Egg and larval development of laboratory-reared gulf flounder, *Paralichthys albigutta*, and southern flounder, *P. lethostigma* (Pisces, Paralichthyidae)

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Abstract.—The egg and larval development of *Paralichthys albigutta* (gulf flounder) and *P. lethostigma* (southern flounder) are described from laboratory-reared and field-collected specimens. *Paralichthys albigutta* eggs and oil globules had a mean diameter of 0.87 mm (range: 0.84–0.90 mm) and 0.18 mm (range: 0.17–0.19 mm), respectively. *Paralichthys lethostigma* eggs and oil globules had a mean diameter of 0.91 mm (range: 0.84–0.96 mm) and 0.18 mm (range: 0.16–0.20 mm), respectively. Recently hatched *P. albigutta* larvae ranged from 1.8 to 2.2 mm in notochord length (NL) and *P. lethostigma* from 2.0 to 2.2 mm NL. Pigment on embryos and newly hatched larvae was relatively more developed for *P. albigutta*. Almost-paired and almost-contiguous ventral midline melanophores occurred on preflexion larvae of both species and remained throughout the larval stages. Pigment on the maxillary, vomer, dorsum of the mid-brain, lateral surface of the caudal area, lateral surface of gut, and extent of pigment along the ventral midline of the isthmus were used to separate laboratory-reared *P. albigutta* from *P. lethostigma*. However, this pigment was less useful in separating field-collected material. The number of cranial spines appeared to be diagnostic in separating laboratory-reared early-preflexion larvae. *Paralichthys lethostigma* consistently had three cranial spines, whereas *P. albigutta* had less than three spines. The development of meristic characters was considered the most useful character in separating *P. albigutta* from *P. lethostigma* because at any given size *P. albigutta* was generally more developed than *P. lethostigma*.

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Four species of the genus *Paralichthys* occur in southeastern U.S. coastal waters. *Paralichthys squamilentus* (broad flounder) is not commonly encountered and is of little commercial or recreational value. *Paralichthys dentatus* (summer flounder), *P. lethostigma* (southern flounder), and to a lesser extent *P. albigutta* (gulf flounder) are common and of great commercial and recreational value (N.C. Div. Marine Fisheries[1]). *Paralichthys dentatus* range from Maine to Cape Canaveral, Florida, and *P. albigutta* and *P. lethostigma* range from North Carolina to Texas (Gutherz, 1967). Eggs and larvae of *P. dentatus* have been described (Smith and Fahay, 1970) whereas those of *P. albigutta* and *P. lethostigma* have not. Comparative taxonomic studies of late larvae and early juvenile (fully formed vertebrae, dorsal, and anal rays) *P. dentatus*, *P. lethostigma*, and *P. albigutta* have been reported. Deubler (1958) showed that late-larval–early-juvenile *P. dentatus* can be distinguished from the other two congenera by differences in pigmentation of the dorsal and anal fins and in vertebral number. The only characters useful for separation of *P. lethostigma* and *P. albigutta* were dorsal-ray and anal-ray counts. Woolcott et al. (1968) showed that 100% separation of the three species could be obtained through a combination of anal-ray and vertebral counts. However, these characters must be fully formed in order to be diagnostic. Vertebral counts alone cannot be used to separate *P. albigutta* and *P. lethostigma*.

The objective of this study is to describe the eggs and larvae of laboratory-reared *P. lethostigma* and *P. albigutta* to provide diagnostic characters for identification of these paralichthyids in field-collected material.

[1] North Carolina Division of Marine Fisheries. 1992. Assessment of North Carolina commercial finfisheries. Completion Rep. for Job 2-IJ-16. North Carolina Div. Mar. Fisheries, Morehead City, NC 28557.

Methods

Adult *P. albigutta* and *P. lethostigma* were collected in the early fall during 1991 and 1992 from commercial pound nets in Core Sound, North Carolina. Individual fish were tagged and meristic data (gill-raker and anal-ray counts) were used to confirm identification (Gutherz, 1967). Spawning was induced with the aid of hormones as described by Smigielski (1975). For *P. albigutta* three different females were fertilized by a single male. Females were injected with carp pituitary hormone (2 mg/kg fish) two or three times to promote hydration. Eggs were fertilized on 14 and 19 February 1992. For *P. lethostigma*, a total of four different males and four different females were spawned. Eggs were fertilized on 17 December 1992, 24 February, and 2 March 1993.

Eggs and sperm from both species were manually stripped into 1-L bowls; eggs were fertilized, and embryos incubated in 100-L black-sided tanks. *Paralichthys albigutta* larvae were reared in uncirculated, filtered water at a mean temperature of 19°C (range: 14–24°C) and a mean salinity of 30 ppt (range: 28–30 ppt). *Paralichthys lethostigma* larvae were reared at a mean temperature of 18°C (range: 16–19°C) and a mean salinity of 32 ppt (range: 23–42 ppt). Rotifers and *Artemia* nauplii were provided to young (≤15 days old) and older (>15 days old) larvae, respectively. Only three *P. lethostigma* larvae were alive after approximately 35 days, limiting the amount of material available for this species. Eggs and larvae were preserved in 10% buffered formalin. Counts of meristic characters were obtained from cleared and stained specimens (Potthoff, 1984). All measurements and observations were made with a Wild M5A stereomicroscope equipped with an ocular micrometer.

The larval period was separated into preflexion, flexion, and postflexion stages—the three stages associated with the development of the caudal fin before, during, and after the upward flexion of the notochord tip. During preflexion and flexion, larval body length was measured from the tip of the snout to the tip of the notochord (notochord length, NL). In postflexion larvae (defined when 6 upper + 7 lower principal rays were formed) body length was measured from the tip of the snout to the base of the hypural plate (standard length, SL).

Postflexion larvae and transforming juveniles of *P. albigutta* and *P. lethostigma* captured in Onslow Bay off North Carolina during the winter of 1990 and 1991 were used for 1) comparison with laboratory-reared specimens and 2) illustrations and investigation of pterygiophore patterns. This material also was used to determine the size when meristic characters develop for *P. lethostigma* because material from laboratory-reared specimens was limited for this species. Observations on pterygiophore patterns and meristic characters were made on cleared and stained specimens. Larval material is deposited at the Beaufort Laboratory under the care of the senior author.

Results

Eggs and recently hatched larvae

Paralichthys albigutta Eggs were spherical and had a mean diameter of 0.87 mm (range: 0.84–0.90 mm, n=44). One oil globule was present and had a mean diameter of 0.18 mm (range: 0.17–0.19 mm, n=44). The yolk was homogenous, the chorion smooth, and the perivitelline space (in live eggs) narrow (ca. 0.2 mm). Pigment was first observed in the caudal area of embryos just after blastopore closure (Fig. 1A). On embryos from late-stage eggs (from tail twisting to hatching), pigment occurred on the oil globule, sparsely in the head region, and was most

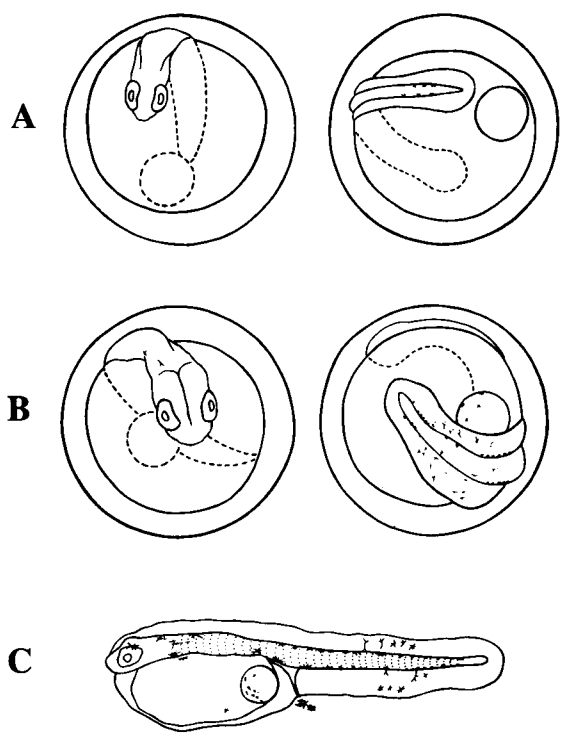

Figure 1

Laboratory-reared eggs and newly hatched larvae of gulf flounder, *Paralichthys albigutta*: (**A**) middle stage: left, anterior view; right, posterior view; (**B**) late stage: left, anterior view; right, posterior view; and (**C**) 2.2-mm-NL newly hatched yolk-sac larva.

developed in the caudal area. In the caudal area, melanophores occurred in the dorsal- and anal-fin folds, dorsal and ventral midlines, and sparsely on the lateral surface (Fig. 1B). Recently hatched *P. albigutta* larvae ranged from 1.8 to 2.2 mm NL. The oil globule was located in the posterior portion of the yolk sac (Fig. 1C).

Paralichthys lethostigma *P. lethostigma* eggs had a mean diameter of 0.91 mm (range: 0.84–0.96 mm, n=154), and the oil globule had a mean diameter of 0.18 mm (range: 0.16–0.20 mm, n=154). Pigment on embryos from middle-stage eggs (just after blastopore closure) was less developed than that observed for *P. albigutta* (Fig. 2A). Like *P. albigutta*, pigment on *P. lethostigma* embryos was only observed in the caudal area. Pigment on embryos from late-stage eggs was less developed than that observed for *P. albigutta* (Fig. 2B). Pigment was observed only in the caudal area on *P. lethostigma* embryos. Recently hatched *P. lethostigma* larvae were similar in size to *P. albigutta* (range: 2.0–2.2 mm NL). Like *P. albigutta*, the oil globule was located in the posterior portion of the yolk sac (Fig. 2C).

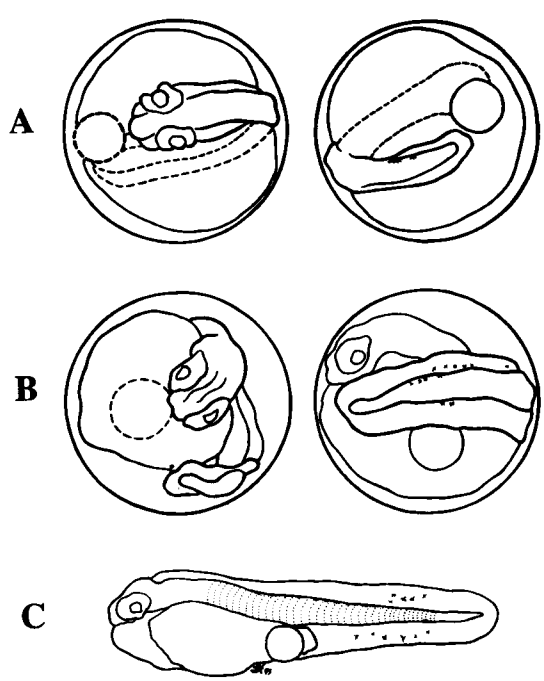

Figure 2

Laboratory-reared eggs and newly hatched larvae of southern flounder, *Paralichthys lethostigma*: (**A**) middle stage: left, anterior view; right, posterior view; (**B**) late stage: left, anterior view; right, posterior view; and (**C**) 2.1-mm-NL recently hatched yolk-sac larva.

Fin development

Paralichthys albigutta Fins began to develop in the following sequence: dorsal and caudal, anal, pelvic, and pectoral (Table 1). The adult complement of rays (Gutherz, 1967; Woolcott et al., 1968; Fahay, 1983) was attained in the following sequence: principal caudal rays (10 upper + 8 lower), dorsal fin (71–85), anal fin (53–63) and pelvic fin (6), and pectoral fin (10–12) (Table 1).

The development of the caudal fin, indicated by a thickening of tissue on the ventral side, began at 5.2 mm NL. Caudal-fin rays (2 upper + 2 lower) were first observed at 5.5 mm NL, indicating the beginning of the flexion stage. The rays began to form at the middle of the fin and developed dorsally and ventrally almost simultaneously. The adult complement of principal caudal rays (10 upper + 8 lower) was attained at 6.9 mm SL.

Dorsal rays were first observed during early flexion (5.5 mm NL, Table 1). Dorsal rays first formed in the head region anterior to the first neural spine. They were accompanied by an extension of the dorsal-fin fold that appeared as a flap (Fig. 3B). Dorsal-fin development proceeded slowly during the flexion stage and more rapidly during postflexion (Table 1). Development proceeded posteriorly; however, the anteriormost ray was not observed until 6.1 mm SL. The anterior rays in the head region, especially the third ray, were elongated (Fig. 3D). The adult complement of dorsal-fin rays consistently was attained at 7.8 mm SL (Table 1).

Anal-fin rays began to form on postflexion larvae at approximately 6.1 mm SL (Table 1). Formation began in the vicinity of the first haemal spine and development proceeded anteriorly and posteriorly simultaneously. Anal-fin-ray development was rapid, and by 7.7 mm SL the adult complement of anal-fin rays consistently was observed (Table 1).

Pelvic-fin rays were first observed on postflexion *P. albigutta* larvae at 6.9 mm SL (Table 1). All specimens ≥8.5 mm SL had a completed pelvic fin.

The pectoral fin persisted as a large rayless blade throughout flexion and early postflexion (Table 1). Rays began to form at 7.1 mm SL at the dorsal position of the blade and developed ventrally (Fig. 4). All specimens ≥8.5 mm SL had the adult complement (10–12 rays) of pectoral fin rays (Table 1).

Paralichthys lethostigma Fins began to develop in the following sequence: dorsal, caudal, anal, pelvic and pectoral (Table 2). The adult complement of rays (Gutherz, 1967; Wolcott et al., 1968; Fahay, 1983) was attained in the following sequence: caudal fin (10 upper + 8 lower), dorsal fin (80–95) and anal fin (63–74), pelvic fin (6), and pectoral fin (11–13) (Table 2).

Table 1

Meristic data from cleared and stained laboratory-reared gulf flounder, *Paralichthys albigutta*. Dashed lines separate preflexion, flexion, and postflexion stage larvae. Postflexion was defined as the stage when 6 upper + 7 lower caudal rays were observed. NL = notochord length; SL = standard length.

Body length (mm)	Days after hatching	Dorsal-fin rays	Anal-fin rays	Pelvic-fin rays	Pectoral-fin rays	Principal caudal rays (upper + lower)
4.5 NL	18	0	0	0	0	0
5.2 NL	19	0	0	0	0	0
5.5 NL	19	3	0	0	0	2+2
5.7 NL	19	2	0	0	0	2+2
5.7 NL	19	2	0	0	0	2+2
5.8 NL	19	3	0	0	0	3+3
5.8 NL	24	5	0	0	0	6+5
6.1 NL	19	2	0	0	0	3+3
6.4 NL	31	4	0	0	0	5+5
5.9 SL	24	9	0	0	0	7+7
6.1 SL	26	15	4	0	0	9+8
6.3 SL	26	16	14	0	0	9+8
6.8 SL	31	55	34	0	0	9+8
6.9 SL	36	72	54	3	0	10+8
7.0 SL	36	68	50	4	0	10+8
7.1 SL	36	68	50	5	1	10+8
7.2 SL	31	62	43	0	0	10+8
7.7 SL	36	74	57	5	3	10+8
7.8 SL	36	69	54	5	5	10+8
7.8 SL	44	75	54	5	9	10+8
8.0 SL	36	72	55	5	5	10+8
8.2 SL	44	72	51	5	5	10+8
8.5 SL	44	71	54	6	10	10+8
8.6 SL	44	74	56	6	10	10+8
10.2 SL	59	74	58	6	11	10+8
10.7 SL	52	73	54	6	13	10+8

The development of the caudal fin began at 5.4 mm NL and was similar to that of *P. albigutta*, but caudal rays were not observed until 6.5 mm NL. The adult complement of principal caudal rays (10 + 8) was attained at 8.2 mm SL (Table 2).

Dorsal rays were first observed on a preflexion specimen but were not consistently observed till early flexion (6.5 mm NL, Table 2). The development of dorsal rays was similar to that observed for *P. albigutta*. Complete development of the dorsal-fin rays occurred at approximately 8.4 mm SL.

Anal-fin rays began to form on postflexion larvae at approximately 7.3 mm SL. Development was similar to that observed for *P. albigutta*. Like *P. albigutta*, anal-fin-ray development was rapid and the anal fin appeared to be completely formed by approximately 8.4 mm SL (Table 2).

Pelvic-fin rays were first observed on postflexion larvae (8.2 mm SL, Table 2). All specimens >9.7 mm SL consistently had a completed pelvic fin.

Pectoral-fin rays began to form at 8.4 mm SL at the dorsal position of the blade and developed ventrally. The largest specimen (11.0 mm SL) still had not attained a completed (11–13 rays) pectoral fin (Table 2).

Pigmentation of laboratory-reared specimens

Paralichthys albigutta Newly hatched *P. albigutta* larvae had dendritic melanophores on the dorsal and anal finfolds midway between the anus and notochord tip (Fig. 1C). Dorsal and anal midline pigment posterior to the anus was well developed. Melanophores occurred on the trunk and head, on the oil globule, and on the yolk sac (Fig. 1C). Melanophores were observed on the snout on a one-day old larva (2.8 mm NL) but not on newly hatched larvae.

Characteristic pigment on preflexion larvae was almost-paired and almost-contiguous dorsal and ventral midline melanophores that remained character-

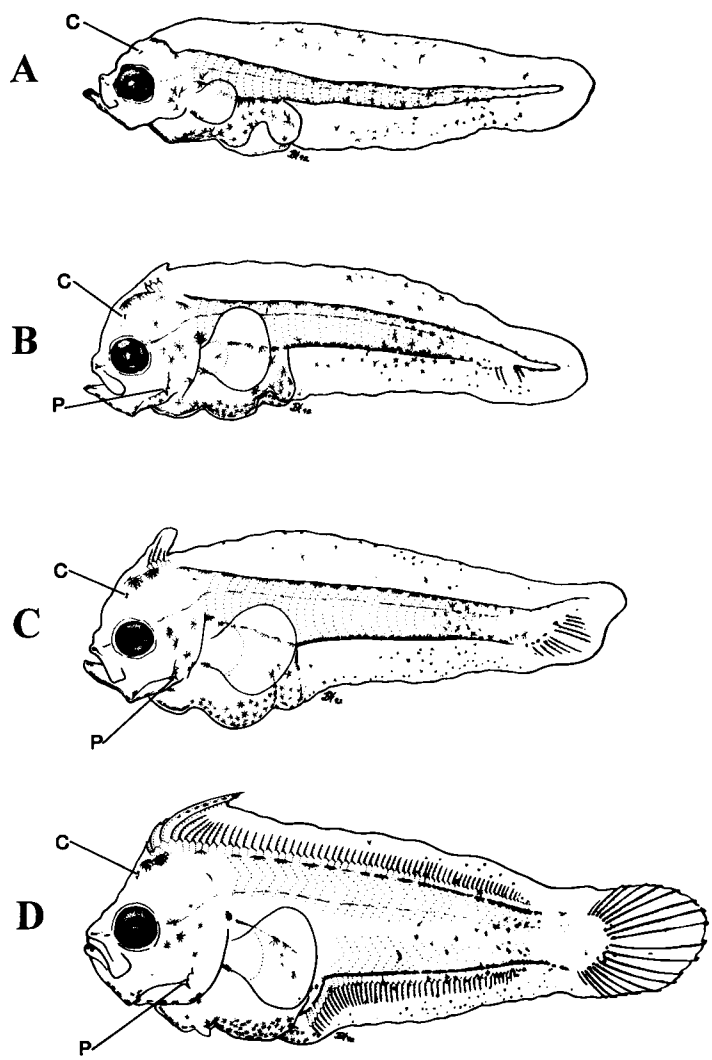

Figure 3

Developmental stages of laboratory-reared gulf flounder, *Paralichthys albigutta* (C=cranial spines, P=preopercular spines). (**A**) 3.5-mm-NL preflexion larva, 10 days old; (**B**) 5.8-mm-NL early flexion larva, 25 days old; (**C**) 6.2-mm-NL late flexion larva, 21 days old; and (**D**) 7.1-mm-SL postflexion larva, 31 days old.

istic throughout the larval stages (Fig. 3A). The almost-paired dorsal and ventral midline melanophores merged into one row of dorsal and ventral punctate melanophores in the future caudal-fin region. The most posterior melanophores generally were opposite each other. The dorsal midline pigmentation extended anteriorly to the forebrain. Melanophores were first observed on the lateral portion of the caudal region at approximately 3.5 mm NL (Fig. 3A). Embedded notochord pigment extended anteriorly ventral to the brain and continued through the eye, giving the appearance of a stripe through the eye. Pigment occurred over most of the ventral finfold and more prevalently in the middle one-third of the dorsal finfold. In the gut region of preflexion larvae, melanophores occurred along the ventral midline, extending anterior of the cleithral symphysis and along the lateral surface of the gut. Melanophores occurred along the dorsal surface of the gut as a continuation of ventral midline pigment and generally terminated in a distinct melanophore at the junction of the operculum (Fig. 3A). In most specimens a distinct melanophore occurred on the ventral area of the base of the pectoral-fin blade. In the head region, melanophores occurred on the operculum and on the dorsum of the midbrain (cranial bump). Pigment was first observed on the dorsum of the cranial bump during early preflexion (2.9 mm NL), but only 20% of the specimens examined (n=10) had a melanophore here at this time. With increasing size the cranial-bump melanophore occurred at a greater frequency, and by 4.0 mm NL all specimens had a melanophore in this region. In the lower-jaw region, melanophores occurred both along the lower-jaw rami and at the lower-jaw angle (Fig. 3A). A punctate melanophore was observed on both sides of the premaxilla or maxilla, or both. This pair of melanophores was first observed on early preflexion larvae (2.9 mm NL) and was observed on every laboratory-reared specimen examined. Vomerine pigment was first observed on 3.2-mm-NL preflexion larvae and occurred thereafter on all specimens examined.

Pigment on flexion larvae was similar to that observed on late preflexion larvae (Fig. 3, B and C). Melanophores occurred on the lateral surface of the body in the caudal area on all specimens. The most anterior melanophore on the dorsal surface of the gut at the junction of the operculum was stellate and was seemingly isolated from the other gut pigmentation (Fig. 3, B and C). Pigment occurred along the proximal base of the developing caudal-fin rays. In the late flexion stage (6.4 mm NL), pigment first appeared on the third elongated dorsal ray. Most of the lateral surface of the hind gut was pigmented. The ventro-lateral surface of the mid- and foregut was pigmented (Fig. 3, B and C).

On early postflexion larvae (Fig. 3D), pigment was observed on the base of the caudal fin and on the

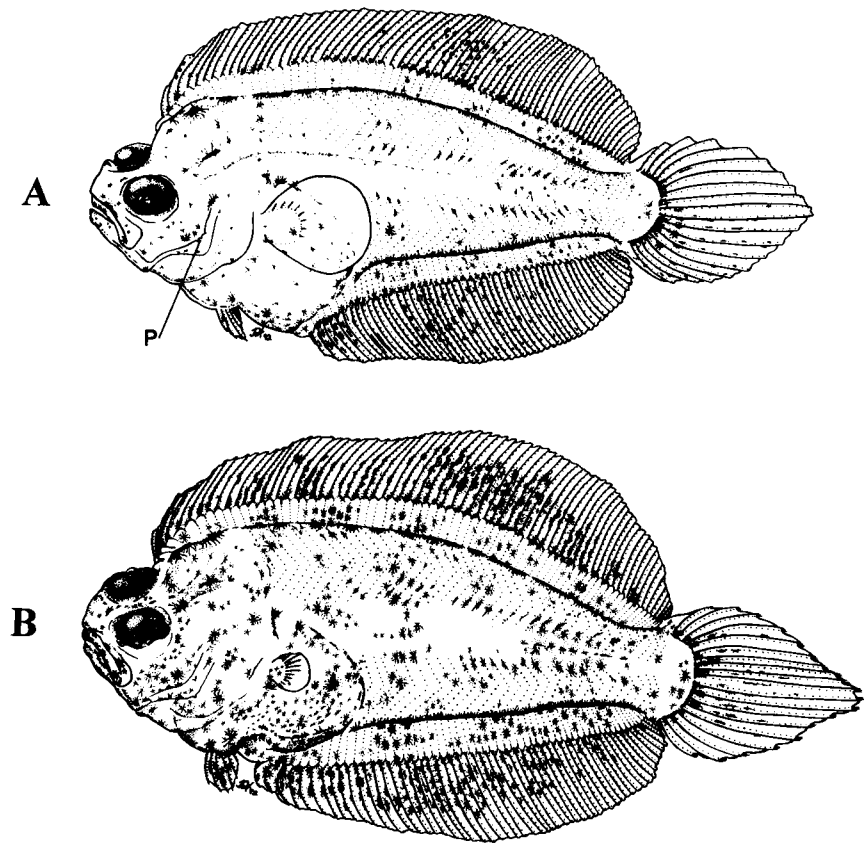

Figure 4

Developmental stages of transforming, postflexion laboratory-reared gulf flounder, *Paralichthys albigutta* (P=preopercular spines). (**A**) 8.2-mm-SL larva, 34 days old; (**B**) 9.0-mm-SL larva, 45 days old.

pelvic fin. Melanophores on the lateral surface of the body in the caudal area began to align with the myosepta. Pigment on the lateral surface of the body increased with increasing size (Fig. 4). The extended third dorsal ray was pigmented, and pigment was scattered on the developing dorsal- and anal-fin rays. This pigment increased in area during development (Fig. 4). Pigment was scattered in no apparent pattern medially on the dorsal- and anal-fin pterygiophores. The anal-fin pterygiophores were relatively more pigmented than those on the dorsal fin. Pigment increased in these areas during development (Fig. 4). The characteristic almost-paired, almost-contiguous dorsal and ventral midline pigment was located at the proximal edge (base) of the dorsal- and anal-fin pterygiophores. Pigment in the head region of early postflexion larvae was similar to that of flexion larvae (Fig. 3D) but increased in intensity during development (Fig. 4).

During transformation, *P. albigutta* larval body pigment increased in intensity (e.g. compare Fig. 4, A and B). Melanophores occurred along the entire base of the caudal fin, but most of the fin was not pigmented. A blotchy pigment pattern was observed on the pterygiophores, caudal peduncle, and lateral line from mid-body anterior to the cleithrum. Melanophores along the lateral line from mid-body to the caudal peduncle formed a streak of pigment in later transforming larvae (ca. 8.7 mm SL). Numerous melanophores on the lateral surface of the caudal area were aligned with the myosepta. This pigment occurred mainly in the posterior two-thirds of the caudal area and extended more anteriorly in the dorsolateral area (Fig. 4). The dorsal and anal fins were pigmented along the entire length of their base (i.e. proximally). On the medial portion of the dorsal- and anal-fin rays on earlier (ca. 7.8 mm SL) transforming larvae, pigment appeared as blotches on the

Table 2

Meristic data from cleared and stained laboratory-reared and field-collected southern flounder, *Paralichthys lethostigma*. Dashed lines separate preflexion, flexion, and postflexion stage larvae. Postflexion was defined as the stage when 6 upper + 7 lower caudal rays were observed. An asterisk indicates field-collected material. NL = notochord length; SL = standard length.

Body length (mm)	Days after hatching	Dorsal-fin rays	Anal-fin rays	Pelvic-fin rays	Pectoral-fin rays	Principal caudal rays (upper+lower)
4.7 NL	20	0	0	0	0	0
4.9 NL	20	0	0	0	0	0
5.4 NL	20	0	0	0	0	0
6.2 NL	31	2	0	0	0	0
6.4 NL	24	0	0	0	0	0
6.5 NL	31	6	0	0	0	5+5
6.6 NL	31	5	0	0	0	4+4
6.8 NL	31	4	0	0	0	4+3
6.9 NL	31	5	0	0	0	6+6
6.9 NL	35	6	0	0	0	6+6
7.0 NL	40	5	0	0	0	5+5
7.1 NL	35	6	0	0	0	6+6
6.7 SL	39	13	0	0	0	7+8
6.7 SL	*	13	0	0	0	9+8
6.7 SL	*	10	0	0	0	9+8
7.3 SL	*	14	13	0	0	7+6
7.3 SL	48	9	0	0	0	7+8
7.8 SL	*	68	41	0	0	9+8
8.2 SL	*	84	65	5	0	10+8
8.4 SL	*	90	71	5	2	10+8
8.7 SL	*	90	70	5	2	10+8
9.2 SL	40	82	62	6	0	10+8
9.2 SL	*	83	64	6	5	10+8
9.7 SL	*	85	67	5	1	10+8
9.8 SL	*	87	63	6	2	10+8
9.8 SL	*	87	65	6	4	10+8
10.2 SL	*	85	64	6	6	10+8
11.0 SL	*	86	63	6	9	10+8

anterior and midbody surfaces of the fins and gradually expanded over the medial portion with development (Fig. 4).

Paralichthys lethostigma Newly hatched *P. lethostigma* larvae had melanophores on the dorsal and anal finfolds that were concentrated in the middle of the body (Fig. 2C). Dorsal midline pigment was well developed and generally occurred from the anterior portion of the caudal region to the head and snout. There were fewer melanophores on the dorsal and ventral midline posterior to the anus (i.e. in the caudal region). Pigment was observed on the oil globule.

Like *P. albigutta*, characteristic pigment on *P. lethostigma* preflexion larvae was almost-paired and almost-contiguous dorsal and ventral midline melanophores that merged into one row of dorsal and ventral melanophores in the future caudal-fin region (Fig. 5A). On very early preflexion larvae (2.8–3.0 mm NL) that still contained vestiges of yolk and oil, the dorsal midline melanophores were continuous over the brain and snout, and the ventral midline melanophores were continuous over the gut. Melanophores on the lateral portion of the caudal region were not observed on early preflexion larvae (ca. <3.6 mm NL) and were not as well developed on later preflexion larvae compared with *P. albigutta* larvae (Figs. 3A and 5A). In the gut region of preflexion *P. lethostigma,* the lateral portion of the gut was typically devoid of pigment (Fig. 5A) and was never as well developed as that observed for *P. albigutta* (Fig. 3A). Usually, ventral melanophores in the gut region

were not continuous because a gap appeared just anterior to the anus. Regularly, we observed a pair of melanophores located ventrally on each side of the hindgut. The pectoral-fin-ray blade was pigmented. Pigmentation of preflexion *P. lethostigma* at the base of the pectoral fin and in the head region was similar to that observed for *P. albigutta* (Figs. 3A and 5A). Pigment on the maxillary and the dorsum of the midbrain (cranial bump) was less frequently observed in preflexion *P. lethostigma* larvae compared with preflexion *P. albigutta* larvae. Cranial bump pigment was observed on 36% of the specimens and maxillary pigment on 64% of the specimens (n=25). Pigment on the vomer of preflexion *P. lethostigma* also occurred less frequently (36% of the specimens had this pigment; n=15); however, this pigment was difficult to discern. It was most effectively seen on fresh, cleared and stained material. Ventral pigment on the isthmus, anterior to the cleithrum, did not extend the full length of the isthmus as was observed on preflexion *P. albigutta* (Figs. 3A and 5A). The number of melanophores observed on the operculum (commonly one) was less than that observed for *P. albigutta*.

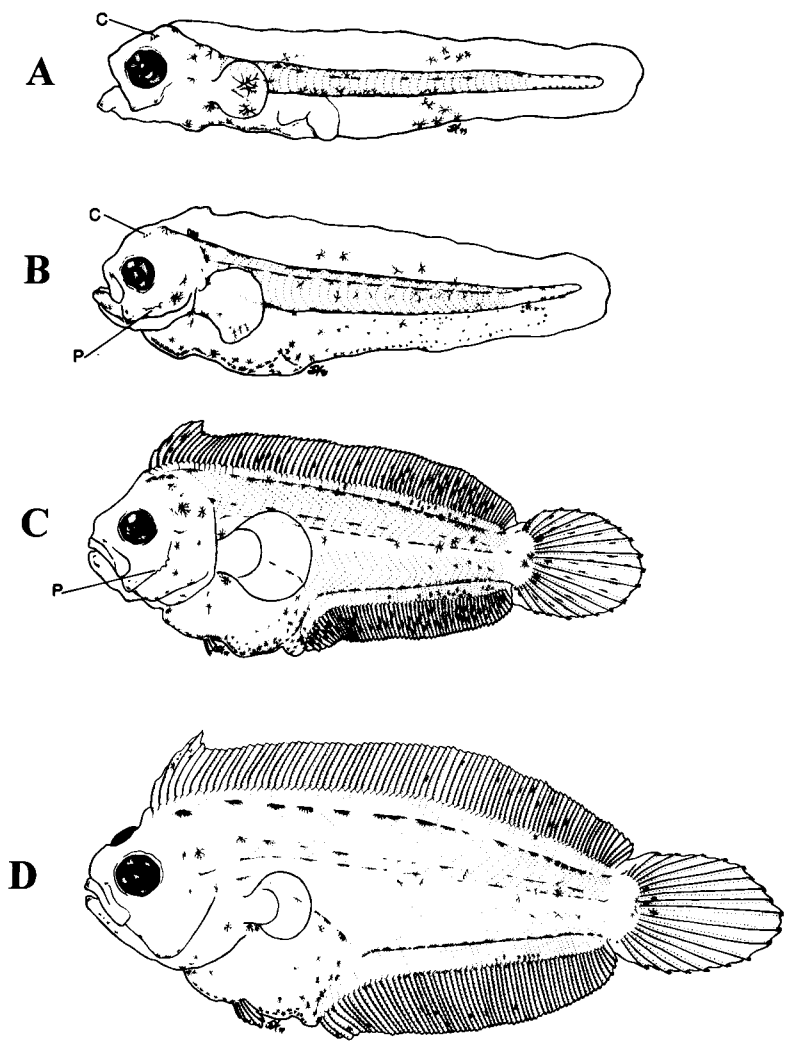

Figure 5

Developmental stages of laboratory-reared southern flounder, *Paralichthys lethostigma* (C=cranial spines, P=preopercular spines). (**A**) 3.3-mm-NL early preflexion larva, 9 days old; (**B**) 6.4-mm-NL late preflexion larva, 24 days old; (**C**) 7.7-mm-SL postflexion larva, 45 days old; and (**D**) 9.1-mm-SL transforming postflexion larva, 40 days old.

Pigment on flexion *P. lethostigma* larvae was similar to that observed on later preflexion larvae (Fig. 5B). Melanophores were observed on the lateral side of the caudal region; however, they were less organized than those observed for *P. albigutta*, which appeared to be more banded in appearance. During flexion, pigmentation increased on the lateral surface of the hindgut. Dorsal finfold pigmentation was sparse and located at midbody. Ventral finfold melanophores occurred along the distal margin and on the finfold surface at midbody. A melanophore was observed at the ventral edge of the pectoral-fin blade, and a distinct melanophore was observed at the junction of the dorsalmost portion of the opercle and the anteriodorsal portion of the gut. This characteristic pigmentation was observed for both species. Pigmentation along the ventral midline of the isthmus increased to cover the entire isthmus. Like *P. albigutta*, melanophores were typically observed on the maxillary and the dorsum of the midbrain, but vomerine pigment was irregularly observed on flexion *P. lethostigma* larvae. Pigmentation (1–4 melanophores) occurred on the opercular region posterior to the eye. Melanophores along the edge of the interopercle and subopercle were first observed on flexion larvae.

On postflexion larvae (6.4–9.3 mm SL), pigment was observed at the base of the caudal fin and on the pelvic fin (Fig. 5, C and D). Melanophores occurred on the lateral surface of the body, but they were never

aligned with the myosepta as was observed for *P. albigutta* (Figs. 3D, 4, and 5).

Other structures and size at transformation

Dorsal and anal fin supports The arrangement of pterygiophores in relation to neural and haemal spines may be valuable for separating *P. albigutta* and *P. lethostigma* larvae because they share similar vertebral counts (hence similar haemal and neural spine counts), but different dorsal and anal fin-ray counts.

The cumulative number of anal-fin-ray pterygiophores between the first haemal spine (which is associated with the first caudal vertebrae) and haemal spines posterior to the fourth haemal spine appeared to be diagnostic (Table 3). Although there was overlap in counts, there is a fair degree of separation in the cumulative number of anal-fin pterygiophores. For example, between haemal spines 1 and 13, from 28 to 31 pterygiophores would aid in identifying *P. albigutta*, whereas ≥28 pterygiophores would aid in identifying *P. lethostigma*. As expected, there was a lesser cumulative number of pterygiophores for *P. albigutta* compared with *P. lethostigma* (Table 3).

The cumulative number of dorsal-fin pterygiophores between the first neural spine (which is associated with the first caudal vertebrae) and following spines appeared less valuable (Table 3). There was considerable overlap between species. The cumulative number of pterygiophores was most valuable when a considerable number were developed. For example, between neural spines 1 and 27, from 46 to 52 pterygiophores would aid in identifying *P. albigutta*, whereas ≥58 pterygiophores would aid in identifying *P. lethostigma* (Table 3).

Cranial spines and preopercle spines Weak, fleshy preopercle spines were observed on both species at all stages, and they decreased in number with increasing size (Figs. 3–5). On both species the exact arrangement and number of preopercle spines generally were difficult to discern but were more readily observed in cleared and stained material. Generally, there were four to five minute spines on the inner shelf of the preopercle, a larger spine at the angle of the outer shelf, and one dorsal to the latter on the outer shelf. Preopercle spines were not observed for specimens of either species ≥ approximately 8.5 mm SL.

Cranial spines may be diagnostic for separating *P. albigutta* and *P. lethostigma* preflexion larvae. We consistently observed three cranial spines on *P. lethostigma* preflexion larvae (2.9–5.4 mm NL). We never observed more than two (zero to two) on *P. albigutta* preflexion larvae of similar size. However, in most instances spines could be discerned only on cleared and stained material. Cranial spines generally were never observed on postflexion larvae of either species.

Paralichthys albigutta began transformation at a smaller size than did *P. lethostigma*. For *P. albigutta*, the migrating eye first appeared at the dorsal midline at 7.8 mm SL. On all specimens >7.8 mm SL, the eye had migrated at least to the dorsal midline. For *P. lethostigma*, the migrating eye first appeared at the dorsal midline at 8.7 mm SL. With one exception, the eye had migrated to at least the dorsal midline on all larvae >8.7 mm SL. On one field-collected larva of 11.2 mm SL, the eye had barely reached the dorsal midline as described for *P. dentatus* (Fahay, 1983). Pigmentation and meristic characters clearly identified this specimen as *P. lethostigma*.

Table 3

The cumulative number (range) of pterygiophores between the first neural and haemal spines and other spines (up to neural and haemal spine number 30 and 20, respectively) for gulf flounder, *Paralichthys albigutta* (n=15 laboratory-reared and 14 field-collected specimens) and southern flounder, *P. lethostigma* (n=1 laboratory-reared and 13 field-collected specimens).

Spine numbers	*P. albigutta*		*P. lethostigma*	
	Dorsal fin	Anal fin	Dorsal fin	Anal fin
1–2	2–3	1–3	2–3	2–3
1–3	4–6	3–5	5–6	4–5
1–4	5–8	5–7	7–9	6–8
1–5	6–9	7–9	8–9	9–10
1–6	8–10	9–11	8–10	11–13
1–7	9–12	10–13	10–12	13–16
1–8	11–13	12–15	12–14	15–18
1–9	13–16	14–17	14–16	17–21
1–10	15–18	16–20	16–18	19–23
1–11	16–21	17–22	18–21	22–26
1–12	18–23	19–25	20–23	24–29
1–13	20–25	21–27	23–26	26–31
1–14	22–28	23–29	25–29	28–34
1–15	24–30	25–31	27–31	30–37
1–16	26–33	27–33	30–34	33–39
1–17	28–35	29–36	32–36	34–42
1–18	30–37	31–38	34–38	37–45
1–19	32–40	33–40	36–41	39–48
1–20	34–42	36–42	38–43	43–51
1–21	36–44		40–45	
1–22	38–46		42–48	
1–23	39–49		44–50	
1–24	41–51		46–53	
1–25	43–53		48–55	
1–26	45–55		51–58	
1–27	46–57		53–61	
1–28	48–60		55–64	
1–29	51–62		58–67	
1–30	53–65		60–70	

Identification of field-collected material Material collected from Onslow Bay off North Carolina was examined to determine the reliability of pigment patterns established from laboratory-reared material (Table 4). We examined the extent of pigment along the isthmus, lateral pigment in the caudal area, vomer and maxillary pigment, and the extent of pigment on the lateral surface of the gut of field-collected material. On the basis of our observations, caution must be used when relating pigmentation of laboratory-reared material to field-collected material. Generally, field-collected specimens had less intense pigmentation than those reared in the laboratory (Figs. 3–6). Our observations of vomer pigment, maxillary pigment, and lateral pigment on the surface of the gut on laboratory-reared larvae were not consistently observed on field-collected larvae (Table 4). Pigment on the lateral surface of the caudal area that aligned with the myosepta and ventral midline pigment that appeared to migrate dorsally on the lateral surface was observed only on *P. albigutta* larvae (laboratory-reared and field-collected). The presence of this pattern might aid in the separation of *P. albigutta* from *P. lethostigma*. However, the absence of this pattern has little value because field-collected *P. albigutta* may lack lateral caudal pigment (Table 4) and when present may not always be aligned with myosepta. On preflexion and flexion field-collected *Paralichthys* larvae, we were unable to define two distinct pigment types but were limited in the number of larvae available. Eight early preflexion larvae (approximately 2.6 mm NL) from Onslow Bay were examined for cranial spines, and only two spines or less were observed. We considered these to be *P. albigutta* on the basis of observations of laboratory-reared material. A collection of material that included early flexion larvae with three cranial spines would be useful 1) to verify the presence of three cranial spines in field-collected material and 2) to categorize larvae into two types (i.e. three cranial spines vs. ≤ two cranial spines) for a detailed examination of pigment patterns. In addition, the use of otolith characteristics produced during the early larval period may be useful in facilitating separation of *P. albigutta* and *P. lethostigma* (Laidig and Ralston, 1995).

Identification of field-collected specimens was based on stage of development of meristic characters (Gutherz, 1967) (Tables 1 and 2). Unless field-collected material is cleared and stained and then measured, the stage of development of meristic characters may not be a valid diagnostic character. The bodies of field-collected material generally are bent, and measurements taken before and after clearing and staining, which tend to straighten the bodies, may vary by 1 mm (Table 4). This is especially pertinent when attempting to identify flexion and early postflexion larvae by size at a developmental stage.

Two specimens of *Paralichthys squamilentus* postflexion larvae were tentatively identified from February collections in Onslow Bay. Pigment at the dorsal and ventral midline resembled *P. dentatus* (Fahay, 1983). One specimen (5.9 mm SL) had 10 postanal melanophores along the ventral midline. Lateral pigment in the caudal area was absent. The

Table 4

Selected field-collected specimens used to examine the effectiveness of pigmentation to identify gulf flounder, *Paralichthys albigutta*, and southern flounder, *P. lethostigma*, larvae. Species identification was determined by relating body length (measured on cleared and stained material) and ray formation. Presence of pigment indicated by a plus sign (+), absence by a minus sign (—).

Specimen number	Body length (mm) preserved	Body length (mm) cleared and stained	Caudal rays	Dorsal rays	Anal rays	Lateral gut pigment	Vomer pigment	Maxillary pigment	Pigment along entire length of isthmus	Lateral caudal pigment	Species identification
1	5.4 NL	6.2 NL	10	8	0	—	—	—	—	—	*P. albigutta*
2	5.2 NL	5.7 NL	8	5–6	0	—	—	—	—	—	*P. albigutta*
3	6.4 NL	7.3 SL	13	13–14	13	—	+	—	—	+	*P. lethostigma*
4	5.4 NL	6.0 SL	12	8	0	+	—	+	+	+	*P. albigutta*
5	5.6 NL	5.7 NL	11	6–7	0	+	+	+	+	+	*P. albigutta*
6	5.0 NL	5.2 NL	0	3	0	—	+	—	—	+	*P. albigutta*
7	5.5 NL	6.5 NL	10	8	0	—	—	—	—	+	Unknown
8	5.0 NL	5.7 NL	4	3	0	+	+	—	—	+	*P. albigutta*
9	5.4 NL	5.9 NL	6	3	0	—	—	—	—	—	*P. albigutta*
10	5.1 NL	5.7 NL	4–5	3	0	—	—	—	—	—	*P. albigutta*
11	7.8 SL	7.8 SL	17	68	41	—	—	—	—	—	*P. lethostigma*
12	6.7 SL	6.7 SL	17	10	0	—	—	—	—	—	*P. lethostigma*

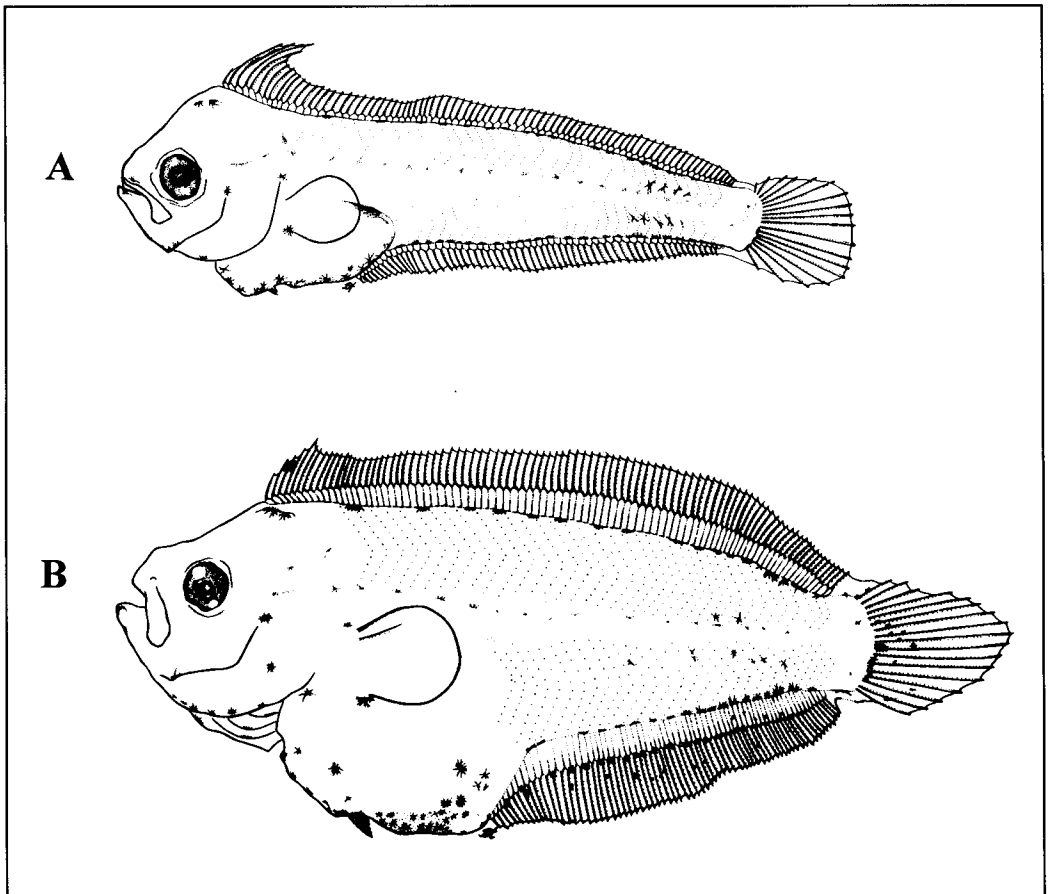

Figure 6

Field-collected (**A**) gulf flounder, *Paralichthys albigutta*, 7.1 mm SL; (**B**) southern flounder, *P. lethostigma*, 8.4 mm SL.

other specimen (6.2 mm SL), had 12 postanal melanophores along the ventral midline. One melanophore occurred on the lateral portion of the caudal area. We considered these specimens to be *P. squamilentus* because they were well developed for their size compared with *P. dentatus* (Fahay, 1983). Approximately 64 dorsal rays and 55 anal rays were formed. Neither the dorsal nor anal fins were completely formed. One specimen had four well-defined cranial spines and five preopercle spines. The other specimen had three cranial spines and five preopercle spines. Both specimens had 40 vertebrae.

Discussion

Paralichthys larvae can be separated from other bothid larvae by ventral midline pigment and the caudal formula when the principal caudal rays are formed (Fahay, 1983). *Paralichthys dentatus* and, tentatively, *P. squamilentus* larvae can be separated from *P. albigutta* and *P. lethostigma* larvae by differences in postanal ventral midline pigment (Fig. 7) and in size at development. *Paralichthys dentatus* has a maximum of 13 postanal melanophores along the ventral midline that are not uniform in size or spacing (Smith and Fahay, 1970). Tentatively, *P. squamilentus* has ventral midline pigment similar to *P. dentatus*. *Paralichthys albigutta* and *P. lethostigma* have approximately 19–31 postanal ventral midline melanophores that are uniform in size and spacing (Fig. 7). Larval *P. dentatus* can tentatively be separated from larval *P. squamilentus* by differences in development. At any given size, *P. squamilentus* appears to be significantly more developed than the other three *Paralichthys* species. However, early preflexion larvae might be difficult to separate from *P. dentatus* because *P. squamilentus* has only been tentatively described from two postflexion specimens (this study). On the other hand, at any given

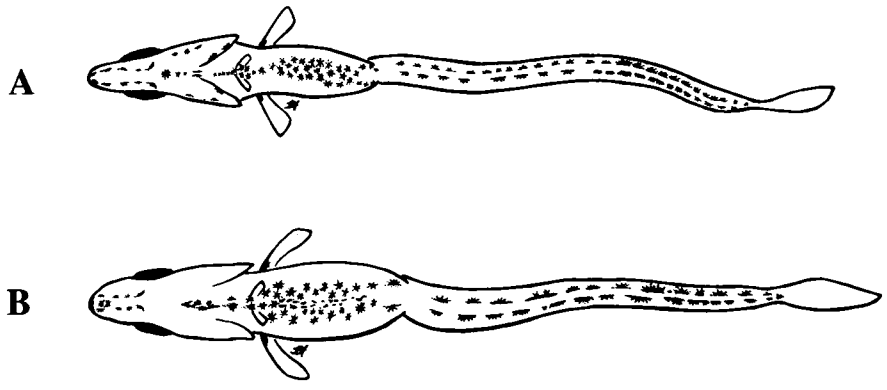

Figure 7
Pigmentation on the ventral side of field-collected, postflexion (**A**) gulf flounder, *Paralichthys albigutta* (9.1 mm SL), and (**B**) summer flounder, *P. dentatus* (9.7 mm SL).

size *P. dentatus* is the least developed of the four paralichthids (Fahay, 1983).

The best diagnostic character for separating *P. albigutta* from *P. lethostigma* is the development of meristic characters, but field-caught specimens must be cleared and stained prior to identification. At any given size, *P. albigutta* is more developed than *P. lethostigma* (Tables 1 and 2). The number of cranial spines could be useful in separating preflexion *P. albigutta* (zero to two spines) from preflexion *P. lethostigma* (three spines) if field-collected specimens are in concordance with those reared in the laboratory. Pigmentation on the lateral surface of the hindgut and caudal area are more developed in *P. albigutta* compared with *P. lethostigma*, but these characters may not be as consistent on wild specimens (e.g. Table 6). Caution should be used when extrapolating pigment patterns on laboratory-reared material to field-collected materials.

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