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Hormone-induced spawning and development of artificially reared larvae of the West Australian dhufish, *Glaucosoma hebraicum* (Glaucosomatidae)

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Abstract. Hormone-induced spawning of the West Australian dhufish, *Glaucosoma hebraicum* (Glaucosomatidae) is reported, and the artificially-reared eggs and larvae are described. Hormones tested were hCG, Ovaprim and LHRH analogue, the last both as implants and in a saline solution. Late-stage fertilized eggs are spherical (960–1200 µm), possess a pigmented oil globule (210–273 µm) anteriorly on the unsegmented yolk, and hatch in about 32 hours at ~23°C. Newly hatched larvae (1.75–2.10 mm) have a prominent yolk sac which is resorbed after 3.3 days. The mouth becomes functional from Day 3, first feeding occurs from Day 3.5 and the inflated gas bladder is first observed from Day 4.3. Larvae examined (1.75–8.13 mm) have a moderately deep body, 24–25 myomeres, early-forming pelvic fins that are heavily pigmented by the postflexion stage, small to moderate head spination, no gap between anus and anal-fin origin, and a distinct pattern of pigmentation. Notochord flexion occurs between 3.42 (15 days) and 4.83 mm (25 days), and the transition to the juvenile stage starts from ~8 mm (45–46 days). Comparisons with taxa with similar larvae show that *Glaucosoma* larvae share some similarities with pempherid larvae, although the evidence is not sufficient to strengthen the view that both groups are closely related.

Extra keywords: eggs, spawning induction

Introduction

The West Australian (WA) dhufish, *Glaucosoma hebraicum* Richardson, 1845 (Family Glaucosomatidae) is predominantly a benthic species endemic to Western Australia, commonly found around caves and ledges on offshore reefs between Shark Bay (25°30′S,114°30′E) and the Recherche Archipelago (33°52′S,121°54′E), to depths of ~200 m (Hutchins and Swainston 1986; Paxton and Hanley 1989; McKay 1997). The dhufish is a highly regarded table fish and one of the most popular recreational fish in Western Australia, attaining a maximum size of 1.22 m total length and 26 kg (Hutchins and Swainston 1986; Kailola *et al*. 1993; McKay 1997). It is caught throughout the year across most of its distributional range, with commercial catches of 100–200 t year^{-1} between 1992–93 and 1996–97 (Kailola *et al*. 1993; Anon. 1997).

A great deal of knowledge of early life history of temperate Australian fishes has been gained from fish culture. Whereas aquaculture research on freshwater species has been aimed mainly at rearing larvae for restocking programmes in inland waterways (e.g. Rowland 1983, 1988), rearing of marine and estuarine fishes has been aimed at species suitable for commercial aquaculture (e.g. Ruwald *et al*. 1991; Battaglene and Talbot 1992, 1994. In Western Australia there has been success in rearing black bream (*Acanthopagrus butcheri*) and snapper (*Pagrus auratus*) and, in the past two years, the WA dhufish *G. hebraicum* (Jenkins and Frankish 1997). Biological data on dhufish suggest that this species may have attributes that make it highly attractive for aquaculture, including fast initial growth and easy handling in captivity (Hesp 1997). In addition, a market survey suggested that an opportunity existed in Australia for its commercial aquaculture (I. Barnetson, unpublished).

The limited information on the reproductive biology of *G. hebraicum* indicates that this species is a multiple spawner, spawning at depths of 40–50 m between late November and April, with a peak in late January and early February. In addition, fecundity estimates range from 0.3 million to 4 million eggs, with females reaching first maturity at 25–30 mm standard length by the end of their fifth year (Cockman and McGuire 1986; Hesp 1997). Despite several ichthyoplankton surveys carried out in waters south of Shark Bay (WA) in recent years (e.g. Jonker 1993; Kendrick 1993), neither eggs nor larvae of *G. hebraicum* have been reported, and no records are available of juveniles <60 mm total length (Sudmeyer *et al*. 1990). The present paper briefly describes the hormone-induced spawning of *G. hebraicum* and provides a description of the early life-history stages of this species based on material reared in the laboratory. The methods and results presented here on the hormone-induced spawning are only preliminary, and controlled quantitative

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studies are required. The descriptions of the eggs, and of the larvae from the yolk-sac through to the postflexion stage, constitute the first for fishes of the family Glaucosomatidae.

Materials and methods

Broodstock

Glaucosoma hebraicum adults (1.6–12.0 kg) were caught by hand-line between 1995 and 1997 off Lancelin (31°0′S,115°18′E), in depths of <25 m. Upon capture, fish were placed directly into a 250-L seawater container and transported to the research centre for acclimatization. Captive dhufish were kept in several fibreglass and concrete 10–50 m^3 tanks at densities of 0.5 to 3.0 kg fish m^{-3} with continuously flowing seawater, and a minimum exchange of 10% h^{-1}. Male:female ratio ranged from 1:1 to 1–2:3–4. A net (500 µm mesh) was installed on each tank overflow to collect naturally spawned eggs.

Hormonal treatment

Broodstock fish were anaesthetized with 300 mL m^{-3} of 2-phenoxy-ethanol. Each fish was tagged intraperitoneally with a passive transponder 11.5 mm long × 2.0 mm diameter. For examination of maturity stage, sperm were sampled by manual stripping and oocytes by using a sterile plastic cannula. Oocytes were examined under a dissecting microscope and the diameters (µm) of the largest oocytes were measured with an eyepiece micrometer.

Males. A total of 17 males were treated with human chorionic gonadotrophin (hCG), and LHRH analogue (LHRH-a; des-Gly^{10},[D-Ala^{6}]-luteinizing hormone-releasing hormone ethylamide) in a 95% cholesterol-base slow-release implant (LHRH-a *imp*) and in a saline solution (Table 1).

Females. A total of 22 females with oocytes ≥400 µm in diameter were injected intraperitoneally with various dosages of hCG, LHRH-a *imp*, LHRH-a in a saline solution, and Ovaprim containing gonadotrophin-releasing hormone (GnRH) and Domperidone (Table 1). The sterile needles used for injection were 18-gauge for liquid hormones and 2 mm diameter for implants. Fish were anaesthetized and their eggs were stripped manually at the first sign of ovulation.

Artificial fertilization

Sperm samples were collected in sterile syringes by manually stripping anaesthetized fish. Each sample was examined under a compound microscope and sperm showing low or no motility was rejected. The selected sperm was kept at room temperature for a maximum of 1 h before it was used.

Eggs from females that had ovulated were manually stripped into plastic containers, transferred into a 1-L container of seawater (filtered to 1 µm absolute) and sterilized by ultraviolet radiation. Sperm from at least two males was added to the suspension of eggs, gently mixed and left for about 5 min, and the floating fraction then disinfected and transferred into an incubator. Fertilized eggs were rinsed with 22–24°C seawater (filtered to 1 µm absolute), sterilized by ultraviolet radiation and incubated.

Table 1. Hormone treatments in male and female *Glaucosoma hebraicum*

	Hormone	Dosage
Males	hCG	770–1130 IU kg^{-1}
	LHRH-a *imp*	29–111µg kg^{-1}
	LHRH-a (saline solution)	67–80 µg kg^{-1}
Females	hCG	870–1200 IU kg^{-1}
	LHRH-a *imp*	5–125 µg kg^{-1}
	LHRH-a (saline solution)	5 µg kg^{-1}
	Ovaprim	6 µg GnRH kg^{-1}

Rearing of larvae

Larvae were reared under continuously flowing seawater that was filtered to 1 µm absolute by cartridge filtration and sterilized by ultraviolet radiation. Water temperature was maintained between 22° and 24°C. Larvae were fed rotifers (*Brachionus plicatilis*) and developmental stages of brine shrimp (*Artemia salina*).

Material examined

Naturally and hormonally induced spawned eggs of *G. hebraicum*, both unfertilized and fertilized, were measured to the nearest 10 µm under a dissecting microscope fitted with an eyepiece micrometer (Table 2). A total of 131 laboratory-reared larvae (1.75–8.13 mm body length, BL) were used (Table 3) to describe morphometrics and pigmentation. Larvae had been fixed in 5% formaldehyde and later preserved in 70% ethanol. Representatives of the larvae examined were lodged with the Australian Museum (Sydney). Two artificially reared juveniles (22.54–24.25 mm BL) were also examined (excluded from Table 3).

Terminology used to describe head spines and morphometric measurements of larvae follows Neira *et al.* (1998). All measurements were made to the nearest 0.01 millimetre under a dissecting microscope fitted with an eyepiece micrometer. Body length (BL) corresponds to notochord length (tip of snout to tip of notochord) in preflexion and flexion larvae, and to standard length (tip of snout to posterior margin of hypurals) in postflexion larvae. Measurements of body depth (BD), head length (HL) and preanal length (PAL) given throughout the text and in Table 3 are expressed as a percentage of body length. Body depth and head length (in terms of % BL) were used to describe a larva as either very elongate (BD <10%), elongate (BD 10–20%), moderate (BD 21–40%) or deep (BD 41–70%), and as having a small (HL <20%), moderate (HL 20–33%) or large head (HL >33%); preanal length was used to characterize a larva as having a moderate (PAL 30–50%), long (PAL 51–70%) or very long gut (PAL >70%). Pigment described refers solely to melanin. Illustrations were made with the aid of a camera lucida.

Results

Natural spawning

The smallest captive male and female dhufish in spawning condition were 47 cm total length (2.2 kg) and 43 cm total length (1.6 kg), respectively. Natural maturation in males was recorded between December and April. Most females underwent gonadal development but did not ovulate or spawn naturally.

Induced spawning

Induced maturation was initially best achieved with implants of about 100 µg kg^{-1} LHRH-a *imp*. Saline injections of hCG and LHRH-a did not induce spawning in males, but LHRH-a *imp* stimulated some sperm production. Sperm produced by males injected with LHRH-a *imp* was similar to that obtained in mature males that were not injected, and contained a high density of highly motile spermatozoa. The three hormones tested on females (Table 1) induced final maturation of oocytes and ovulation, particularly LHRH-a which induced egg production at all dosages. However, although treatments with either LHRH-a

Table 2. Diameter (µm) of eggs of *Glaucosoma hebraicum* spawned naturally and after hormone treatment
n, no. of eggs measured. F, fertilized; UF, unfertilized

	Naturally spawned UF	hCG 870 IU kg^{-1} UF	hCG 1200 IU kg^{-1} UF	hCG 1200 IU kg^{-1} F	LHRH-a *imp* 100 µg kg^{-1} UF	LHRH-a *imp* 109 µg kg^{-1} F
Range	1000–1200	1000–1120	960–1060	1000–1080	1000–1060	1000–1100
Mean ± 2 s.e.	1074 ± 21	1047 ± 24	1014 ± 7	1041 ± 15	1035 ± 10	1046 ± 5
n	20	14	51	14	17	76

Table 3. Body length, age and body proportions of larval stages of *Glaucosoma hebraicum* reared in the laboratory
n, no. larvae; BL, body length; HL, head length; BD, body depth; PAL, preanal length. Values for HL, BD and PAL given as percentage of body length and shown as range and mean ± 2 s.e.

	Larval stage			
	Yolk sac (*n* = 36)	Preflexion (*n* = 47)	Flexion (*n* = 23)	Postflexion (*n* = 25)
BL (mm)	1.75–2.60	2.30–3.95	3.42–4.80	4.83–8.13
Age (days)	0–3	3–15	16–27	25–46
HL (% BL)	9.2–25.0 (16.3 ±1.6)	18.8–39.1 (29.0 ± 1.5)	34.9–50.0 (40.8 ± 1.5)	35.2–59.1 (43.1 ± 1.8)
BD (% BL)	7.8–21.7 (13.0 ± 1.3)	16.5–27.3 (20.0 ± 0.7)	33.5–42.4 (37.9 ± 1.0)	36.8–50.7 (44.8 ± 1.4)
PAL (% BL)	48.9–65.4 (56.2 ± 1.4)	46.1–66.5 (54.1 ± 1.2)	59.3–68.7 (64.0 ± 0.9)	64.1–73.5 (68.3 ± 1.0)

or Ovaprim also resulted in hormonally assisted natural spawning of viable eggs, females treated with hCG did not spawn naturally and eggs had to be stripped manually. In addition, eggs released by females treated with either LHRH-a or Ovaprim were fertilized by the males in the tanks.

Description of eggs

Eggs are spherical and positively buoyant, have a smooth, unpigmented chorion, and range between 960 and 1200 µm in diameter (1039 ± 5 µm, mean ± 2 s.e.; *n* = 192). No significant differences were found in the diameters of naturally and hormonally induced spawned, fertilized and unfertilized eggs (ANOVA, $P > 0.05$; Table 2). Late-stage fertilized eggs possess a large pigmented oil globule (240 ± 6 µm diameter, mean ± 2 s.e.; *n* = 24) located anteriorly in the unsegmented yolk. Eggs hatched in ~32 h at ~23°C.

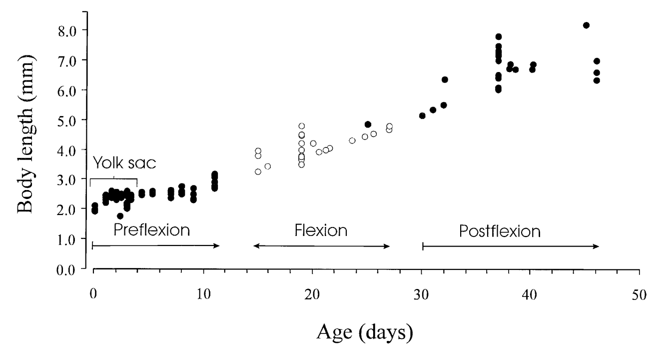


Fig. 1. Body length (mm) v. age (days) of reared larvae of *Glaucosoma hebraicum.* Open circles (○) show larvae undergoing notochord flexion.

Identification of larvae

Identification of the reared larvae as those of *G. hebraicum* Richardson, 1845 was confirmed by comparing the fin meristics obtained from the largest larva in our series with that provided for adults of this species in the literature (D VIII, 11; A III, 9; P_1, 16; P_2 I, 5; McKay 1997). A developmental series from the largest to the smallest larva was assembled using body shape, head spines, gut shape, sequence of fin development, and pattern of pigmentation.

Description of larvae

Age and size range of developmental stages. Newly hatched yolk-sac larvae (0 h) measured between 1.75 and 2.10 mm (Figs 1, 2). The yolk sac was completely resorbed after 3.3 days. The largest yolk-sac larva measured 2.60 mm and the smallest larva without a yolk sac measured 2.30 mm (Table 2). The preflexion stage, i.e. after yolk-sac resorption and before the flexion stage, lasted about 15 days, with larvae ranging between 2.30 and 3.95 mm (Fig. 1). Flexion of the notochord commenced around Day 15 and was complete between Days 25 and 30. The smallest and largest larvae undergoing flexion measured 3.42 (Day 15) and 4.80 mm

(Day 27), respectively. The smallest postflexion larva measured 4.83 mm and corresponded in morphology to a 25-day-old larva (Table 3; Fig. 1).

Morphology. Larvae are very elongate to moderate in depth during the yolk-sac to preflexion stage (BD 7.8–27.3%), gradually becoming deep by the postflexion stage as the gut enlarges and becomes voluminous (BD 36.8–50.7%; Table 3). The snout is round and short, and the head becomes large from the flexion stage (HL 34.9–50.0%), reaching to ~60% of the body length in postflexion larvae (mean 43.1%). Both the relative depth of the body and the head length remain similar and increase at a similar rate during development. The relative distance between the snout and the anus remains long throughout development (PAL 54.1–68.3%, Table 3), as the gut does not lengthen with growth.

Newly hatched larvae possess a moderately large, unsegmented yolk sac (length across 48.8–63.8% BL) with a large, pigmented oil globule located anteriorly in the sac, and they have no formed eyes, mouth or pectoral-fin buds (Fig. 2*A*). The eyes are barely distinguishable and unpigmented in yolk-sac larvae but are developed, round and fully pigmented in early preflexion larvae from 2.30 mm (3 days; Fig. 2*B*). The mouth becomes functional in preflexion larvae from Day 3 and first feeding (rotifers) occurs after 3.5 days. Small villiform teeth develop along the premaxilla in preflexion larvae from 2.60 mm (7 days) and along the dentary from 3.12 mm (11 days; Fig. 2*C*). The gill membranes are free from the isthmus. A small inflated gas bladder is evident from the late preflexion stage from 2.45 mm (4.3 days). The gut in newly hatched larvae has a distinctly coiled foregut and an uncoiled, elongate hindgut; the gut becomes voluminous in late preflexion larvae, and triangular and compact in late postflexion larvae (Figs 2*B*–2*H*). A short preanal membrane is present until the late flexion stage (Figs 2*B*–2*F*), and there is no gap between the anus and the origin of anal fin. There are 24–25 myomeres (10 + 15 = 25 vertebrae; McKay 1997). Scales start to form in late postflexion larvae from about 7.00 mm (37 days) and were still developing in the largest larva examined (8.13 mm; 45 days) (Fig. 2*H*).

Larvae possess small to moderate head spination comprising anterior and posterior preopercular spines, opercular, subopercular, interopercular, posttemporal and supracleithral spines, a small sphenotic ridge and a smooth supraocular ridge (Figs 2*D*–2*H*). Two posterior preopercular and 3–5 anterior preopercular spines appear during the late preflexion and flexion stage, respectively. Posterior and anterior preopercular spines increase to 7–10 and 8–10 spines from 6.02 mm (37 days), respectively, and remain small during development; both sets of spines are present in the largest larva examined (Fig. 2*H*). A small subopercular spine forms by 4.20 mm (19 days), and one small interopercular spine by 4.80 mm (19 days); the subopercular spine remains single while the number of interopercular spines increases to 4–5 by 6.02 mm (37 days). An opercular spine develops from 4.60 mm (19 days). The smooth supraocular ridge forms during the flexion stage (Figs 2*E*, 2*F*) and becomes prominent from 5.16 mm (30 days). The small sphenotic ridge develops from 5.16 mm (30 days) and becomes blunt from 7.00 mm (45–46 days). A low posttemporal ridge forms during the flexion stage and is present in the largest larva examined with up to four peaks (Fig. 2H). The supracleithral spine begins to form soon after notochord flexion is complete and develops 2–4 small peaks from 5.16 mm (30 days). All spines, and the supraocular and sphenotic ridges, were present in the largest larva and two juveniles examined (22.54–24.25 mm).

Development of fins. Pectoral-fin buds appear in early preflexion larvae from 2.30 mm (3 days), when the yolk sac is almost completely resorbed. Incipient pectoral-fin rays appear in late flexion larvae from 4.60 mm (19–20 days) and all rays (16) are formed in postflexion larvae from 6.10 mm (37 days; Figs 2*E*, 2*F*, 2*H*). Pelvic-fin buds, and dorsal-, caudal- and anal-fin anlagen appear simultaneously in late preflexion larvae from 3.82 mm (15 days; Fig. 2*D*). Incipient pelvic-fin rays are present in flexion larvae from 4.00 mm (19 days) and all elements (I, 5) are developed in early postflexion larvae from 5.16 mm (30 days). Incipient dorsal- and anal-fin rays appear in flexion larvae from 4.00 mm (19 days), and all elements are present in early postflexion larvae from 5.16 mm (30 days) except for the posterior-most spines of both the dorsal (VIII) and anal (III) fins which at that size are still soft rays (Fig. 2*G*). The full complement of dorsal-fin spines and rays (VIII, 11) is present in late postflexion larvae from 6.60 mm (45 days), and the third anal-fin spine is visible in the largest larva examined (8.13 mm, 45 days; Fig. 2*H*). Caudal-fin rays begin to form during notochord flexion, and the full complement (9+8) is evident in postflexion larvae from 5.16 mm (30 days). On the basis of the timing of development of all elements in each fin, the sequence of fin formation can be expressed as P_2, C → P_1 → D → A.

Pigmentation. Larvae are initially moderately pigmented, becoming heavily pigmented with growth. Pigment intensity, as well as types of melanophores present (e.g. punctate and/or stellate), varied considerably among the larvae examined (Figs 2*A*–2*H*). Newly hatched yolk-sac larvae possess pigment on the anteriorly located oil globule, diffuse melanophores scattered dorsally along the body, a melanophore on the foregut and the hindgut above the anus, and one large melanophore midway ventrally on the tail (Fig. 2*A*). In live, newly hatched yolk-sac larvae (not

illustrated), two of the dorsal melanophores, i.e. the one above the foregut and the one midway along the tail, are prominent and spread into the dorsal finfold, whereas the large ventral tail melanophore spreads into the ventral finfold.

Head pigment is restricted to one melanophore on the lower jaw in preflexion larvae, increasing to up to five distinct melanophores along the ventral midline of the lower jaw in flexion larvae (Figs 2*B*–2*F*). Pigment on the head intensifies with growth and becomes heavy in late postflexion larvae, with melanophores densely distributed all over the head including the upper and lower jaw tips and lower jaw angle, along the premaxilla and dentary, along the isthmus and at the cleithral symphysis, and on the subocular and opercular areas (Figs 2*G*, 2*H*). The characteristic diagonal dark band across the eye was forming in the two juveniles examined (22.54–24.25 mm) and is present in juveniles >30 mm (>100 days) (Fig. 3). Internal pigment on the head is visible until the early postflexion stage and consists of a few melanophores around the upper jaw tip, the forebrain, ventrally along and on the junction of the mid and hindbrain, and along the cleithrum (Figs 2*C*–2*G*).

Dorsal body pigment in preflexion larvae consists of three prominent melanophores — on the nape, midway along the trunk and on the anterior portion of the tail — all of which remain prominent until the late preflexion stage (Figs 2*B*–2*D*); the nape melanophore becomes internal by the flexion stage. Ventral body pigment in preflexion larvae consists of a single melanophore on the gut, one at the anus, and up to four ventrally along the tail. Pigment dorsally on the trunk and tail, and ventrally on the gut and tail, intensifies with growth and expands laterally over the body including the trunk myosepta, covering all except the last 6–7 caudal myomeres in late preflexion to late flexion larvae, and the last 3 caudal myomeres in postflexion and late postflexion larvae (Figs 2*E*–2*H*). The entire caudal peduncle was pigmented in the juveniles examined. The gut becomes heavily pigmented in late postflexion larvae (Fig. 2*H*). Internal pigment is present over the gas bladder in preflexion larvae from 2.45 mm, and dorsally along the gut and notochord, and remains visible until the late flexion stage (Figs 2*E*, 2*F*) before becoming obscured by the external pigmentation. The characteristic six horizontal dark bands along the body, which usually disappear in older adults, were present in the juveniles examined (Fig. 3).

Pigment on the pelvic-fin buds starts to form during the late flexion stage and the fins become heavily pigmented from the early postflexion stage (Fig. 2*G*); both fins were also heavily pigmented in the juveniles examined. All other fins remain unpigmented during development except for pigment on dorsal-fin spines I-VII and the interconnecting fin membrane, and the bases of the second dorsal and anal fin (Fig. 2*H*). The pectoral-fin base becomes pigmented during the late postflexion stage. No pigment was present on the second dorsal, caudal, anal and pectoral fins in the juveniles examined.

Discussion

Hormone-induced spawning

Preliminary results of this study showed that hormonal maturation and ovulation of WA dhufish, *Glaucosoma hebraicum*, as well as the artificial rearing of eggs through to the juvenile stage, could be successfully achieved. The minimum total lengths of sexually mature dhufish found in this study, i.e. 47 cm for males (m) and 43 cm for females (f), were greater than those described by Hesp (1997) (m, 35–40 cm; f, 25–30 cm). Whereas most male dhufish matured naturally, wild-caught females did not readily spawn in captivity but responded well to hCG, LHRH-a and Ovaprim. Both hCG and LHRH-a have been successfully used to induce oocyte maturation in several other marine finfish species, including hCG in grouper (*Epinephelus striatus*) and snapper (*Pagrus auratus*), and LHRH-a in milkfish (*Chanos chanos*) and barramundi (*Lates calcarifer*) (Lee *et al.* 1986; Garcia 1989; Battaglene and Talbot 1992; Watanabe *et al.* 1995). In addition, Ovaprim has been used to induce ovulation in several freshwater fish species (Thomas and Boyd 1989). The dosage of LHRH-a *imp* that provided best results for inducing maturation in dhufish lies within the range employed in other marine fishes (Garcia 1989; Mayes *et al.* 1993).

Although females injected with low dosages of either LHRH-a or Ovaprim (≤13 μg kg^{-1}) spawned unaided, those treated with high dosages ovulated but did not spawn naturally, and eggs had to be stripped manually. Manual stripping of eggs resulted in spawns being wasted because of difficulty in assessing the exact ovulation time, poor fertilization rates, and high (30%) loss of female broodstock through repeated handling. A delay of even a few hours in stripping mature eggs can affect fertilization success in some temperate fishes (Shelton 1989), and finfish species that are susceptible to handling stress during the spawning period may fail to ovulate or may ultimately die as a result of the treatment (Billard *et al.* 1981). Although hormonal induction leading to unaided spawning is relatively uncommon (e.g. Kuo *et al.* 1988), it can result in single ovulations per season and poor fertilization rates in species such as snapper and milkfish (Lee and Tamaru 1987; Battaglene and Talbot 1992; Pankhurst and Carragher 1992).

Larval development and relationships

This paper presents the first account of the early life-history stages of the percoid family Glaucosomatidae. As with most cases in which the description of larval stages of a fish species is based solely on artificially reared material,

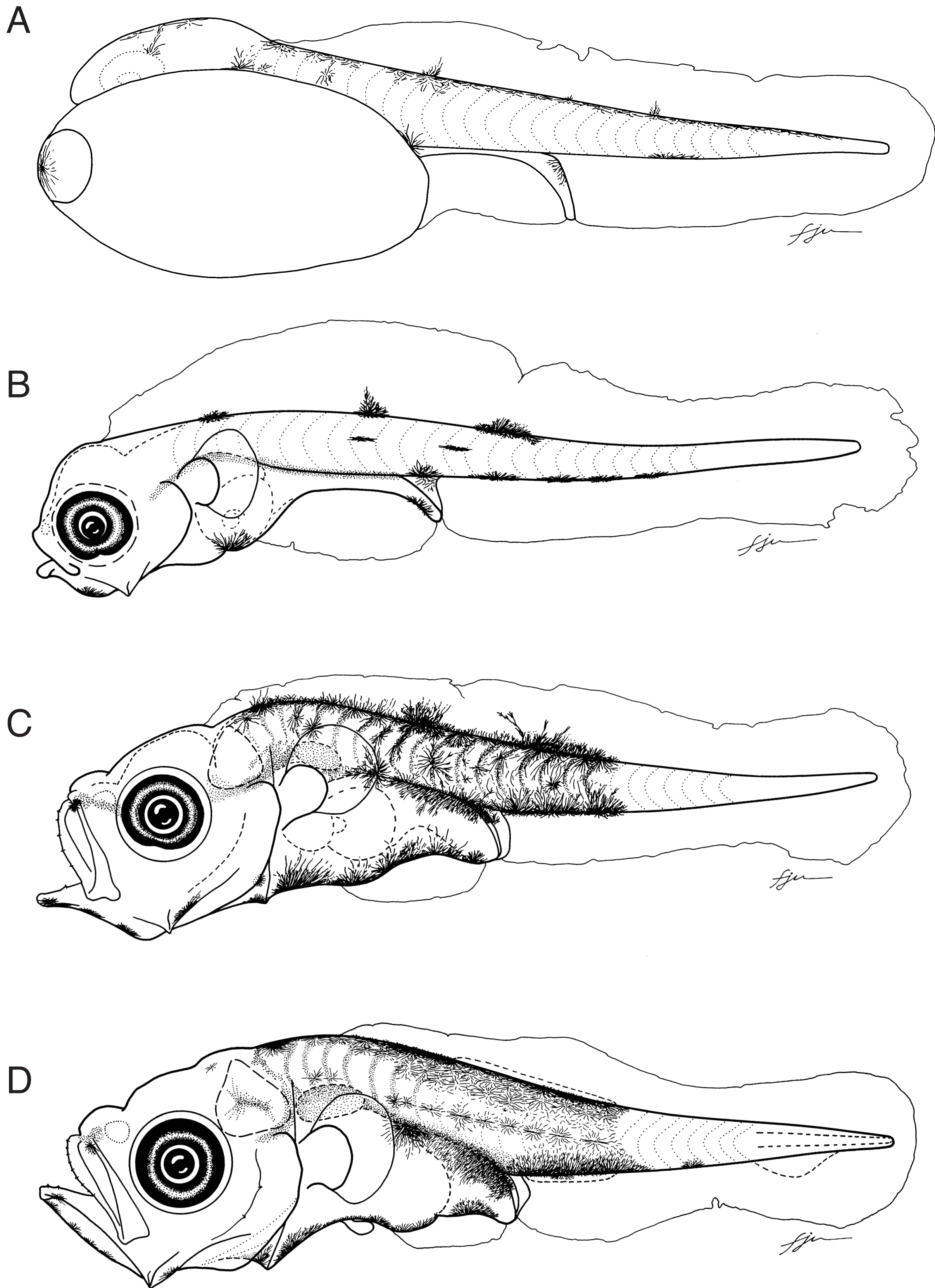
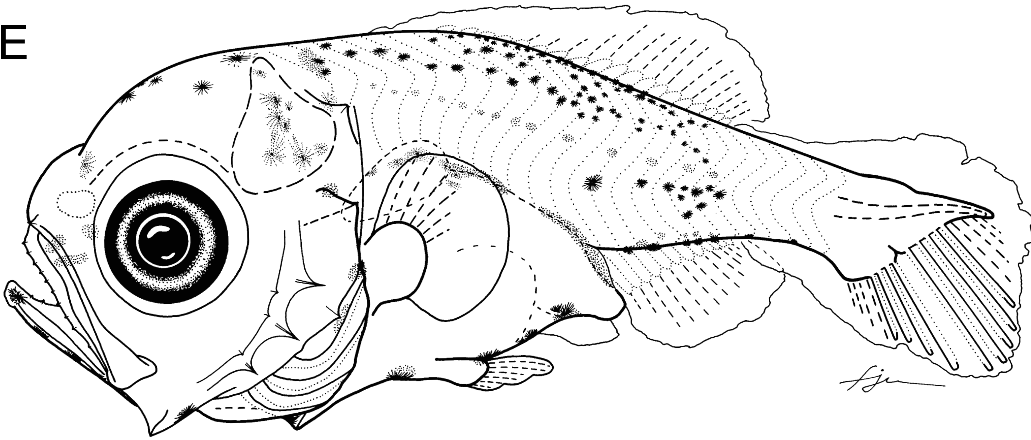
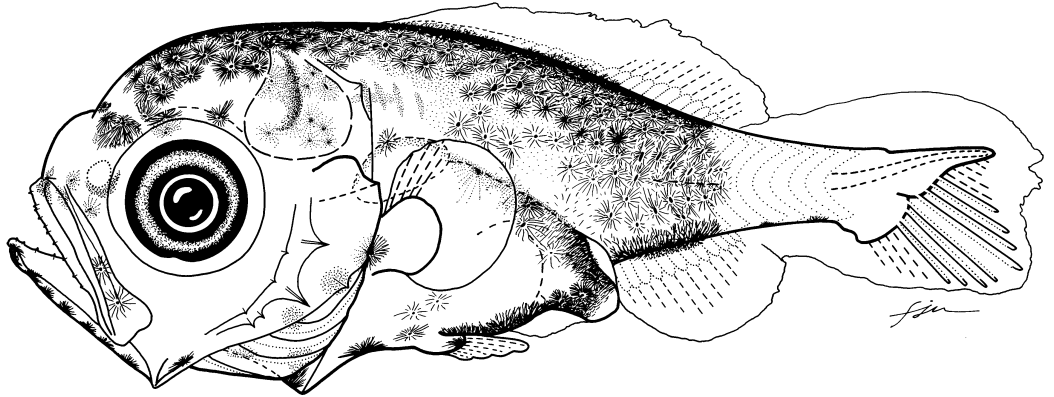
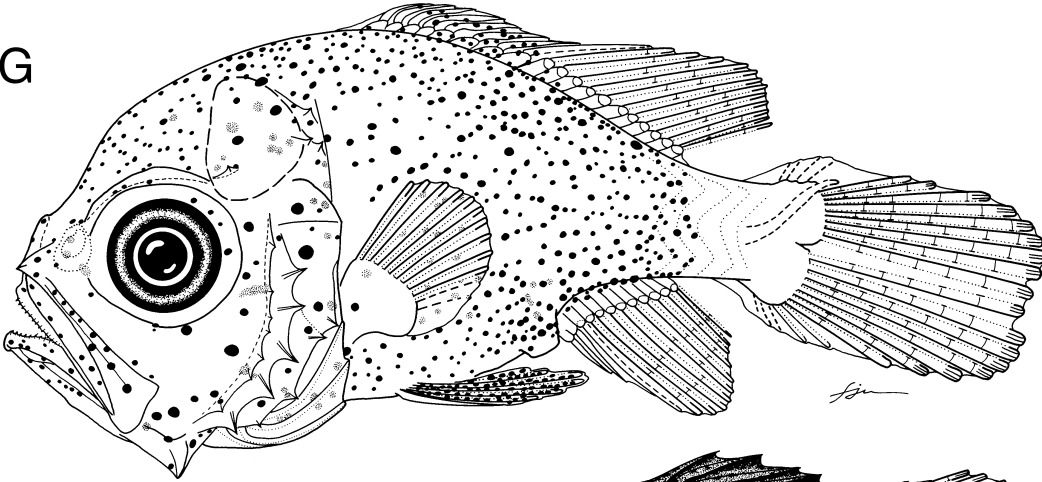


Fig. 2. Larvae of *Glaucosoma hebraicum* reared in laboratory. *(A)* 2.05 mm BL newly hatched, yolk sac (0 h). *(B)* 2.43 mm BL preflexion larva (3.3 days). *(C)* 3.16 mm BL preflexion larva (11 days). *(D)* 3.82 mm BL late preflexion larva (15 days). *(E)* 4.62 mm BL flexion larva (19 days). *(F)* 4.65 mm BL flexion larva (19 days); note expanded melanophores compared with larva illustrated in E. *(G)* 5.35 mm BL early postflexion larva (35 days); only last five caudal myomeres drawn. *(H)* 8.13 mm BL late postflexion larva (45 days); developing scales and last caudal myomeres not drawn. Illustrated by F. J. Neira.







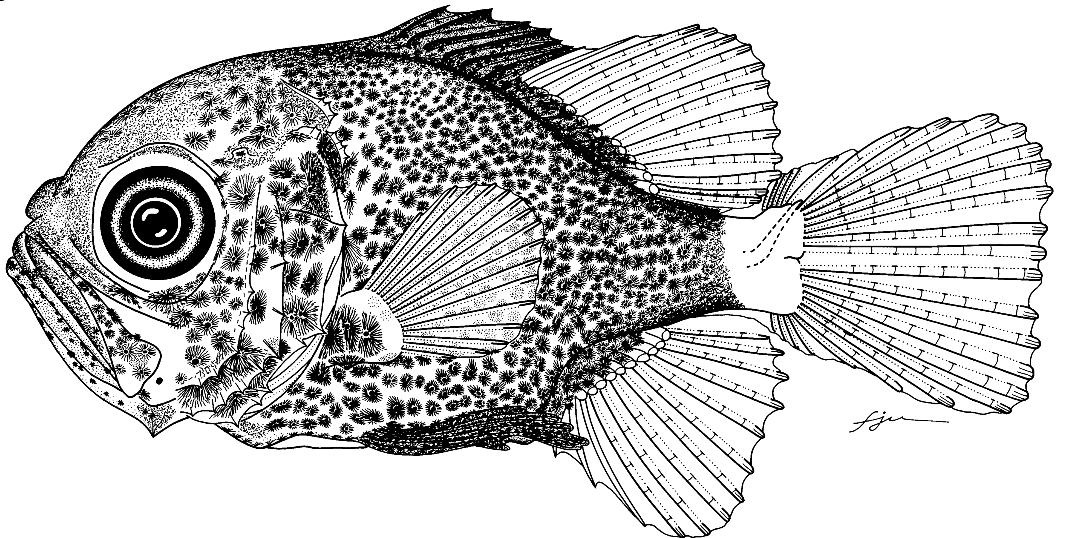


Fig. 2. *continued*

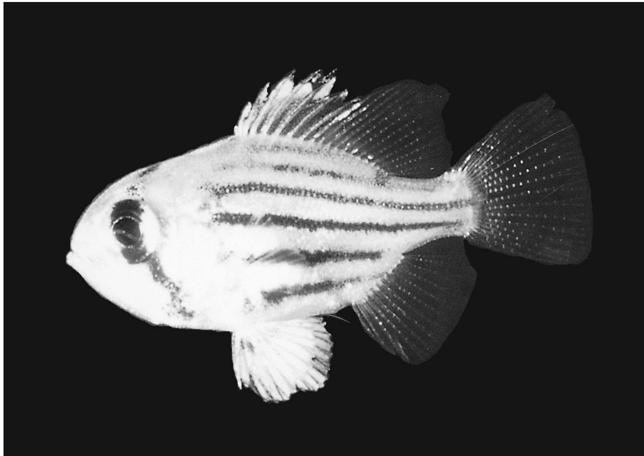


Fig. 3. 31.0 mm BL reared juvenile of *Glaucosoma hebraicum* (105 days) (photographed by J.B.Hutchins, Western Australian Museum, Perth).

it should be borne in mind that *G. hebraicum* larvae described herein may differ to some extent from field-caught larvae, given the range of conditions in rearing tanks (e.g. light intensity, temperature, larval density, food supply) and fixation time (e.g. before or after death). For example, the marked difference in pigment intensity between the two 19-day flexion *G. hebraicum* larvae drawn in our series (cf. Fig. 2*E*, 2*F*) illustrates the variability that can occur even at the same developmental stage with reared material. However, the difference between these flexion larvae (and also among several other larvae examined) seems to be associated with the expanded nature of the melanophores and not, as is often the case with reared larvae (Hunter 1984), with the presence of more pigment cells in the heavily pigmented specimen. In addition to pigment intensity, reared larvae may also differ from wild-caught larvae in those morphological characteristics that are partially controlled by environmental conditions during rearing, such as fin-ray counts (Hunter 1984). However, counts of all fin spines and rays in our reared material were identical to those provided for adult *G. hebraicum* in the literature (McKay 1997).

Reared larvae can be assigned ages (hours, days) to describe developmental stages and the duration of developmental events. During this study, both the preflexion (after yolk-sac resorption) and flexion stages lasted for several days: 3–15 and 16–27 days, respectively. In contrast, the size range of these stages was markedly narrow: 2.30–3.95 and 3.42–4.80 mm, respectively (Table 3; Fig. 1). The small variation in length compared with duration (days) of these stages implies that it could be highly misleading to assign ages to field-caught larvae based on reared material. In addition, 37–40-day-old postflexion larvae ranged between 6.02 and 7.48 mm, whereas 45–46-day-old late postflexion larvae ranged between 6.35 and 8.13 mm, suggesting that size increments of larvae near transition to the juvenile stage decreased with increasing days after birth. A decrease in larval growth near the juvenile stage has also been described for other artificially reared marine fishes, including red sea bream (*Pagrus major*) and starry flounder (*Platichthys stellatus*) (Policansky 1982; Fukuhara 1991). As with sea bream, size rather than age of larval *G. hebraicum* is probably more suitable for estimating the onset of transition to the juvenile stage. Although the precise size range at which this transition takes place in *G. hebraicum* could not be determined from our reared series, it possibly starts at around 8 mm (45–46 days) because the largest postflexion larva examined (8.13 mm) had attained the full complement of external meristic characters, except for the scales which were still developing.

Except for the early-forming pelvic fins, the development of larval *G. hebraicum* follows a pattern similar to that found in most generalized percoids (Johnson 1984). This includes the formation of the caudal, dorsal and anal fin anlagen shortly before the start of the notochord flexion, a size at flexion between 3 and 5 mm which ends with the attainment of the full complement of caudal-fin rays, and the presence of spines on bones of both the opercular (preopercle, opercle, subopercle and interopercle) and pectoral (supracleithrum and posttemporal) series (Johnson 1984). In addition, the last dorsal-fin spine (VIII) and the last anal-fin spine (III) are initially soft rays but become spines during the postflexion stage, as with the larvae of most percoids (Leis and Trnski 1989; Neira *et al.* 1998). The precocious development of the pelvic fins constitutes the only apparent specialization of *Glaucosoma* larvae to pelagic life. This pattern of early-forming pelvic fins has also been observed in larvae of a few other percoid families, including the Lutjanidae, Monodactylidae (*Monodactylus* only), Pempheridae and Serranidae (Leis and Rennis 1983; Miskiewicz 1989), and in larvae of some members of non-percoid taxa such as beryciforms and stromateoids (Horn 1984; Neira *et al.* 1998).

Glaucosoma, the sole genus of the Glaucosomatidae, shares several anatomical similarities with members of the percoid family Pempheridae, including the structure of the gas bladder, the foramen on the frontal bone and the lack of direct articulation between the second epibranchial and the pharyngobranchial (Katayama 1954; Tominaga 1986; Johnson 1993). These, including the similarities between the saccular otoliths (sagittae) of *G. magnificum* and those of *Pempheris* (McKay 1997), seem to further support the close relationship *Glaucosoma*–Pempheridae that led Johnson (1993) to recommend the inclusion of the Glaucosomatidae as a subfamily of the Pempheridae. *Glaucosoma* larvae described here share several similarities with pempherid larvae, including the moderately deep body, myomere number (24–25), the early-forming pelvic fins, a similar size range at notochord flexion (3.6–4.3 mm in pempherids), the pigment pattern on the body and gut in the early stages, the

compact triangular gut, and the lack of a gap between the anus and the anal-fin origin. However, they also differ in several respects: the pelvic fins in *Glaucosoma* are initially unpigmented and form ventrally on the gut during the late preflexion stage, whereas in pempherids they are pigmented and appear first laterally on the gut in the yolk-sac stage and then migrate ventrally and anteriorly; the foregut is coiled in early preflexion *Glaucosoma* larvae, but it only starts to coil by the mid-preflexion stage in pempherid larvae; *Glaucosoma* larvae possess a more extensive head spination that includes more spines in the preopercular series, and also suborpercular, interopercular and posttemporal spines, and supraocular and sphenotic ridges, all of which are absent in pempherid larvae; and *Glaucosoma* larvae lack the prominent dorsal finfold of pempherid larvae, and are generally much more heavily pigmented than pempherid larvae (Leis and Rennis 1983; Kinoshita 1988). Since there is no evidence to suggest that the similarities between *Glaucosoma* and pempherid larvae are derived, no conclusions can be drawn as to whether these support the view that both groups are closely related; this in turn illustrates the difficulty in assessing relationships among percoids by using larval characters alone (e.g. Neira *et al.* 1997).

Taxa with similar larvae

Larval *G. hebraicum* can be identified by the body shape, the presence of 24–25 myomeres, the early-forming abdominal pelvic fins that become heavily pigmented by the early postflexion stage, the gut that is compact and triangular-shaped from the flexion stage, the head spines, and the distinct body pigment from the late flexion stage. Field-caught *G. hebraicum* larvae are likely to be confused with those of the two other species of *Glaucosoma* found in Western Australia, namely *G. buergeri* and *G. magnificum*. Larvae of those species are undescribed but would be unlikely to co-occur with those of *G. hebraicum* since both are tropical species that are found mostly north of Shark Bay along northern Australia, including off Papua New Guinea. In addition, while dorsal and anal fin counts in *G. hebraicum* are identical to those of *G. buergeri* (D VIII,11; A III, 9), gill rakers could be used to separate late postflexion larvae of both species (4–6 + 11–13 *v*. 6–9 + 13–15), whereas dorsal and anal fin counts will separate postflexion larvae of both species from those of *G. magnificum* (D VIII,14; A III,12) (Allen and Swainston 1988; Paxton and Hanley 1989; McKay 1997).

Glaucosoma larvae can also be confused with larvae of members of the Pempheridae and with *Monodactylus* (Monodactylidae), which are found within the distributional range of *Glaucosoma* (Allen and Swainston 1988) and which possess similar body shapes and early-forming pelvic fins. However, in addition to the distinguishing features between *Glaucosoma* and pempherid larvae mentioned above, larvae of both groups can be further separated by the higher number of anal-fin rays in pempherids (17–45 *v*. 9–12), whereas *Monodactylus* larvae possess a distinct wide band of heavy pigment between the snout and the anus, and a higher number of dorsal and anal-fin rays (27–32) (Leis and Rennis 1983; Kinoshita 1988; Miskiewicz 1989). Larvae of *Glaucosoma* could also be confused with larvae of non-percoids that have early-forming pelvic fins, including members of the Berycidae and Trachichthyidae (Beryciformes), and some stromateoids. However, berycids are lightly pigmented and have I, 7–13 pelvic-fin elements (Mundy 1990; Miskiewicz *et al.* 1998), trachichthyids possess 26–30 myomeres and have I, 6 pelvic-fin elements (Jordan and Bruce 1993), and stromateoids usually have 30 or more myomeres (Horn 1984).

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