# DEVELOPMENT OF *PHOLIS CRASSISPINA* (PHOLIDAE) LARVAE FROM THE WATERS OF PETER THE GREAT GULF, SEA OF JAPAN

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The article describes the larvae and provides the main meristic features of juvenile and mature *Pholis crassispina* specimens from the waters of Peter the Great Gulf, Sea of Japan. An original scheme for designating melanophores is proposed. The dynamics of melanophore coloration development in larvae is described. The cleithral pigment in this species is present from the preflexion stage. At the same stage, after reaching an absolute body length of 14.3 mm, a superficial melanophore row appears on the sides of the abdomen; melanophores can have a ray structure or appear as dots. After a body length reaches approximately 18.4 mm, each postanal ventral melanophore corresponds to one segmented ray of the anal fin. From this larval length, the number of segmented rays in the anal fin can be determined by counting the number of melanophores at their bases. A set of features is proposed that allows reliable identification of *P. crassispina* larvae at each stage of development.

*Keywords*: *Pholis crassispina*, larva, development, melanin coloration, caudal fin skeleton, Peter the Great Gulf, Sea of Japan.

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## INTRODUCTION

The butterflyfish family (Pholidae) in the hall. Peter the Great of the Sea of Japan is represented by five species: Pholis crassispina (Temminck et Schlegel, 1845), P. nebulosa

(Temminck et Schlegel, 1845), P. picta (Kner, 1868), P. fasciata (Bloch et Schneider, 1801), and

Rhodymenichthys dolichogaster (Pallas, 1814) (Lindberg and Krasyukova, 1975; Chereshnev and

Nazarkin, 2008; Sokolovsky et al. et al., 2011; Parin et al., 2014). Of these, only P. nebulosa and P. crassispina, which was relatively recently described for the ichthyofauna of the Bay, have scales on their heads (Yatsu, 1981; Chereshnev and Nazarkin, 2008). From the waters of the hall. Only P. nebulosa larvae are listed by Peter the Great (Sokolovsky and Sokolovskaya, 2008). Also, according to literature data, P. nebulosa is resident and widespread in the world. Peter the Great is a species, and P. crassispina has the status of a small species (Sokolovsky et al., 2011).

However, when studying the traits of scaly-headed butterfish from Srednyaya Bay and mass butterfish larvae from Zhitkova Bay (Peter the Great Bay), it was revealed that the trait values for the entire sample go beyond the variability of the species.*P. nebulosi.* The present article is devoted to the description of these larvae and identification of the species of the studied scaly-headed butterfish.

## MATERIAL AND METHODOLOGY

The material used was larvae, juveniles and mature individuals.*P. crassispina*from Peter the Great Bay, Sea of Japan (Fig. 1). In the coastal waters of Zhitkova Bay (Balanov et al., 2022), larvae with an absolute length (*TL*) 12.2–30.8 mm (*n*= 42 specimens) were caught in March–June 2022.Juveniles and mature individuals*TL*91–175 mm (*n*= 22 specimens) were caught in Srednyaya Bay in 2007–2022. Larvae were collected at light stations using a net (Balanov et al., 2020). Juvenile and sexually mature fish were caught using a fry seine. All material was fixed in a 4% formaldehyde solution and then transferred to 60% isopropyl alcohol for storage. The body length of all individuals was measured on fixed material; the Zen Pro program (Carl Zeiss Microscopy GmbH, Germany) was used to measure the length of larvae.

All measurements and counts were performed according to the schemes proposed by Makushok (1958), Yatsu (1981), and Voskoboynikova (2005). The following character

designations are used in the description: *D* , *A* , *P* , *V* , *C* – dorsal, anal, pectoral, pelvic, and caudal fins; *TL* – absolute length from the anterior edge of the snout to the end of the fin fold (until the postflexion stage) or to the end of the middle rays of the caudal fin (in other specimens).

Pigmentation was studied in fixed specimens. The description of changes in pigmentation and the degree of skeletal development in larvae was performed using photographs taken with a SteREO Discovery.V12 stereomicroscope ("Carl Zeiss AG", Germany). Each larva was photographed before and after staining with alizarin red and clearing in a 1.5% potassium hydroxide solution (Yakubovski, 1970). Photographs of unstained larvae were used to study external pigment. Photographs of stained specimens were used to describe internal pigment and to study differences in alizarin staining of the skeleton at various stages of larval development. A total of 176 photographs were examined. Paired melanophores were counted on the left side of the body. In the anal fin, melanophores at the base of the segmented rays were examined; the first two melanophores at the beginning of the fin correspond to the spiny rays, so they were not counted. Live larvae were examined for the presence of erythrophores and xanthophores.

There is no generally accepted methodology for designating elements of melanin coloration for fish of the gunnel family. We propose an original scheme for designating melanophores (Fig. 2) based on previously published works on species of this family (Kendall et al., 1984; An atlas..., 1988; Matarese et al., 1989; Watson, 1996) and our own data, by analogy with melanophore schemes for prickleback fishes (Stichaeidae) (Balanov et al., 2020).

Meristic characters of juvenile and mature fish were counted using radiographs obtained on a Faxitron MX-20 digital X-ray machine ("Faxitron", USA). To count the rays of *P* , they were stained with alizarin in a 1.5% potassium hydroxide solution.

For the description of larval development, stages were identified depending on the presence or absence of a yolk sac and the degree of notochord flexion (Kendall et al., 1984): yolk-sac – the

yolk sac stage, preflexion – before notochord flexion, flexion – formation of rays in the caudal fin and notochord flexion, postflexion – after completion of notochord flexion and formation of rays in the caudal, anal, and dorsal fins.

The studied specimens are stored in the collection of the Laboratory of Ichthyology at the NSCMB FEB RAS (Vladivostok).

## RESULTS

### *Pholis crassispina* (Temminck et Schlegel, 1845)

(Fig. 3–5)

#### Description of larvae at different developmental stages

Larvae ***TL* 12.2–13.0 mm** ( *n* = 3 specimens), at the yolk-sac stage, were caught in mid-March 2022. A characteristic feature of this stage is the presence of a yolk sac. The notochord is straight, *D* and *A* are in the form of fin folds (Fig. 3a). Only the jaws and cleithrum stain with alizarin. The tail is represented by a symmetrical fin fold (Fig. 4a).

The larvae are lightly pigmented, with large melanophores without a distinct shape. On the yolk sac near the future pericardial sac, there is a large paired stellate melanophore *CSM* (here and below: for designations and general scheme of melanophore arrangement, see Fig. 2). Above the intestinal tube runs a row *RG* of 11–12 paired stellate melanophores, which become less distinct and merge into a solid line closer to the end of the intestine. Three to five closely positioned stellate melanophores *DMEG* are located at the end of the intestinal tube. Along the middle of the abdomen from the head to the end of the intestinal tube runs a row *VR* , represented by a dashed line. Along the base of *A* is a row *PVM* consisting of stellate melanophores, the number of which does not correspond to the number of future rays (Tables 1, 2). On the tail under the notochord, there are one to two stellate melanophores *CM* (Fig. 4a).

**Table 1.** Occurrence of the main elements of melanin pigmentation in the larvae of *Pholis crassispina* at different developmental stages, %

| Trait | Y olk-sac | P reflexion | F lexion | P ostflexion |
|---|---|---|---|---|
| *PVM* | 100 | 100 | 100 | 100 |
| *RG* | 100 | 100 | 100 | 100 |
| *ELR* * | | 66.7 | 92.9 | 100 |
| *CA* , 25 | | 50.0 | 7.7 | |
| *CA* , 50 | | 33.3 | | |
| C *A* , 75 | | | 30.8 | 18.8 |
| *CA* , 100 | | 16.7 | 61.5 | 81.2 |
| *CM* | 100 | 100 | 100 | 100 |
| *CLM* | 33.3 | 66.7 | 100 | 100 |
| *MOC* | | 11.1 | 21.4 | 43.8 |
| *CSM* | 100 | 100 | 100 | 100 |
| *MA* | | | 28.6 | 81.3 |
| *IM* | 33.3 | 33.3 | 85.7 | 43.8 |
| *MBV* | | 11.1 | 100 | 75.0 |
| *DMEG* | 100 | 100 | 100 | 100 |
| *PCM* | 100 | 100 | 100 | 100 |
| *n* | 3 | 9 | 14 | 16 |

**Note.** *This row shows the occurrence of the trait, the following four rows show how far from the cleithrum the last melanophore of the *ELR* row is located; *CA* – distance from cleithrum to anus, %. Here and in Table 2: designations and melanophore arrangement scheme are shown in Fig. 2. Here and in Tables 2, 3: *n* – number of examined specimens, ind.

**Table 2.** Number of melanophores in some pigment rows in larvae *Pholis crassispina* at different developmental stages

| Character | Y olk-sac | | | P reflexion | | | F lexion | | | P ostflexion | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min–max | *M* ± *m* | *n* | min–max | *M* ± *m* | *n* | min–max | *M* ± *m* | *n* | min–max | *M* ± *m* | *n* |
| *PVM* | x | x | 3 | x | x | 9 | 35–39 | 37.0 ± 0.3 | 14 | 36–38 | 36.8 ± 0.3 | 16 |
| *RG* | 10–11 | 10.7 ± 0.3 | 3 | 9–11 | 9.8 ± 0.2 | 9 | 8–12 | 10.0 ± 0.4 | 14 | 8–12 | 10.5 ± 1.0 | 4 |
| *ELR* | – | – | 3 | 1–4 | 1.3 ± 0.5 | 9 | 4–28 | 15.7 ± 2.0 | 14 | 7–28 | 19.6 ± 1.6 | 16 |

**Note.** min–max – variation limits; *M* ± *m* – mean value and its error; "–" – character not detected; x – melanophores present but not counted.

Larvae ***TL* 14.2–18.4 mm** ( *n* = 9 specimens), at the preflexion stage were caught on 14.04–06.05.2022 (Fig. 3b). The notochord is straight. Alizarin stains the skull bones, gill arches, cleithrum, all vertebrae of the trunk region, the bases of rays *A* and spines *D* ; neural arches of vertebrae at the boundary between the trunk and caudal sections of the spine are detected. By the end of the preflexion stage, almost all vertebrae in the caudal section are stained with alizarin except for seven precaudal vertebrae. The caudal fin is asymmetrical in shape. The lower lobe is larger, oval-shaped and directed downward and backward, with up to 11 principal rays stained with alizarin (Fig. 6a).

Only one larva at this stage had a weakly pigmented melanophore *MOC* in the form of a small dot in the otic capsule. All specimens had one melanophore *CLM* in the form of a stripe. *IM* was observed rarely – in 33.3% of the sample (Table 1) and appears as a cluster of melanophores on the throat. *MBV* appears as a cluster of melanophores at the base of *V* .

The *ELR* row runs slightly below the midline of the abdominal wall. It has one to four melanophores (modal class - 1), which usually (in 50.0% of individuals with this pigment) are located no further than 25% of the distance from the cleithrum to the anus; only in one larva this row extends to the anus (Tables 1, 2). Melanophores *ELR* have the form of dots or radial structure. The *RG* row runs along the dorsal surface of the intestinal tube and consists of 9-11 paired large stellate melanophores (modal class - 9). At the end of the intestinal tube, there are three to seven stellate melanophores *DMEG* (modal class - 7), which sometimes merge into a single spot (Fig. 5). At *TL* 14.2, in the studied larvae at the beginning of *A* , melanophores *PVM* are located at the base of future rays and extend to the proximal part of the pterygiophore (Fig. 3b), however, closer to the end of *A* , the melanophores become looser, positioned very close to each other, forming a continuous line, so their number does not correspond to the number of rays *A* . When larvae reach

*TL* 18.4 mm, melanophores *PVM* move away from each other, become more structured and appear as large dots. From this length value onwards, one melanophore *PVM* corresponds to one ray *A* .

Between *C* and the last melanophores of *A* , melanophores *PCM* are located (Fig. 4b). Unlike melanophores *PVM* , they are represented by thin loose branched lines or small spots. Additionally, melanophores *PCM* are not tied to the rays *A* and are positioned more chaotically.

Melanophores *CM* are located at the base of the rays and on the rays of the caudal fin. At the early preflexion stage, *CM* consists of three stellate melanophores. In larger larvae, a characteristic cluster *CM* (more than five melanophores) is observed at the base of the rays and on the rays up to 1/3 of their length (Fig. 4b).

Characteristic features of the preflexion stage: straight notochord, all trunk vertebrae and up to 11 principal rays stain with alizarin *C* , caudal fin is asymmetrical and its principal rays are directed downward and backward, hypural bones do not stain, *ELR* contains no more than four melanophores.

Larvae ***TL* 20.0–25.0 mm** ( *n* = 14 specimens), at the flexion stage, were caught on 06–18.05.2022 (Fig. 3c). The larvae have a bent notochord (Fig. 4c). At the beginning of the stage, four precaudal vertebrae do not stain with alizarin, but by the end of the stage, the urostyle and vertebrae are almost completely stained, as well as almost all hemal and neural processes of the vertebrae (except for neural arches and the first–third precaudal processes). At this stage, larvae begin to show alizarin staining of the hypaxial and two epaxial hypurals. The rays of *A* , *D* and *P* stain well. The caudal fin is asymmetrical. The lower lobe is larger, oval-shaped and directed downward and backward. In the caudal fin, up to four upper and up to three lower marginal rays stain with alizarin, as well as seven principal rays on each of the upper and lower hypurals (Fig. 6b).

At the flexion stage, 28.6% of the sample have melanophores *MA* at the joint of the dentale and anguloarticulare. In the otic capsule, 21.4% have melanophores *MOC* , their number varies from one to six (modal class – 1). In larvae, *CLM* may be represented by one melanophore in the form of a stripe or one to four in the form of dots. The melanophore *CSM* is more rounded and smaller in size compared to previous stages. The cluster of melanophores *IM* in the throat area is weakly expressed and occurs in 85.7% of larvae in the sample. The entire sample has pigment *MBV* at the base of *V* (Table 1).

Row *ELR* consists of 4-28 melanophores (modal class - 19) (Table 2). In 61.5% of the sample, the *ELR* row reaches the anal opening. This feature was absent in only one larva from the sample. In specimens of the same length, the *ELR* row can vary greatly in the number and distribution of pigment. The paired *RG* row consists of 8-12 melanophores (modal class - 10), and the *DMEG* cluster includes four to eight (modal group - 5-6) melanophores.

In the predominant part of the sample, the melanophores *PVM* appear as dense, large dots, however, in some individuals, a breakdown into several small dots can be observed. On *C* there is a cluster of small melanophores *CM* (Fig. 4g). On the body, randomly distributed small, dot-like melanophores *BM* can be seen.

Characteristic features of the flexion stage: the chord is bent; hypural bones and all vertebrae, rays of *A* , *D* , and *P* are stained with alizarin. In the caudal fin, up to four upper and up to three lower marginal rays, as well as 14 principal rays are stained; *C* is asymmetrical and its principal rays are directed downward and backward, the *ELR* row contains up to 28 melanophores, each *PVM* melanophore corresponds to a ray of *A* .

Larvae ***TL* 25.0-30.8 mm** ( $n$ = 16 specimens), in the postflexion stage, were caught on 12.05-16.06.2022 (Fig. 3g). The chord shortens, with its upper end bent and directed backward (Fig. 4g). In larvae, the pterygiophores of *A* and *D* , epurals in *C* , pelvic bones and rays *V* are well

stained with alizarin. The upper marginal rays of *C* are located close to the principal rays of the epaxial hypurals. In the caudal fin, up to six upper and up to five lower marginal rays, as well as 14 principal rays are stained. The caudal fin is symmetrical and its rays are directed backward (Fig. 6c).

In the auditory capsule *MOC* is present in 43.8% of individuals (Table 1). Melanophore *CSM* has significantly decreased in size, visible only after clearing (Fig. 6). Melanophore *CLM* breaks down into two to five smaller melanophores (modal class – 2; in one specimen the number of melanophores reached 12).

The number of melanophores in the row *ELR* varies from seven to 28 (modal group – 21-24). In 81.2% of larvae, melanophores extend to the anal opening (Table 2). The row *RG* consists of 8-12 melanophores (modal class – 12). *DMEG* is represented by one to seven melanophores (modal class – 3).

At the end of the stage, melanophores *PVM* are represented by a cluster of small dots. On *C* there is a cluster of melanophores *CM* , which along the rays can be represented as dots or stripes.

At *TL* 29.1 mm, the juvenile coloration begins to form: brown rectangular spots appear on *A* , *D* and along the median line of the body; on the head there are four stripes – from the middle of the anterior edge of the eye to the snout, from the lower edge of the eye downward, diagonally from the posterior edge of the eye to the pectoral fin, from the right upper edge of the eye to the occiput (Fig. 5a).

Characteristic features of the postflexion stage: the tip of the notochord is bent backward, the skeleton is completely stained with alizarin and corresponds to the juvenile state; the caudal fin is symmetrical, including up to six upper and up to five lower marginal rays, as well as 14 main rays directed backward; juvenile coloration is formed.

Neither erythrophores nor xanthophores were found in larvae at any developmental stage.

### Juvenile and mature fish

Values of the main meristic characteristics of individuals from Peter the Great Bay are given in Table 3. The number of soft rays in *A* varies from 35 to 39, rays in *P* – from 11 to 13. There are scales on the head.

**Table 3.** Values of some meristic characters of juvenile and mature individuals *Pholis crassispina* in Srednyaya Bay of Peter the Great Bay and throughout the range

| Character | Peter the Great Bay (our data, *n* = 22) | Entire range (according to: Yatsu, 1981; *n* = 38) |
|---|---|---|
| Number of: | | |
| spiny rays in dorsal fin | 77–81 | 73–81 |
| soft rays in anal fin | 35–39 | 34–41 |
| vertebrae: | | |
| – abdominal | 39–41 | 37–42 |
| – caudal | 44–48 | 42–49 |
| – total | 84–87 | 80–88 |
| rays in pectoral fin | 11–13 | 11–13 |

## DISCUSSION

The first question that should be discussed: to which species of gunnels do the larvae belong that were found in large numbers in Zhitkova Bay of Peter the Great Bay of the Sea of Japan? All examined specimens – both larvae (Table 2) and juvenile and mature fish (Table 3) – have the number of soft rays in *A* varying from 35 to 39. Recall that in larvae, we counted melanophores *PVM* at the base of *A* , the number of which corresponds to the number of soft rays in this fin. In Peter the Great Bay, out of five known species of the gunnel family, such number of soft rays in *A* can be found only in two species – *P. crassispina* and *P. nebulosa* . Other species have more rays: 40–51 in *R. dolichogaster* , 45–48 in *P. picta* and 41–48 in *P. fasciata* (Yatsu, 1981).

Depending on the literary source, for the waters of Peter the Great Bay, either one species of scaled eelpout (formerly genus *Enedrias* ) is indicated – *P. nebulosa* (Soldatov, Lindberg, 1930;

Lindberg, Krasyukova, 1975; Fedorov, 2004; Sokolovsky et al., 2007) or two – *P. crassispina* and *P. nebulosa* (Chereshnev, Nazarkin, 2008; Sokolovsky et al., 2011; Parin et al., 2014). The latest systematic description of species of the genus *Enedrias* was conducted by Yatsu (Yatsu, 1981), who proved the validity of both species and showed that they differ well in the number of rays in *P* : 11-13 in *P. crassispina* and 14-15 in *P. nebulosa* . In the studied fish from the waters of Peter the Great Bay (larvae from the flexion stage, juvenile and mature fish), the number of rays varied from 11 to 13 (Table 3). Thus, all studied specimens belong to the species *P. crassispina* . Juvenile and mature fish were caught in the western part of Peter the Great Bay, larvae - in the central part; based on this fact, we can assume the widespread distribution of *P. crassispina* in this area. Additional research is needed to confirm the presence/absence of *P. nebulosa* here.

There is very little published data on the larvae of *P. crassispina* . The known sizes of larvae of this species at different developmental stages ( *TL* 10.8-28.4 mm - according to: An atlas ..., 1988) are close to our data - *TL* 12.0-31.0 mm. In the waters of Peter the Great Bay, larvae of this species are found from March to May-June.

It was previously assumed (Tokuya, Amaoka, 1980; Kimura et al., 1988) that the presence or absence of melanophores *CLM* near cleithral bones is a diagnostic feature for distinguishing between *P. crassispina* and *P. nebulosa* . Tokuya and Amaoka (Tokuya, Amaoka, 1980), when studying plankton samples collected near Hokkaido Island (Japan), divided the larvae of pricklebacks into two groups based on the presence or absence of *CLM* . Since the larvae did not differ in countable features, the authors suggested that the larvae could belong either to *P. crassispina* , or to *P. nebulosa* . Kimura et al. (Kimura et al., 1988), studying the larvae of *P. nebulosa* raised in aquarium conditions, found cleithral melanophores in them at all larval stages (at the yolk-sac stage, *CLM* was found in some specimens). They suggested that the group of fish with melanophores on the cleithrum was *P. nebulosa* , and without them – *P. crassispina* .

According to our data, larvae of *P. crassispina* from the waters of Peter the Great Bay have *CLM* at all larval stages (Fig. 3, 5; Table 1), furthermore, the presence of these melanophores in this species has been noted by other researchers (An atlas ..., 1988). Thus, *CLM* is not a diagnostic feature for distinguishing between these species.

Despite the similarity in the external appearance of larvae in Gymnocanthus species with scales on the head, when comparing descriptions of larvae *P. nebulosa* (Kimura et al., 1988; An atlas ..., 1988) with our own data on larvae *P. crassispina* , differences in pigmentation were found. However, it should be noted that we studied melanophores in fixed larvae, while Japanese ichthyologists probably observed coloration in live or unfixed material. Perhaps this is why we did not find pigmentation along the vertebral column and on the sides of the posterior part of the body in the studied larvae of *P. crassispina* (present in *P. nebulosa* - according to: Kimura et al., 1988). Larvae of *P. crassispina* from Peter the Great Bay lack colored melanophores in the auditory capsules and along the spine (present in *P. nebulosa* ). Based on data from the works of Japanese authors (Kimura et al., 1988; An atlas ..., 1988), it is unclear whether *P. nebulosa* has *ELR* melanophores and how they are developed (in *P. crassispina* they appear from the preflexion stage) - this cannot be understood from the provided descriptions and drawings.

When describing the larvae *P. crassispina* from the waters near the Japanese archipelago, the presence of melanophores *CLM* , *RG* , *ELR* , *VR* and *PVM* (An atlas ..., 1988) is noted for this species. The description of *RG* , *VR* and *PVM* almost completely coincides with our data. In larvae caught in waters near Japan, at early stages *CLM* is represented by a single melanophore, and as development progresses, it breaks up into two-three melanophores, but the stage at which this pigment appears is not specified. According to data for the waters of Peter the Great Bay, in larvae of *P. crassispina CLM* is observed already from the yolk-sac stage; initially it appears as a small dot, then as an elongated oval not completely covering the cleithrum (preflexion stage), and later

usually breaks up into two-five rounded melanophores (Table 1). In larvae from waters near Japan with body length > 20 mm, stellate melanophores *ELR* begin to appear, the row is highly variable. According to our data, the *ELR* row appears at the preflexion stage with *TL* 14.3 mm, melanophores may have a radial structure or be in the form of dots. Also, larvae of *P. crassispina* from Japanese waters are noted to have a low body height, which coincides with our data.

It should be noted that fish larvae from the family Pholidae have been studied rather fragmentarily (Rass, 1949; Fahay, 1983; Matarese et al., 1984, 1989; Watson, 1996). Works examining the complete developmental cycle of larvae of individual species are extremely rare (Russell, 1976; Kimura et al., 1988; An atlas ..., 1988). In these works, each author described pigmentation in their own way, and therefore it is sometimes difficult to understand exactly which melanophores are being considered in different articles (see above about melanophores *ELR* in *P. nebulosa* ).

In order to standardize the notation of melanophores, we proposed their original scheme using the example of *P. crassispina* (Fig. 2). For *P. crassispina* the composition and dynamics of melanophore appearance on the body and fins have also been clarified. It is shown that the melanophore row *ELR* appears at the preflexion stage at *TL* 14.3 mm, and melanophores can have a radial structure or be in the form of dots. It was found that in larvae of *P. crassispina* from approximately *TL* 18.4 mm, one melanophore *PVM* corresponds to one segmented ray *A* . From this length value, it is possible to determine the number of segmented rays *A* , by counting the number of melanophores *PVM* . Previously, Russell (1976) reported this for *P. gunnellus* .

It was previously shown (Kimura et al., 1988) that in *P. crassispina* determining the developmental stage of larvae may be difficult due to the weak notochord flexion (transition between preflexion and flexion) and the absence of precise criteria for the transition from flexion to postflexion. Analysis of the placement of rays and bones of the caudal fin, as well as their

staining with alizarin in a 1.5% potassium hydroxide solution, provided a set of features that more reliably characterize each stage of larval development. Before notochord flexion at the end of preflexion, up to 11 principal rays of *C* are stained with alizarin, the caudal fin is asymmetrical, its principal rays are directed downward and backward, and hypural bones are not stained. At the flexion stage, the notochord is bent, hypural bones are stained with alizarin, the caudal fin is asymmetrical, and its principal rays are directed downward and backward. At the postflexion stage, the tip of the notochord is bent backward, the caudal fin is symmetrical, and its rays are directed backward.

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## COMPLIANCE WITH ETHICAL STANDARDS

The Biomedical Ethics Commission of the NSCMB FEB RAS considers that this work does not contradict Directive 2010/63/EU of the European Parliament and of the Council of the European Union dated 22.09.2010 on the protection of animals used for scientific purposes (https://ruslasa.ru/wp-content/uploads/2017/06/Directive_201063_rus.pdf). Extract No. 1-170724 from meeting minutes No. 7 dated 17.07.2024.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE CAPTIONS

**Fig. 1.** Sampling locations *Pholis crassispina* in Peter the Great Bay, Sea of Japan: ( ● ) – Srednyaya Bay (juvenile and mature fish), ( ▲ ) – Zhitkova Bay (larvae).

**Fig. 2.** Designations and general scheme of melanophore arrangement in larvae of the Pholidae family using the example of *Pholis crassispina* , view: a – from above, b – from the side, c – from below. Here and in Fig. 5: *BM* – melanophores on the sides of the body, *CLM* – cleithral melanophores, *CM* – melanophores on the caudal fin, *CSM* – pericardial sac melanophores, *DMEG* – dorsal melanophores at the end of the intestinal tube, *ELR* – superficial melanophore row on the sides of the abdomen, *IM* – isthmus melanophores, *MA* – melanophores at the joint of dentale and anguloarticulare, *MBV* – melanophores under the pelvic fins, *MOC* – melanophores in the auditory capsule, *PVM* – postanal ventral melanophores, *PCM* – precaudal melanophores, *RG* – row on the dorsal surface of the intestinal tube, *VR* – ventral row.

**Fig. 3.** Larvae of *Pholis crassispina* at different developmental stages: a – yolk-sac ( *TL* 12.7 mm), b – preflexion ( *TL* 16.6 mm), c – flexion ( *TL* 23.1 mm), d – postflexion ( *TL* 30.2 mm).

**Fig. 4.** The arrangement of melanophores at the base of caudal fin rays in larvae of *Pholis crassispina* at different developmental stages: a – yolk-sac ( *TL* 12.7 mm), b – preflexion ( *TL* 16.0 mm), c – flexion ( *TL* 24.4 mm), d – postflexion ( *TL* 30.2 mm). *X* – notochord, other designations see in Fig. 2.

**Fig. 5.** Trunk section of larva *Pholis crassispina* ( *TL* 30.2 mm) before (a) and after (b) staining with alizarin.

**Fig. 6.** Alizarin-stained caudal fins of *Pholis crassispina* at different stages of larval development: a – preflexion ( *TL* 18.4 mm), b – flexion ( *TL* 23.7 mm), c – postflexion ( *TL* 30.2 mm). a.n. and a.hae. – neural and hemal processes of vertebrae, in – interneural, ih –

interhaemalia, hy.ha. and hy.ea. – hypaxial and epaxial hypurals, e – epurale, vert.c. – caudal vertebrae, CP – urostyle, PR – principal rays, UR and LR – upper and lower marginal rays.