Development of larval and early juvenile penpoint gunnel (*Apodichthys flavidus*) (family: Pholidae)

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The penpoint gunnel (*Apodichthys flavidus*) is a member of the perciform family Pholidae. Pholids, commonly referred to as gunnels, are eel-like fishes that inhabit the rocky intertidal and subtidal regions of the northern oceans and are often associated with macroalgae, such as *Fucus* spp. or kelp (Watson, 1996). Gunnels are ecologically important forage fishes that form part of the diet of birds and commercially important groundfish species (Hobson and Sealy, 1985; NMFS[1]; Golet et al., 2000). The diet of *A. flavidus* and other pholids comprises primarily harpactacoid copepods, gammarid amphipods, isopods, and other crustaceans (Cross, 1981). *Apodichthys flavidus* ranges along the west coast of North America from southern California to the Gulf of Alaska (Mecklenburg et al., 2002). Adult *A. flavidus* are distinguished from other pholids by their total vertebral counts, the presence of a thick and grooved first anal spine, a preanal length that is approximately 60% standard length (SL), and a dark green to light olive coloration (Yatsu, 1981). It is one of the largest pholids (up to 46 cm) and is important in the live fish trade for both home and public aquaria (Froese and Pauly[2]).

In late winter to early spring months (January–April), adult *A. flavidus* spawn in nearshore waters. A single female lays clusters of demersal, adhesive eggs onto substrate that a male will guard until hatching (Clemens and Wilby, 1961; Wilkie, 1966; Marliave, 1975). The eggs are 3 mm in diameter and the incubation period is approximately 2.5 months (Wilkie, 1966; Marliave, 1975). Larvae are about 12–13 mm total length (TL) at the time of hatching, well developed, and have pigmented eyes, an elongated body, and very little to no yolk sac (Wilkie, 1966; Marliave, 1975). After about 50 days, the larvae settle as juveniles and are approximately 25 mm SL (Marliave, 1975). Although *A. flavidus* reproduction has been well-studied, there has not been a complete description of larval development. Wang[3] provided a summary of life history information and pencil illustrations of early flexion, postflexion, and juvenile stages. Matarese et al. (1989) published an illustration of a 15.0-mm flexion larva and some characters distinguishing *A. flavidus* from *Pholis* spp.

In the present study we describe development of *A. flavidus* from recently hatched larvae to newly settled juveniles, including some general aspects of osteological development. Larval *A. flavidus* are compared with larvae of other pholid species included in the genus *Apodichthys* by Yatsu (1981, 1985): *Xererpes fucorum* and *Ulvicola sanctaerosae*. This classification was not followed by Matarese et al. (1989) or Watson (1996) and is not followed in the present study. This work will aid in the accurate identification of *A. flavidus* larvae in samples taken during nearshore ichthyoplankton surveys and in ecological studies and will contribute to a better understanding of pholid systematics.

Materials and methods

We examined 58 larval and juvenile *A. flavidus* (11.9–42.3 mm) collected in dip-net surveys by scientists of the Alaska Fisheries Science Center (AFSC; Busby et al., 2000) and the University of Washington (UW) from four sites: Clam Bay (47°34.5′N, 122°32.5′W), Sequim Bay (48°2.3′N, 123°2.0′W), Iceberg Point (48°42.4′N 122°53.3′W), and Friday Harbor (48°54.5′N, 1230.7°W), all located in

[1] NMFS (National Marine Fisheries Service). 1998. Final environmental assessment and regulatory impact review for Amendment 36 to the Fishery Management Plan for the groundfish fishery of the Bering Sea and Aleutian Islands Area and Amendment 39 to the Fishery Management Plan for groundfish of the Gulf of Alaska to create and manage a forage fish species category, 76 p. NOAA/NMFS Alaska Regional Office PO Box 21668 Juneau, Alaska 99802-1668.

[2] Froese, R., and D. Pauly (eds.). 2004. Fishbase. World wide web electronic publication http://www.fishbase.org [accessed November 2004].

[3] Wang, J. C. S. 1986. Fishes of the Sacramento-San Joaquin estuary and adjacent waters, California: a guide to the early life histories, 602 p. Technical Report 9 of the Interagency ecological study program for the Sacramento-San Joaquin Estuary. [Available from Ecological Analysts, Inc. 2150 John Glen Drive, Concord, CA 94520.]

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Puget Sound, WA, and from adjacent waters. Specimens were initially preserved in 3.5% buffered formalin solution and later transferred to 70% ethanol (Busby et al., 2000). A dissecting stereomicroscope was used to examine pigmentation, general body size and structure, and to obtain meristic counts. Morphological measurements were made on 55 suitable specimens by using a digital image analysis system consisting of a video camera attached to a dissecting stereomicroscope and a computer with image analysis software. All measurements were taken from the left side of the specimen. Standard length was used throughout the study unless otherwise indicated. During flexion stage, notochord length (NL) was measured and recorded as SL. Measurements included standard length, head length, eye diameter, body depth, snout to anus length, and pectoral-fin length, as described by Moser (1996).

To describe osteological development of *A. flavidus*, with emphasis on the development of the caudal skeleton, 12 specimens were cleared and stained by using the technique described by Potthoff (1984). The terms "unossified precursor" or "element" are used to describe unossified elements that took up alcian blue stain but not alizarin red-s stain. From the cleared and stained specimens, stages of larval development were identified from landmarks of caudal-fin development. Caudal skeletons of six specimens representing distinct stages were used to create illustrations. Developmental stage terms follow Kendall et al. (1984) and Neira et al. (1998). The flexion stage was divided into three additional stages: early-, mid- and late-flexion. Early-flexion begins at hatching, mid-flexion begins with the formation of the forth hypural and epurals, and late flexion begins with the development of the fifth hypural and ends with complete notochord flexion. Nomenclature of caudal skeleton elements follows Fujita (1989).

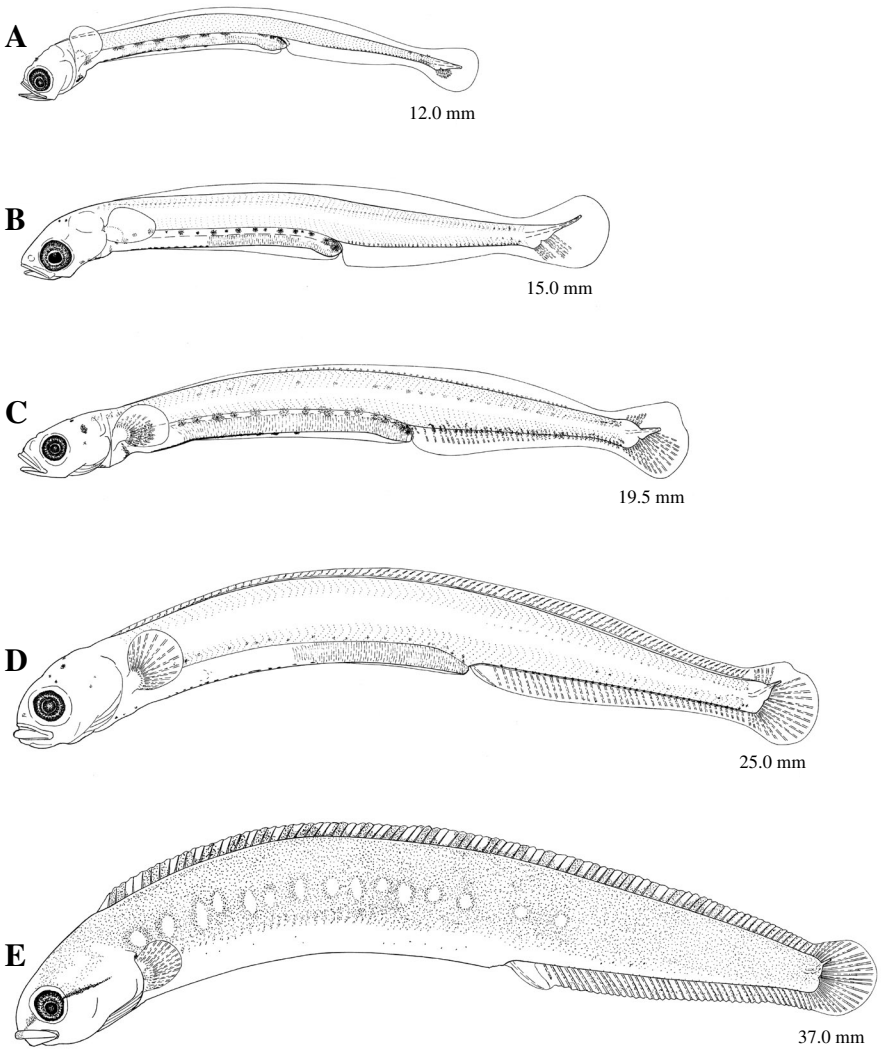

Figure 1

Developmental series of penpoint gunnel (*Apodichthys flavidus*).(**A**) Early-flexion larva, Clam Bay, 6 April 1989 (UW 104928); (**B**) mid-flexion larva (from Matarese et al., 1989); (**C**) late-flexion larva, Friday Harbor, 20 April 1994 (UW 104930); (**D**) postflexion larva, Clam Bay, 20 April 1989 (UW 104932); (**E**) juvenile, Sequim Bay, 25 April 1989 (UW 104934). Illustrations by Beverly Vinter.

Results

Morphology

Apodichthys flavidus larvae are approximately 12.0–13.0 mm at hatching and in early flexion stage and have little or no yolk sac present (Fig. 1A). The early-flexion stage occurs between hatching and 14.0 mm. Mid-flexion begins at approximately 14.0 mm (Fig. 1B), late-flexion at 17.0 mm (Fig. 1C), and postflexion at 20.0 mm (Fig. 1D). Transformation to the juvenile stage occurs between 25.0 mm and 30.0 mm. Juveniles examined ranged from 30.1 to 42.3 mm and looked like small

Table 1

Body proportions of larval and early juvenile penpoint gunnel (*Apodichthys flavidus*). Values given for each body proportion are expressed as percentages of standard length (SL) or head length (HL): mean ± standard deviation, and range.

Sample size, standard length, and body proportion	Flexion	Postflexion	Juvenile
Sample size	33	18	4
Standard length (SL)	14.9 ±2.1 (11.9–19.2)	21.9 ±1.3 (20.0–24.0)	34.2 ±5.6 (30.1–42.3)
Snout to anus length/SL	62.9 ±1.8 (58.0–66.1)	63.3 ±1.2 (61.8–66.1)	63.5 ±1.8 (62.5–65.2)
Body depth/SL	8.3 ±0.9 (6.4–9.9)	9.3 ±0.7 (8.2–10.4)	11.6 ±1.1 (11.2–12.9)
Head length/SL	13.2 ±0.7 (12.1–14.8)	14.1 ±0.7 (12.9–15.5)	16.4 ±1.1 (15.3–17.3)
Eye diameter/HL	33.6 ±4.7 (22.3–44.1)	27.4 ±3.1 (23.5–35.4)	20.0 ±3.9 (15.8–24.8)
Pectoral fin length/SL	7.6 ±1.2 (5.4–10.2)	7.9 ±1.0 (6.2–10.1)	6.2 ±1.4 (4.9–7.1)

adults (Fig. 1E). Larvae are slender bodied throughout development and body depth increases from 8% SL during flexion to 12% SL in juveniles (Tables 1 and 2). Head, snout-to-anus, and pectoral-fin lengths are consistent throughout development at approximately 14%, 63%, and 7% SL, respectively. Eye diameter decreases from 34% head length (HL) in flexion larvae to 20% HL in juveniles.

Pigmentation

Early-flexion larvae have a few faint melanophores located dorsally on the head and nape and a single melanophore on the isthmus (Fig. 1A). Along the dorsal surface of the gut, a row of large melanophores is present—irregularly spaced anteriorly and posteriorly, regularly spaced medially. Another row of smaller, evenly spaced melanophores is present along the anterior ½ to ¾ length on the ventral surface of the gut. A row of postanal ventral melanophores (PVMs) extends from the anus to the caudal peduncle. Generally there is one PVM per myomere but in many individuals one or more are missing from the row. In addition, there are small patches of melanophores along the dorsal and ventral margins of the caudal peduncle. In mid-flexion larvae, patches of melanophores on the head, nape, isthmus, and caudal peduncle are more defined, and a row of internal melanophores is present above the notochord (Fig. 1B). These melanophores develop simultaneously. In addition, the number of melanophores along the dorsal surface of the gut nearly doubles. The PVMs in late-flexion larvae are larger and more slashlike (Fig. 1C). During postflexion, pigmentation previously noted now appears very faint (Fig. 1D). Juvenile pigmentation resembles that of adults (Fig. 1E). Most notably, a horizontal streak of melanophores extends from the snout to the anterior margins of the eye and continues from the posterior margin of the eye to the mid-operculum. The body in live specimens is a uniform bright green to olive and has white spots located above the gut and anterior portion of the anal fin. The gut is generally unpigmented, with the exception of a few very small irregularly spaced melanophores on the lateral surface.

Osteology

Head region In early-flexion larvae the maxilla, mandible, two mandibular teeth, branchiostegal rays, and cleithrum are ossified. The premaxilla ossifies during mid-flexion. The remaining bones in the head region are not ossified until the juvenile stage.

Fins Unossified precursors of five principal caudal-fin rays are present at hatching and throughout early flexion. During mid-flexion, 12 unossified precursors of pectoral-fin rays and 13 unossified caudal-fin elements are present, both first appearing at 15.0 mm. Also at this size, a few faint unossified anal-fin elements are present in the anterior portion of the anal finfold, which are not visible in unstained specimens. Beginning at 17.0 mm, unossified precursors of dorsal-fin spines are first present in the area of the dorsal finfold above vertebrae 45–50 and then develop anteriorly and posteriorly from this position. Unossified anal-fin elements are also added from anterior to posterior. In addition, the scapula, coracoid, and radials of the pectoral fin are ossified and the adult complement caudal-fin elements is present, but still unossified (13 principal rays and 11 procurrent rays). By the end of late flexion (about 20.0 mm), elements of the dorsal, anal, and pectoral fins finally become ossified (Table 2). The single spine is the first element in the anal fin to become ossified. Pterygiophores of all fins are ossified in juveniles and the pectoral- and caudal-fin rays become ossified and branched. The number of fin elements present at any particular stage and length is somewhat variable as we noted slight differences in the numbers present between our stained and illustrated specimens.

Vertebral column At hatching, all neural and haemal spines are present and ossified (Table 2). In addition, vertebral centra begin to differentiate from anterior to

posterior during the early- and mid-flexion stages. At 17.0 mm, the vertebral centra are completely differentiated but remain unossified. In juveniles, the vertebral centra are completely ossified.

Caudal skeleton In early flexion larvae, the notochord begins to bend upward and the haemal spine of the second preural centrum, the fused parhypural plus first and second hypurals, and the third hypural are present (Fig. 2A). Caudal skeleton elements begin to ossify at this stage, beginning at the base of each. Ventral elements that develop at mid-flexion are the haemal spine of the third preural centrum and the fourth hypural (Fig. 2B). Dorsally, epurals 1–3 and neural spines of the second and third preural centra form during this stage. Elements present in early-flexion are now fully ossified. At the beginning of late-flexion (about 17.0 mm), a fifth hypural and a fourth epural are present but not ossified (Fig. 2C). In the 19.2-mm late-flexion specimen examined, the third and fourth epurals were fused and the first epural was fused to the neural spine of the second preural centrum (Fig. 2D). In postflexion larvae, the distal margins of hypurals 3–5 are oriented vertically and the first epural separates from the neural spine of the second preural centra (Fig. 2E). All other elements in the caudal skeleton have grown and are fully ossified. In juveniles, the caudal fin has the adult form and the final element, the uroneural, is present just ventral to the second and fused third and fourth epurals (Fig. 2F). A ventral caudal radial, present from mid-flexion to postflexion (Figs. 2, C–E), is absent in juveniles.

Discussion

Our description of *A. flavidus* development can be used to distinguish larvae of this species from co-occurring species of *Pholis* spp., *Apodichthys*, *Ulvicola*, and *Xererpes* along the West Coast of the United States. As with *Ulvicola sanctaerosae*, *A. flavidus* does not have a preflexion stage and larvae hatch at an advanced developmental state (Watson, 1996). Larval *Pholis* spp. differ from larval *A. flavidus* by the presence of pelvic fins in the former and by differences in pigmentation. The melanophores along the dorsal surface of the gut in *Pholis* spp. are more numerous (about 25 vs. about 18 in postflexion larvae) and closer in spacing anteriorly (Matarese et al., 1989), and *Pholis* spp. do not develop an internal row of melanophores along the dorsal margin of the notochord. *Apodichthys flavidus* develops a series of internal melanophores along the entire length of the notochord by the mid-flexion stage. Larvae of *U. sanctaerosae* can be distinguished from *A. flavidus* by pigmentation and by the number and persistence of pectoral-fin rays in postflexion larvae and juveniles. Larval *U. sanctaerosae* have fainter melanophores that are more irregularly spaced along the dorsal

Table 2

Meristics of cleared and stained larval and early juvenile penpoint gunnel (*Apodichthys flavidus*). Counts are of ossified elements only. Specimens above dashed line (– – –) were undergoing notochord flexion.

Standard length (mm)	Dorsal-fin spines	Anal-fin elements	Pectoral-fin rays	Branchiostegal rays	Neural spines			Haemal spines	Centra			Caudal-fin rays
					abdominal	caudal	total		abdominal	caudal	total	
12.2				5	50	43	93	44				
12.8				5	50	42	92	43				
13.4				5	51	45	96	44				
14.6				5	52	43	95	42				
15.0				5	51	43	94	44				
17.0				5	52	42	94	45				
19.2	XC	I	15	5	50	41	91	44				
21.2	XCI	I	15	5	50	43	93	45				
23.5	XCI	I	15	5	51	44	95	45				
30.1	XCII	I, 39	14	5	51	42	93	45	51	45	96	24
33.2	XCI	I, 41	15	5	50	43	93	43	50	45	95	24
42.3	XCI	I, 40	15	5	50	42	92	42	50	44	94	24

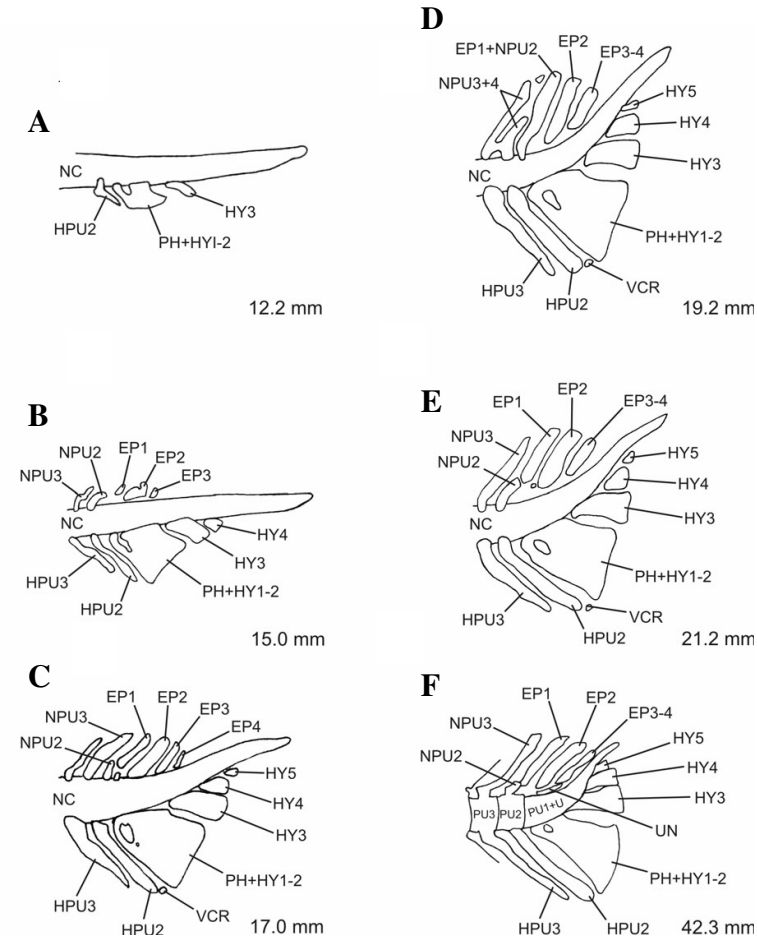

Figure 2

Caudal skeleton development of penpoint gunnel (*Apodichthys flavidus*). (**A**) Early-flexion larva, Clam Bay, 27 February 1995 (UW 104943); (**B**) mid-flexion larva (note irregularly shaped EP2), Clam Bay, 10 May 1989 (UW 104937); (**C**) late-flexion larva, Clam Bay, 19 April 1988 (UW 104939); (**D**) late-flexion larva, Clam Bay, 15 May 1989 (UW 104940); (**E**) postflexion larva, Friday Harbor, 8 April 1993 (UW 104943); (**F**) juvenile, Iceberg Point, 18 July 1963 (UW 018016). Caudal-fin element abbreviations: EP=epural; HPU=haemal spine, preural; HY=hypural; NC=notochord; NPU=neural spine, preural; PH=parhypural; PU=preural centra; U=urostyle; UN=uroneural; VCR=ventral caudal radial. Illustrations by Lisa De Forest.

surface of the gut than in *A. flavidus*, and in early- and mid-flexion larvae, these melanophores are restricted to the posterior ¼ to ½ of the gut. Another distinguishing characteristic is that *U. sanctaerosae* does not fully develop pectoral-fin rays and the pectoral fin does not persist after the juvenile stage. A pectoral finfold is present during the larval stage of this species; however, only the uppermost pectoral-fin rays (6 or 7 vs. 14 or 15 for *A. flavidus*) partially develop but do not persist, and the pectoral finfold decreases in size during the latter part of development. Larvae of *Xererpes fucorum* can be distinguished from *A. flavidus* by the presence of a preflexion stage and by having fewer total (84–93 vs. 96–101) and postanal (35–40 vs. 47–52) myomeres. In addition, during the later stages of development, *X. fucorum* has fewer pectoral-fin rays than *A. flavidus* (12 vs. 14–15).

Yatsu's (1985) revision of the family Pholidae placed *U. sanctaerosae* and *X. fucorum* in the genus *Apodichthys*, but this classification was not followed by Matarese et al. (1989), Watson (1996), or in the present study. Larvae of both these species are quite similar

to *A. flavidus*; however, we recommend a more detailed study of larval *U. sanctaerosae* and *X. fucorum,* including a description of caudal skeleton development, before concluding that larval characters do, or do not, support Yatsu's (1985) classification. In particular, it would be interesting to investigate whether either species develops a fourth epural that fuses with the third, or a first epural that fuses with the neural spine of the second preural centrum during flexion. In another cleared and stained individual we observed fusion of the neural spines on the third and fourth preural centra (NPU4 and NPU3, Fig. 3D). However, only one specimen of *A. flavidus* was examined at each of these fusions and more specimens, when available, should be cleared and stained to determine if these fusions occur in all larval *A. flavidus*. Presence or absence of a ventral caudal radial may also be of interest. Although it is unclear what becomes of the ventral caudal radial between postflexion and juvenile stages from our study of *A. flavidus*, we hypothesize that it fuses with the tip of the haemal spine of the second preural centrum. Taking all of these unusual aspects of *A. flavidus* larval development into account, we suggest that development of *U. sanctaerosae* and *X. fucorum* should be further investigated to clarify the systematic relationships among the genera.

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