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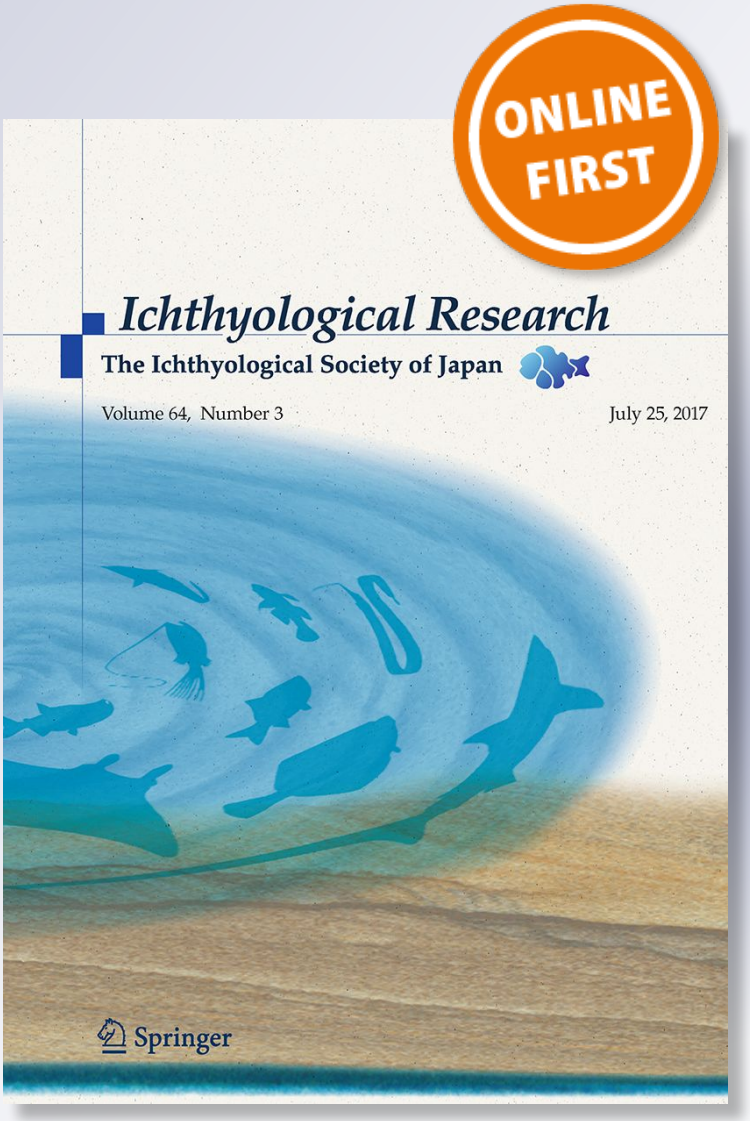

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SHORT REPORT

Preliminary observations of the skeletal development in pre-flexion larvae of sablefish *Anoplopoma fimbria*

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Abstract
Sablefish *Anoplopoma fimbria* support a lucrative fishery in the Gulf of Alaska, but their numbers have been declining despite a regulated fishery. Recruitment in *A. fimbria* is poorly understood due to its unusual early life history relative to many other deep-water fishes. Developmental patterns can identify critical periods during ontogeny that influence foraging and swimming abilities among individual larvae. External development in *A. fimbria* has been described, but the data presented here are the first examination of the skeletal development of *A. fimbria* during the transition to first feeding.

Keywords Black cod · First-feeding larvae · Neuston · Recruitment bottlenecks

Introduction

Sablefish *Anoplopoma fimbria* has an extensive range in the eastern Pacific Ocean, extending from northern Mexico to the Bering Sea, with the greatest abundance centered in the Gulf of Alaska (McFarlane and Nagata 1988; Shotwell et al. 2014). *Anoplopoma fimbria* is an extremely valuable commercial species in the United States, with an annual ex-vessel value of over $92 million US and is considered the highest valued finfish exploited in Alaskan waters (Hanselman et al. 2017; Shotwell et al. 2018). However, population size in the Gulf of Alaska has been in decline since the 1980s, commercial harvests have decreased, and female spawning stock biomass is projected to decline through 2019. Despite these trends, production of young *A. fimbria* improved in 2016 and 2017, though the processes underlying successful recruitment remain unresolved.

Recruitment bottlenecks for *A. fimbria* are hypothesized to occur early in the first year of life (Wing 1997; McFarlane and Beamish 1992; Sigler et al. 2001; Shotwell et al. 2014) when larvae and juveniles are susceptible to oceanographic and biological variability that mediate growth and survival rates. Young of *A. fimbria* may be uniquely vulnerable to environmental variability relative to other species due to their distinct early life history. Larvae undergo an extreme ontogenetic vertical migration from hatching at depth (>300 m) in cold water (<6 °C) in the early spring to a near-surface (neustonic) existence (<3 m) by late spring (Mason et al. 1983). The rapid ascent of sensitive, newly hatched larvae through the water column exposes offspring to significant thermal shifts, which may be exacerbated by recent climate warming and higher sea surface temperatures (12–15 °C). The neustonic phase is prolonged (>6 months; Mason et al. 1983; Wing 1997; Kendall and Matarese 1987) so individuals are exposed to variability in temperature, salinity, flow regime, wind stress, prey field, as well as near-surface predators including seabirds and pelagic fishes over an extended period (Sigler et al. 2001; Shotwell et al. 2014; Coffin and Mueter 2016; Siddon et al. 2016). This extended vulnerability increases the time over which small losses from the population can accumulate, and small fluctuations in mortality can influence recruitment strength over several orders of magnitude (Peck et al. 2012).

It is clear that *A. fimbria* have an unusual early life history that influences their growth, survival, and recruitment. Information about skeletal development and developmental state can be used to identify critical periods during ontogeny that influence foraging and swimming abilities among individual larvae. However, the early osteology of this species has not been described despite the commercial importance of *A. fimbria*. The developmental state of the skeletal system constrains many behaviors that can be used by early stage fishes to reduce mortality, either

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directly through predator avoidance and prey capture or indirectly through starvation (Østergaard et al. 2005; Anto and Turingan 2010). Our objective in this study is to present the first examination of the skeletal development of *A. fimbria* using laboratory-reared specimens. By presenting these data, we will be poised to examine how environmental variability may impact survival in early stage *A. fimbria* by assessing deviations in the onset and timing of osteological development.

Materials and methods

Specimens were obtained from a laboratory study conducted during the winter of 2017 to assess the feasibility of a small-scale rearing operation and to modify the rearing protocols developed by Cook et al. (2015) for large-scale aquaculture operations. Eggs were obtained from the NOAA Northwest Fisheries Science Center Laboratory in Manchester, Washington. Laboratory conditions were used that simulate "natural" conditions closely. Specimens were reared at 5.6 °C in complete darkness from egg fertilization to hatching, and light level was gradually increased between hatching and first feeding. After first feeding, temperatures and light levels were increased to 6.8 °C and 10 µmol photo m^{-2} s^{-1}, respectively. The rearing operation was continued to 66 days post-hatch (dph); at that time, we ceased rearing operations due to few larvae remaining. All procedures performed in our study involving animals were in compliance with the ethical standards of the institution where the studies were conducted. For the examination of skeletal anatomy, three to five individuals at hatching to 59 dph were removed each day and fixed in buffered formalin. After two weeks, fixed specimens were transferred to 70% ethanol for an additional two weeks.

To examine the internal anatomy, larvae were cleared and double-stained ($n = 315$) according to the protocols of Taylor and Van Dyke (1985). A subset of specimens ($n = 17$) were stained with a modified protocol of Taylor and Van Dyke (1985), skipping the acidic cartilage-staining step to reduce the potential of de-calcification and to identify the earliest onset of calcification using only the bone stain. Larvae were examined, imaged, and measured using a dissecting microscope equipped with a high-resolution digital camera and image analysis software. Skeletal elements were identified in the specimens using the following references: Cubbage and Mabee (1996), Hilton (2002), and Kubicek and Conway (2016). All figures were prepared using Adobe Illustrator Creative Cloud 2018.

All specimens examined for this project are archived at the University of Washington Fish Collection, Burke Museum of Natural History and Culture: UW188699-UW188778.

Results

External Development At 5.6 °C, eggs hatched approximately 14 days after fertilization (mean, 3.26 mm notochord length (NL); range, 3.00−3.52 mm NL). Larvae were unpigmented with a large yolk and no mouth at hatching. A hatch mark was present on the otoliths, which had not been previously confirmed in *Anoplopoma fimbria*. Early eye pigmentation was noted at eight dph (mean, 6.69 mm NL; range, 6.57−7.06 mm NL) and was fully pigmented at 16 dph (mean, 7.59 mm NL; range, 7.42−7.68 mm NL). The anus opened at 12 dph, but the mouth was not apparent until 22 dph and likely not functional until first feeding at 27 dph (mean, 7.18 mm NL; range, 6.97−7.62 mm NL). Larvae became surface oriented at 30 dph (mean, 8.07 mm NL; range, 7.37−8.74 mm NL) and completely reliant on exogenous feeding after the yolk was exhausted at 38 dph (mean, 8.92 mm NL; range, 8.39−9.50 mm NL).

Internal Development Internally, minimal ossification of the skeleton of *A. fimbria* occurred at the examined stages. At the temperatures used during the 2017 study, the first dermal elements to appear were the cleithrum at 19 dph (mean, 6.71 mm NL; range, 6.21−7.25 mm NL) and the maxilla at 28 dph (mean, 7.14 mm NL; range, 5.96−8.14 mm NL), which were weakly ossified as indicated by the lack of strong alizarin staining.

Similar to the external morphology, at hatching, no internal structures were discernible, except for the otoliths. By 3 dph (mean, 3.82 mm NL; range, 3.24−4.57 mm NL), the notochord was apparent, but no cartilaginous structures were present yet. One of the first cartilaginous elements to form was Meckel's cartilage at 15 dph (mean, 6.47 mm NL; range, 6.27−6.89 mm NL) (Fig. 1a), although the mouth was not apparent externally until 22 dph (mean, 7.12 mm NL; range, 6.39−7.86 mm NL). Much of the development between 26 dph and 45 dph (range, 7.36−8.55 mm NL) was isolated to the structures of the head that promote feeding or respiration.

Oral jaws Meckel's cartilage elongated throughout the early development of the oral jaws. At 21 dph (mean, 7.17 mm NL; range, 6.43−7.85 mm NL), the palatoquadrate formed, followed by the hyosymplectic cartilage at 22 dph (mean, 7.12 mm NL; range, 6.39−7.86 mm NL) (Fig. 1b, c). A very thin, rod-like maxilla was present in individuals 28 dph (mean, 7.14 mm NL; range, 5.96−8.14 mm NL). The hyomandibular foramen in the dorsal part of the hyosymplectic cartilage was present at 44 dph (mean, 8.59 mm NL; range, 7.99−8.94 mm NL). At 59 dph (mean, 9.44 mm NL; range, 9.31−9.57 mm NL), the articular region of Meckel's cartilage was present at the posterior tip of the lower jaw.

Visceral Arches The first elements of the visceral arches that developed were the ceratohyals, which were

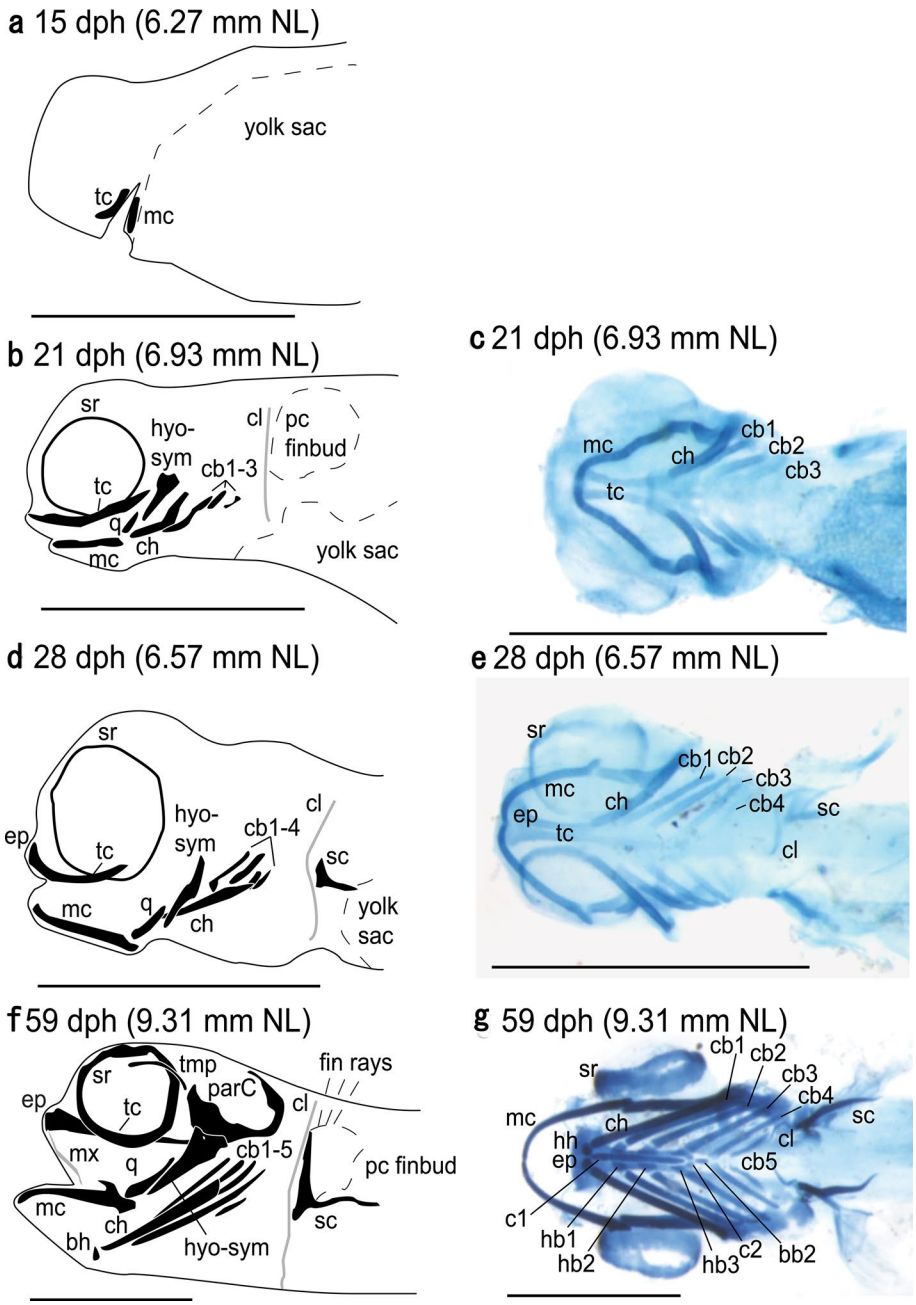

Fig. 1 Development of cephalic structures in *Anoplopoma fimbria* at 15 days post-hatch (dph; **a**), 21 dph (**b**, **c**), 28 dph (**d, e**), and 59 dph (**f**, **g**). Lateral views of the internal anatomy are shown in panels **a**, **b**, **d**, and **f**, and ventral views are depicted in **c**, **e**, and **g**. In the line drawings, cartilaginous structures are denoted by *the black fill*. *All scale bars* represent 1 mm. *bh* basihyal; *c1* anterior copula; *c2* posterior copula; *ch* ceratohyal; *cl* cleithrum; *ep* epibranchial; *hb* hypobranchial; *hh* hypohyal; *hyo-sym* hyosymplectic; *mc* Meckel's cartilage; *NL* notochord length; *parc* parachordal cartilage; *q* palatoquadrate; *sc* coraco-scapular cartilage; *sr* sclerotic ring; *tc* taenia communis; *tmp* taenia marginalis posterior

cartilaginous at 17 dph (mean, 6.95 mm NL; range, 6.7−7.34 mm NL), followed by ceratobranchials 1 and 2 at 19 dph (mean, 6.71 mm NL; range, 6.21−7.25 mm NL). At 21 dph (mean, 7.17 mm NL; range, 6.43−7.85 mm NL), ceratobranchial 3 formed. The 4th ceratobranchial cartilage and the anterior copula were present at 26 dph (mean, 7.66 mm NL; range, 7.36−8.06 mm NL). Development along the gill arches continued to 29 dph (mean, 7.33 mm NL; range, 6.17−8.01 mm NL) with the appearance of hypobranchials 1–3 (Fig. 1d, e). After 29 dph, very few developmental events were observed in the gill arches of pre-flexion *A. fimbria* until 43 dph. At 43 dph (mean, 8.63 mm NL; range, 8.39−8.98 mm NL), ceratobranchial 5 was present. The first elements of the dorsal gill arches were the cartilages of pharyngobranchials 2 and 3 with epibranchials 1–3, forming between 56 and 59 dph (mean, 9.49 mm NL; range, 8.57−9.57 mm NL).

Neurocranium The first cartilage elements of the neurocranium (ethmoid plate and trabecula communis) were present at 15 dph (mean, 6.47 mm NL; range, 6.27−6.89 mm NL). The otic capsule is apparent by 21 dph (mean, 7.17 mm NL; range, 6.43−7.85 mm NL), but remains relatively unchanged throughout pre-flexion and flexion. By 59 dph (mean, 9.57 mm NL; range, 9.31−9.57 mm NL), the taenia marginalis

posterior and parachordal cartilage are present (Fig. 1f). Overall, very little development of the neurocranium was observed in the examined stages.

Pectoral Fins The pectoral fin bud was supported by a single skeletal element, the cleithrum, at 19 dph (mean, 6.71 mm NL; range, 6.21−7.25 mm NL). At 23 dph (mean, 6.83 mm NL; range, 4.63−7.94 mm NL), the pectoral fin bud was supported by a second element, the scapulocoracoid cartilage (Fig. 1d, e).

Body We defined this region as posterior to the pectoral fin buds extending to anterior of the caudal region. In the size ranges examined, we observed no ossification along the notochord or the pelvic fins and the absence of basidorsals. At 56 dph (mean, 9.40 mm NL; range, 8.57−9.87 mm NL), the basiventrals began forming anterior to the hypural plates and progressed from posterior to anterior.

Caudal Fin Development in the caudal region was first observed at 45 dph (mean, 8.49 mm NL; range, 8.39−8.55 mm NL) with the presence of hypurals 1–2 (Fig. 2). Hypural 3 and the parhypural are present at 51 dph (mean, 9.51 mm NL; range, 8.62-10.09 mm NL). The appearance of the basiventrals accompanied flexion in three individuals (mean, 9.72 mm NL; range, 9.31−10.09 mm NL).

Discussion

The development of external features generally agreed with what has been described in the literature (Kendall and Matarese 1987; McFarlane and Nagata 1988; Alderdice et al. 1988; McFarlane and Beamish 1992; Jensen and Damon 2002). The maxilla is present in the upper jaw but remains rudimentary and not strongly ossified throughout the sizes examined, suggesting that only elements of the lower jaw are involved in feeding by the end of the study (59 dph; mean, 9.57 mm NL; range, 9.31−9.57 mm NL). Although the maxilla may provide support during the feeding process, the lack of ossification and a premaxilla would reduce the probability of upper jaw protrusion during feeding (Anto et al. 2009) at this stage. Feeding is likely accomplished by a combination of suction and ram feeding where pre-flexion *Anoplopoma fimbria* overtake prey in the water column while using some suction pressure to overcome the challenges of foraging in a viscous environment (Hunt von Herbing et al. 1996; Osse and van der Boogaart 1999). However, suction feeding is not the sole feeding mode at this stage due to the lack of ossified structures in the head, which reduce the efficiency of force transmission needed to support suction feeding (Anto and Turingan 2010).

Anoplopoma fimbria migrate toward the surface near the end of the yolk-sac stage (Kendall and Matarese 1987), which in our experimental setup occurred between 30 and 38 dph (~7.30−8.60 mm NL), and then develop quickly once in the neuston (Doyle 1992). From the examination of the internal anatomy, the development of structures was saltatory (Balon 1981), with a pulse of development taking

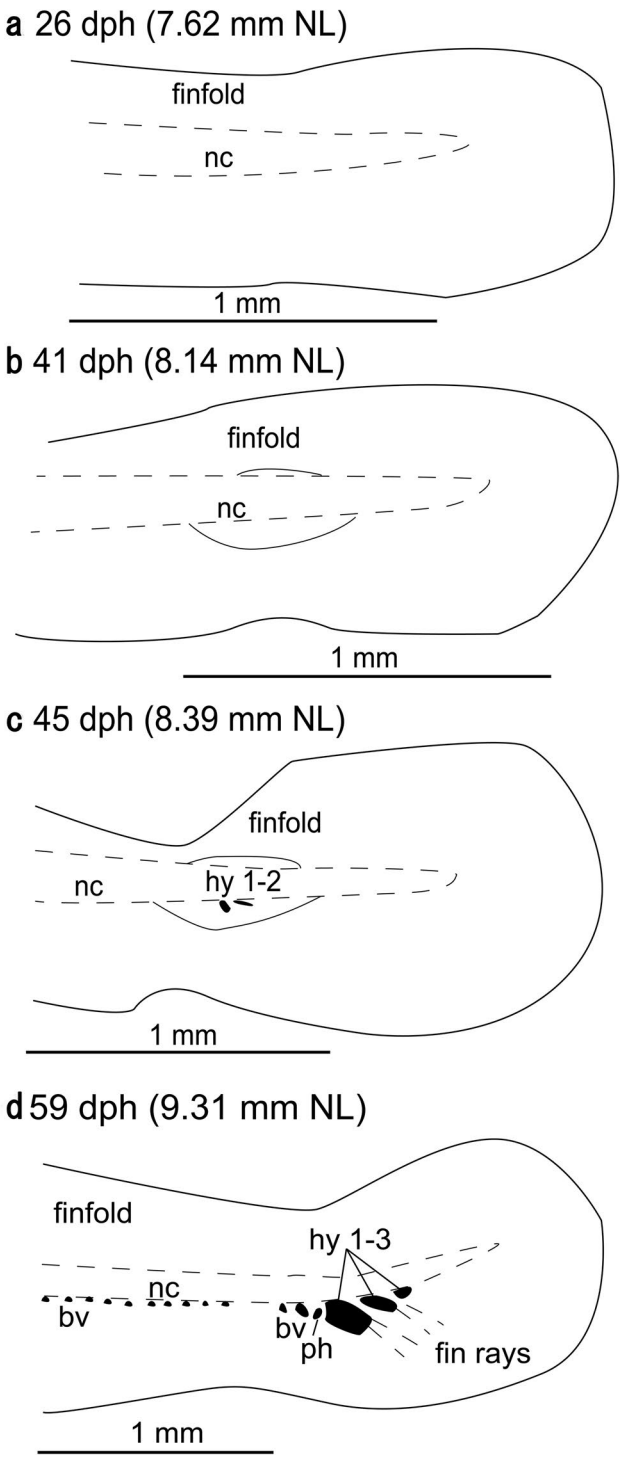

Fig. 2 Ontogenetic changes in the caudal fin of early stage *Anoplopoma fimbria* 26 days post-hatch (dph; **a**), 41 dph (**b**), 45 dph (**c**), and 59 dph (**d**). *bv* basiventral; *hy* hypural; *nc* notochord; *NL* notochord length; *ph* parhypural

place, followed by many days of no observable developmental changes. For example, many elements of the gill arches formed between 17 and 29 dph (range, 6.75−8.01 mm NL), but after 29 dph (mean, 7.33 mm NL; range, 6.17−8.01 mm NL), no new elements of the gill arches developed until ceratobranchial 5 at 43 dph (mean, 8.63 mm NL; range, 8.39−8.98 mm NL). This may be a consequence of yolk-sac utilization, in which individuals are allocating resources to support growth rather than development before the yolk sac is exhausted at 38 dph.

The delayed and weak ossification of skeletal elements was unexpected in this species, especially considering its unique life history and rapid growth rates once in its neustonic phase. One caveat of this finding is that the acetic acid step in the clearing and staining process can de-calcify calcified structures (Walker and Kimmel 2006), even if the time in this step is minimized. The three specimens that possessed a well-stained cleithrum were cleared and stained with a modified protocol that did not use acetic acid. It is likely that the cleithrum and maxilla are weakly calcified in pre-flexion specimens (~49 dph; mean, 8.77 mm NL; range, 8.70−8.84 mm NL), and the addition of acetic acid during the clearing and staining protocol was enough to de-calcify this structure. A re-examination of the specimens supports this hypothesis because the cleithrum and maxilla were present but unstained in specimens after 19 dph and 28 dph, respectively. Other than the cleithrum and maxilla, no other dermal structures were present or ossified in the specimens examined using the modified protocol (Walker and Kimmel 2006), which suggests that even with the de-calcification that we experienced using the standard clearing and staining process, we are capturing the onset of ossification for early stage *A. fimbria*.

For future studies on the development of *A. fimbria*, it will be important to use the modified protocol proposed by Walker and Kimmel (2006) to reduce the risk of de-calcification. Another caveat is that specimens were fixed in buffered formalin, which can also de-calcify structures if specimens are stored in this solution for too long or it is not buffered correctly (Schnell et al. 2016). We selected this preservation method because our ultimate goal is to compare the developmental state of our laboratory specimens to historical or recently collected wild specimens. Therefore, we wanted to reduce any potential variability in the staining of structures due to the preservation method.

The first examination of the internal anatomy of *A. fimbria* revealed some potential bottlenecks related to the developmental state of feeding and swimming structures. For instance, first feeding was observed at 27 dph (~7.30 mm NL) after several structures developed that contribute to the hyoid–mandible musculoskeletal linkage (e.g., Meckel's cartilage, palatoquadrate, hyosymplectic, cleithrum) (Anto and Turingan 2010). In *A. fimbria*, exogenous feeding is likely initiated when these elements develop because they establish the necessary muscle attachments for the hyoid–mandible musculoskeletal linkage that opens and closes the lower jaw during foraging. Therefore, if these structures have not developed, larvae will not be able to adequately feed exogenously and will be more susceptible to predation, starvation, and inter- and intraspecific competition for food in the neuston. Given the commercial importance of *A. fimbria* in the North Pacific, a description of early development provides the foundation needed to identify potential bottlenecks in early development when recruitment is likely set. Early developmental studies also provide the baseline data needed to understand how physical and biological parameters, such as temperature, prey quality, and prey quantity, modify vital rates that can influence skeletal development.

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