase to a maximum of 63 in larvae 3.5-4.0 mm in length. These melanophores decrease to a minimum of 25 in larvae 5.5 mm or larger. The ventral

FIGURE 2.—Field-collected and reared *Icelinus quadriseriatus* larvae: A) 2.8 mm (LACM 025-RB-36-AU-01); B) 3 days old (reared), 3.9 mm (LACM 44160-1); C) 4.6 mm (LACM 012-88-36-BB-01); D) 6.6 mm (LACM 012-88-36-BB-01); E) Ventral view, 6.6 mm.



TABLE 1.—Morphometrics of *Icelinus quadriseriatus* larvae, represented as a mean percentage (\bar{x}_p) of body length, with a range (r) of percentages and a standard deviation (SD). Specimens between dashed lines are undergoing flexion of the notochord. * = reared larvae.

| Size | | Preanal length | | | Body depth | | | Pectoral fin length | | | | Head length | | Eye diameter | | |
|----------|---|------------------|-------------|-----|--------------------|-------------|-----|---------------------|-------------|-----|------------------|-------------|-----|----------------|-------------------------|-----|
| (mm) | N | \overline{x}_p | r | SD | \overline{x}_{p} | r | SD | Σ _p | r | SD | \overline{x}_p | r | SD | Χ _ρ | r | SD |
| 2.5-2.9 | 4 | 42.0 | (38.5-46.1) | 4.1 | 29.6 | (25.2-31.9) | 3.0 | 5.9 | (5.2-6.8) | 0.9 | 24.0 | (20.4-26.2) | 2.5 | 11.3 | (9.6-12.2) | 1.2 |
| *2.5-2.9 | 4 | 47.5 | (41.1-51.7) | 4.7 | 25.3 | (23.5-27.5) | 1.7 | 8.4 | (4.3-13.1) | 3.9 | 25.3 | (22.1-28.5) | 2.6 | 12.2 | (11.1-14.1) | 1.3 |
| 3.0-3.4 | 4 | 41.2 | (39.1-45.0) | 2.7 | 23.8 | (21.8-25.5) | 2.0 | 9.1 | (7.9-11.0) | 1.5 | 23.0 | (21.8-24.7) | 1.3 | 10.9 | (10.3-11.3) | 0.4 |
| *3.0-3.4 | 7 | 45.9 | (41.2-51.3) | 3.8 | 21.7 | (19.1-24.2) | 2.1 | 9.4 | (7.1-12.1) | 1.7 | 23.6 | (18.2-26.7) | 2.8 | 11.3 | (10.0-12.4) | 0.9 |
| 3.5-3.9 | 4 | 43.0 | (41.0-44.0) | 1.4 | 22.9 | (21.1-26.6) | 2.5 | 7.4 | (6.6-8.7) | 0.9 | 22.8 | (21.1-25.1) | 1.7 | 10.2 | (9.7-10.9) | 0.5 |
| *3.5-3.9 | 8 | 39.5 | (34.4-50.5) | 4.9 | 19.0 | (15.8-28.5) | 4.2 | 10.7 | (7.4-16.7) | 2.7 | 21.3 | (17.6-31.5) | 4.6 | 9.7 | (8.2-13.6) | 1.8 |
| 4.0-4.4 | 4 | 43.0 | (40.0-47.5) | 3.3 | 24.9 | (21.0-28.2) | 3.0 | 9.3 | (7.3-10.3) | 1.4 | 24.5 | (23.0-26.1) | 1.3 | 9.9 | (9.0-10.8) | 0.7 |
| *4.0-4.4 | 3 | 39.7 | (36.8-43.2) | 3.2 | 20.4 | (15.2-25.2) | 5.0 | 10.5 | (6.6-14.8) | 4.1 | 23.1 | (19.1-26.6) | 3.8 | 9.9 | (8.2-11.1) | 1.5 |
| 4.5-4.9 | 4 | 46.0 | (41.9-47.8) | 3.2 | 23.8 | (22.1-27.3) | 2.4 | 7.4 | (6.8-8.0) | 0.6 | 22.4 | (19.4-25.9) | 2.8 | 9.3 | (8.9-10.0) | 0.9 |
| *4.5-4.9 | 2 | 45.0 | (40.9-49.1) | 5.8 | 24.6 | (23.1-26.0) | 2,1 | 12.5 | (10.7-14.2) | 2.5 | 27.5 | (27.3-27.6) | 0.2 | 11.7 | (11.6-11.8) | 0.1 |
| 5.0-5.4 | 4 | 45.6 | (44.0-48.7) | 2.1 | 24.9 | (21.1-27.7) | 2.8 | 7.4 | (5.5-9.2) | 1.6 | 23.4 | (20.4-26.2) | 2.6 | 9.1 | (8.5-10.0) | 0.7 |
| 5.5-5.9 | 4 | 44.9 | (43.6-48.0) | 2.1 | 26.5 | (25.3-28.0) | 1.2 | 8.7 | (6.5-10.5) | 1.7 | 25.4 | (22.5-27.5) | 2.3 | 9.3 | (8.7-10.0) | 0.6 |
| *5.5-5.9 | 1 | 48.6 | • | | 26.6 | | | 14.8 | • | | 29.7 | | | 11.0 | | |
| 6.0-6.4 | 4 | 49.2 | (46.1-54.7) | 3.7 | 27.3 | (25.9-28.3) | 1.4 | 9.1 | (7.5-11.4) | 1.8 | 27.5 | (24.4-30.2) | 2.4 | 8.7 | (8.3-9.2) | 0.4 |
| 6.5-6.9 | 4 | 46.0 | (44.3-49.9) | 2.7 | 27.2 | (24.4-31.2) | 3.0 | 11.5 | (8.1-15.7) | 4.6 | 29.4 | (25.3-33.9) | 3.9 | 8.9 | (8.4-9.4) | 0.4 |
| 7.0-7.4 | 4 | 46.7 | (44.1-52.2) | 3.2 | 27.8 | (25.4-29.1) | 1.5 | 12.1 | (9.3-14.9) | 2.9 | 27.9 | (24.7-31.5) | 2.5 | 8.8 | (8.5-9.2) | 0.3 |
| 7.5-7.9 | 3 | 48.7 | (47.0-49.7) | 1.5 | 27.5 | (27.2-27.9) | 0.4 | 17.2 | (16.3-18.3) | 1.0 | 30.2 | (29.5-31.2) | 0.9 | 9.3 | (8.7-9.6) | 0.5 |
| 8.0-8.4 | 2 | 50.7 | (47.7-53.6) | 4.2 | 29.0 | (27.3-30.7) | 2.4 | 1 9 .7 | (15.9-23.5) | 5.4 | 29.8 | (29.6-30.0) | 0.3 | 9.6 | (8. 9- 10.2) | 0.9 |
| 8.5-8.9 | 4 | 51.5 | (49.4-53.2) | 1.6 | 30.8 | (27.4-33.9) | 2.7 | 22.3 | (21.1-24.2) | 1.3 | 34.4 | (32.4-37.3) | 2.4 | 9.5 | (8.7-9.9) | 0.5 |
| 9.0-9.4 | 4 | 52.2 | (50.9-54.0) | 1.3 | 29.7 | (29.3-30.0) | 0.3 | 23.9 | (22.5-25.1) | 1.2 | 33.8 | (31.1-36.7) | 2.3 | 9.3 | (8.7-10.2) | 0.7 |
| 9.5-10.4 | - | | | | _ | | | — | | | — | | | — | | |
| 10.5 | 1 | 51.4 | | | 25.6 | | | 32.2 | | | 39.1 | | | 11.1 | | |
| 16.3 | 1 | 51.5 | | | 22.7 | | | 28.8 | | | 36.8 | | | 11.0 | | |
| 18.7 | 1 | 46.5 | _ | _ | 26 .7 | | | 24.6 | | | 37.9 | | | 9.1 | | |

abdominal pigment consists of 1-6 rows of melanophores aligned anteroposteriorly (Fig. 2E). The dorsal peritoneal pigment consists of 2-5 melanophores in a double row over the posterior half of the gut, increasing to a maximum of 17 in a 5.8 mm reared larva. There are usually 3-5 melanophores surrounding the anus in newly hatched larvae and up to 12 in the larger larvae. There is always a distinct melanophore on the lower jaw angle.

As the larvae grow they develop melanophores on the isthmus (throat), chin, pectoral insertion, anterior gut (usually 2 melanophores slightly internal from the ventral abdomen), head, and dorsal body (Table 2). Reared larvae >4.0 mm possess considerably more pigment (based on the number of melanophores) on the dorsal body and the anterior gut areas than field larvae of the same size.

The larvae undergo flexion between 5.2 mm and 7.6 mm (reared larvae $4.5 \cdot 5.8 + \text{ mm}$). Melanophores (1-4) are usually present on the caudal fin anlage and later at the base of the caudal fin. In flexion and postflexion larvae the caudal fin base melanophores are present in over 95% of the specimens.

Postflexion larvae (8.0-9.3 mm SL) develop numerous small punctate melanophores over the midbrain portion of the cranium (Fig. 3A). Melanophores form between the otic capsule and the hindbrain. Melanophores occur on the preopercle between the eye and the fourth preopercular spine. There are 1-3 melanophores at the pectoral insertion, 3-6 melanophores along the pectoral base, and a circle of 7-9 small melanophores dorsal to the pectoral origin. There are 4-11 small melanophores ventral to the eye and dorsal to the maxillary bone and 1 melanophore at the posteroventral edge of the maxillary. Several melanophores occur along the edge of the mandible from the articular to the dentary bone. Four to five minute melanophores are situated between the eye and premaxillary bone. The ventral abdomen becomes sprinkled with 40-45 melanophores.

Transition from larval to juvenile pigmentation starts at about 9.2-9.5 mm SL. Transforming larvae develop four patches of melanophores on the dorsal trunk and three patches on the lateral trunk (Fig. 3B). Melanophores appear on the dorsal and caudal fins. In larger specimens (Fig. 3C) the head and dorsolateral pigment takes on the juvenile pattern. The dorsal and lateral trunk melanophores merge forming three dorsolateral bars on the body. Distinct patches of melanophores cover the hypural

FIGURE 3.—Field-collected *Icelinus quadriseriatus* postlarvae and juvenile (D): A) 9.3 mm (LACM 012-88-36-BB-01); B) 9.2 mm (MEC I48 Stn. E Rep. #2); C) 10.5 mm (MEC I-89, E-LS EPI); D) 18.7 mm (LACM 21639).









TABLE 2.—Presence of melanophores at described locations in *Icelinus quadriseriatus* larvae, represented as a percentage of larvae showing the melanophoes. * = reared larvae.

| Size range (mm) | N | Lower jaw angle | lsthmus (throat) | Chin | Pectoral inser- tion | Anterior gut | Head (dorsal) | Dorsal trunk |
|-----------------------|---|-----------------------|---------------------|-------|----------------------------|-----------------|------------------|-----------------|
| 2.5-2.9 | 4 | 100.0 | 0.0 | 0.0 | 0.0 | 25.0 | 0.0 | 0.0 |
| *2.5-2.9 | 4 | 100.0 | 25.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3.0-3.4 | 4 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *3.0-3.4 | 7 | 100.0 | 14.3 | 42.9 | 14.3 | 0.0 | 14.3 | 14.3 |
| 3.5-3.9 | 4 | 100.0 | 75.0 | 50.0 | 25.0 | 0.0 | 0.0 | 0.0 |
| *3.5-3.9 | 8 | 100.0 | 75.0 | 62.5 | 50.0 | 12.5 | 37.5 | 25.0 |
| 4.0-4.4 | 4 | 75.0 | 25.0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *4.0-4.4 | З | 100.0 | 100.0 | 100.0 | 100.0 | 66.7 | 33.3 | 0.0 |
| 4.5-4.9 | 4 | 100.0 | 25.0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *4.5-4.9 | 2 | 100.0 | 100.0 | 100.0 | 100.0 | 50.0 | 50.0 | 50.0 |
| 5.0-5.4 | 4 | 100.0 | 50.0 | 25.0 | 25.0 | 0.0 | 0.0 | 0.0 |
| 5.5-5.9 | 4 | 100.0 | 100.0 | 0.0 | 25.0 | 25.0 | 0.0 | 0.0 |
| *5.5-5.9 | 1 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 6.0-6.4 | 4 | 100.0 | 100.0 | 25.0 | 50.0 | 25.0 | 0.0 | 0.0 |
| 6.5-6.9 | 4 | 100.0 | 100.0 | 25.0 | 50.0 | 0.0 | 0.0 | 0.0 |
| 7.0-7.4 | 5 | 100.0 | 80.0 | 40.0 | 0.0 | 20.0 | 0.0 | 20.0 |
| 7.5-7.9 | 3 | 100.0 | 100.0 | 0.0 | 66.7 | 0.0 | 0.0 | 0.0 |
| 8.0-8.4 | 2 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| 8.5-8.9 | 3 | 100.0 | 100.0 | 66.7 | 100.0 | 33.3 | 33.3 | 33.3 |
| 9.0-9.4 | 5 | 100.0 | 100.0 | 80.0 | 100.0 | 60.0 | 80.0 | 20.0 |

plates. The ventral abdomen and postanal ventral melanophores begin to fade and become less numerous.

Transformed juveniles (including a 16.3 mm specimen, LACM 056-OB-75-JA01) display most adult characters (Bolin 1944) including a double row of scales just ventral to the dorsal fin (Fig. 3D). A gap in the scale row is located below the insertion of the second dorsal fin. Melanophores almost disappear from the postanal and ventral midline and concentrate on the head, dorsal body, and fins. There is a concentration of pigment between the eye and the lower jaw angle and at the base of the caudal fin.

Meristic Elements

Ray elements of the pectoral and caudal fins start developing in larvae 6.8-7.0 mm long. Spine and ray elements of the dorsal and anal fins develop at about 7.4-7.6 mm. The pelvic fin elements are countable in specimens that are double stained and at least 8.2 mm SL (Table 3).

The fin and vertebral counts of 10 double stained field-collected larvae 7.4-8.9 mm long and one juvenile 27.8 mm SL correspond closely to the counts of 36 x-rayed adults from the LACM collection. These counts are consistent with x-ray count frequencies given in Howe and Richardson (1978) except for the dorsal spine counts. The most frequent dorsal spine count listed in Howe and Richardson was eight whereas in the LACM specimens it was nine. It is possible that there is some slight variability in this meristic character or the last (inconspicuous) dorsal spine may have been overlooked in the former's x-ray counts.

TABLE 3.—Meristics of *lcelinus quadriseriatus* larvae. ND = no data (specimens between the dashed lines are undergoing flexion of the notochord). * = reared larvae; + = cleared and stained larvae; $^{\circ}$ = x-rayed.

| _ | | | | | | | | | | | | | | |
|-----|--------------|-------------------|-----------------|---------------|-------------------|-------------------|------------------------------|---------------------------------------|-------------------|---------------------|------------------|-----------------|----------|----------------------------------|
| | Size (mm SL) | Dorsal fin spines | Dorsal fin rays | Anal fin rays | Pectoral fin rays | Pectoral fin rays | (right) Pelvic fin spines | Pelvic fin spines and rays (right) | Preopercie spines | Precaudal vertebrae | Caudal vertebrae | Total Vertebrae | Myomeres | Postanal ventrat melanophores |
| | 2.7 | - | _ | _ | _ | | | _ | _ | _ | _ | _ | 34 | 33 |
| | 3.5 | | _ | _ | _ | _ | _ | _ | | - | | | 34 | 42 |
| | 4.7 | _ | _ | _ | _ | _ | _ | _ | | _ | _ | _ | 34 | 32 |
| | | | | | | | | | | | • • | | | • |
| + | 5.3 | - | - | — | — | - | | — | | - | — | — | 34 | 44 |
| | 5.7 | - | — | — | — | - | | | | - | — | — | 36 | 38 |
| • | ′5. 8 | - | | — | — | - | — | — | | - | — | — | 34 | 34 |
| | 6.6 | - | — | — | — | _ | — | — | | — | — | — | 34 | 34 |
| + | 6.8 | - | — | - | 11 | 11 | buds | buds | 2 | ND | ND | ND | 35 | 26 |
| + | 7.4 | IX | 12 | 11 | 16 | 16 | — | — | 4 | 10 | 24 | 34 | ND | 30 |
| | | | | | | | | | | | | | | |
| + | 7.0 | ~ | | 12 | 12 | 14 | Duds | sbuds | 3 | | | ND | 30 | 27 |
| | 1.1 | <u>۲</u> | 14 | 10 | 10 | 10 | Duas | souas | 4 | | | | 00 | 20 |
| + | 0.2 | <u>.</u> | 15 | 13 | 10 | 10 | 1,2 | 1,2 | 4 | 11 | 22 | 33 | | 34 |
| + | 0.0 | | 14 | 12 | 10 | 10 | 1,2 | 1,2 | 4 | 11 | 23 | 34 | | 31 |
| | 9.2 | IX I | 14 | 12 | 10 | 10 | 1,2 | 1,2 | 4 | ND | ND | 34 | 35 | 29 |
| | 9.3 | | 14 | 12 | 16 | 16 | 1,2 | 1,2 | 4 | | ND | 34 | 34 | 32 |
| ~1 | 0.5 | | 13 | 12 | 15 | 16 | 1,2 | 1,2 | 4 | NU | UN 00 | 33 | 33 | 28 |
| - 1 | 0.3 | IX. | 14 | 12 | 16 | 16 | 1,2 | 1,2 | 4 | 11 | 23 | 34 | ND | 8 |
| -1 | 8.7 | VIII | 13 | 11 | 16 | 76 | 1,2 | 1,2 | 4 | 10 | 24 | 34 | ND | _ 4 |

Ossification

Ossification begins in larvae at least 5.3 mm NL long. The cleithrum, premaxilla, maxilla, mandible, parts of the neurocranium, and 3 of 6 branchiostegal rays on each side are ossified. The first 14 vertebrae, the middle 10 out of 12 principal caudal rays, 11-13 pectoral rays, 2-3 preopercular spines, and opercle bone, and all of the branchiostegal rays become ossified at 6.8-7.0 mm.

Ossification is well developed in specimens 7.4 mm FL long or greater. The nasal, quadrate, dorsal spines, and the first 28 of 33 vertebrae become ossified as well as the hypural plates and all of the principal caudal rays.

A double row of teeth appears on the lower jaw of double-stained larvae between 6.8 and 7.4 mm FL in length. On the upper jaw a single row of teeth is seen in larvae 8.5 mm SL or greater in length.

Spination

One to three preopercular spines appear at 6.5-7.0 mm FL. Four preopercular spines are visible in larvae over 7.0 mm FL. Nasal and parietal spines form at 9.0-9.3 mm SL. Double-stained material shows the parietal spine arising from two arcs of bone that fuse distally into one spine before breaking the surface of the skin. A foramen remains in the center of the spine and is retained in the parietal spines of juveniles and adults.

Development of the Caudal Complex

The caudal fin anlage begins to form in preflexion larvae of about 5.3 mm NL (Fig. 4A). During flexion three non-ossified hypural elements form ventral to the notochord (Fig. 4B). The first hypural (identified as $HY_{1.3}$) may be a remnant of a fusion process of two to three elements. Matarese and Marliave (1982) described three elements that fuse to form the inferior hypural plate in Ascelichthys rhodorus; a foramen is left where the parhypural (counted as HY_1) fuses with HY_2 and HY_3 . In *I.* quadriseriatus a foramen is present in $HY_{1.3}$.

The second and third hypural elements (HY₄ and HY₅) form separately (Fig. 4B-C) and fuse to form the ossified superior hypural plate (HY₄₋₅) (Fig. 4D). Finally, the two plates (HY_{1.3} and HY₄₋₅) begin to fuse anteriorly in juveniles (Fig. 4D).

Cartilaginous neural spines begin to form during flexion (Fig. 4B). By about 8.7 mm SL the neural spine on the first preural centrum (PU₁) appears (on one specimen) as two distinct elements (Fig. 4C).



FIGURE 4.—Caudal complex of *Icelinus quadriseriatus*: A) 5.3 mm (LACM 012-88-36-BB-01); B) 6.8 mm (LACM 012-88-36-BB-01); C) 8.7 mm (LACM 012-88-36-BB-01); D) 27.8 mm (LACM 43579-1). EP = epural; HS = hemal spine; HY = hypural; NC = notochord; NS = neural spine; PU = preural centra; U = urostyle; UN = uroneural.

This condition has been illustrated in one other larval sculpin (Matarese and Marliave 1982, Fig. 2E), but may be variable. By the juvenile stage the neural element(s) appear as one broad, ossified spine (Fig. 4D).

Hemal spines form in unison with the neural spines. The hemal spine on the first pre-ural centrum develop an elongate, descending process to which several procurrent rays articulate. A similar elongate structure was identified as the parhypural in *Icelus spiniger* (Nelson 1984). The present study agrees with Materese and Marliave (1982) and Yabe (1985) in identifying the process as part of the hemal spine because it is always associated with the spine during development.

Twelve principal caudal rays develop and articulate with the hypural plates during flexion (Fig. 4B). The number of these rays varies in other sculpins from 11 to 13 (Materese and Marliave 1982; Nelson 1984).

Procurrent rays form after flexion (Fig. 4C) and increase in number to a maximum of 10 for the upper procurrent rays and 8 for the lower procurrent rays. The last lower procurrent ray appears to articulate with a radial cartilage between the posterior tip of the descending hemal spine process and the anteroventral edge of the inferior hypural plate.

Three epurals form dorsal to the ural-centrum in the postflexion larvae and are also present in juveniles and adults. A uroneural appears dorsal to the reduced urostyle in the juveniles (Fig. 4D).

Occurrence

Icelinus quadriseriatus larvae were collected in every month of the year with peak occurrence from May to June in 1979, which is in agreement with peak occurrence of prespawning females (Goldberg 1980) for 1977, allowing for a time lag between prespawning condition and hatching of the larvae; the larvae are assumed to be planktonic for about 2 months. Peak spawning periods may vary yearly. Goldberg (1980) reported a winter peak of oocyte development that was earlier in 1978 (January-April) than in 1977 (March-June).

In the Southern California Bight, *I. quadriseriatus* larvae seemed to be concentrated in deeper waters away from the intertidal and shallow subtidal areas. Of the 33 bightwide discrete depth samples (nueston, middepth, and epibenthic) collected in 1979 in which *I. quadriseriatus* occurred, only 2 samples were taken at the 8 or 15 m depth stations. Ten samples were taken at the 22 m stations and 21 samples were taken at the 36 m station.

Icelinus quadriseriatus larvae were generally found near the bottom of the water column. Of the 33 discrete depth tows taken in which *I. quadriseri*atus larvae were found, 31 were epibenthic tows and two were middepth tows.

The bottom temperature at stations where I. quadriseriatus larvae occurred ranged from 11° to 14°C. Since the larvae were usually found at the bottom, this suggests that these are the temperatures under which they normally develop in the field. Reared larvae were observed to survive aquarium temperatures of at least 18°C.

DISCUSSION

Fertilization

Many cottids, including Chitonotus and Oligocottus snyderi, practice internal fertilization facilitated by an intromittent male sex organ (Morris 1956; Hubbs 1966; Stein 1973; Misitano 1980). Bolin (1941) reported that Orthonopias triacis has internal fertilization even though the males do not have an external penis. Apparently the female has a "protrusile oviduct" which is probably smeared with sperm and then retracted. Fertile eggs have been removed from females of Scorpaenichthys marmoratus and Icelinus filamentosus (Hubbs 1966). Icelinus quadriseriatus males possess no external penis and since no females were found with fertile eggs, it is still questionable as to what mode of fertilization these sculpins possess. It is possible that I. quadriseriatus has external fertilization (a rarity among sculpins), but until the mating process is observed or an internally fertilized female is found, this question will remain unanswered.

Comparison with Other Sculpin Eggs

The eggs of *I. quadriseriatus* are adhesive, demersal, and share many characteristics with other cottid eggs. The presence of a flocculant mass inside the yolk has been observed in several other genera such as *Chitonotus*, *Orthonopias*, *Clinocottus*, and *Leptocottus* (Bolin 1941; Morris 1951; Jones 1962; Misitano 1980). Multiple oil globules that coalesce to one globule are common in other sculpins; however, *I. quadriseriatus* does have the highest initial number of oil globules (15) recorded in the literature. The diameters for *I. quadriseriatus* eggs are closest to the diameters of *Artedius lateralis* and *Clinocottus analis* eggs (Budd 1940).

The pale-green color of the yolk and the pigmentation of the late-stage embryo are most similar to *Chitonotus pugetensis* eggs (Goldberg 1980; Misitano 1980). (The eggs of other *Icelinus* species have not been described and could not be used for comparison.)

The appearance of tubercles on the head of the late-stage embryos has been observed in several other cottid species. Budd (1940) described them in Artedius lateralis and Clinocottus analis as a "patch of minute nodules" which are "believed to aid as a rasp in breaking through the shell... allowing the larva to escape anterior end foremost". Bolin (1941) referred to them in Orthonopias as a "large number of small granular patches". Morris (1951) described them in Clinocottus recalvus as "minute convexities of low elevation which grade into the dorso-anterior surface of the head" and are formed due to the "the extreme pressure which it suffers during its late stages of confinement". In the case of I. quadriseriatus the head does flatten in the late-stage embryos and may be under pressure, but whether these structures form in response to that pressure is not known. The tubercles may serve only to reinforce the area of the head which pushes its way through the chorion.

Distinguishing Larval Characters

Distinguishing characters include 1-6 rows of ventral gut melanophores, 25-63 postanal ventral melanophores, and a distinct lower jaw angle melanophore on either side. An increasing percentage (25-100%) of larvae over 3.5 mm in each size class develop isthmus, chin, and pectoral insertion melanophores.

Icelinus quadriseriatus larvae are characteristic of the Paricelinus/Triglops/Icelus/Chitonotus/Icelinus group of Richardson (1981) which is distinguished by four preopercular spines, a pointed snout, moderately slender body, and postanal pigment restricted to ventral midline.

Paricelinus, Triglops, and Icelus all have a higher number of vertebrae: 40-42, 44-54, and 37-44 respectively (Washington et al. 1984), than I. quadriseriatus. Paricelinus hopliticus in addition has pigment on the snout, nape, and anterior gut in preflexion larvae.

Chitonotus pugetensis larvae, while similar in appearance to yellowchin larvae, differ in having several anterior gut melanophores, an early development of head pigment and a slightly higher range of vertebrae (35-36). Larger (6.0-9.4 mm) C. pugetensis are more slender-bodied (23-25% SL) (Richardson and Washington 1980) than I. quadriseriatus SL (27-30%). In transforming C. pugetensis larvae there are usually three pelvic fin rays and a nuchal spine next to the parietal spine while *I. quadriseriatus* has only two pelvic rays and no nuchal spine. The fin ray counts of *C. pugetensis* (Howe and Richardson 1978) are somewhat higher than the yellowchin, especially the anal fin rays (14-17, x = 15.7, in *C. pugetensis*; 10-13, x = 12.1, in *I. quadriseriatus* larvae).

Artedius creaseri and A. meanyi, recently added to this group (Washington 1986), have anterior gut pigment, pigment in the ventral finfold and fewer postanal ventral melanophores (<13). Postflexion A. creaseri and A. meanyi larvae have nuchal spines next to the parietal spines.

Seven species of *Icelinus*, all with undescribed larval stages, co-occur with *I. quadriseriatus* in the Southern California Bight, including one undescribed species (R. Rosenblatt fn. 2). Several larvae have recently been collected at a 75 m depth station (LACM) that are very similar to field-collected *I. quadriseriatus* larvae including possession of lower jaw angle, chin, isthmus, and pectoral insertion melanophores. A cleared and stained specimen has a count of 38 vertebrae that identifies it tentatively as *I. tenuis*. These larvae differ from *I. quadriseriatus* in having a higher myomere count (37-40), anterior peritoneal pigment similar to *Chitonotus*, and an absence of caudal melanophores.

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