Updated Morphological Descriptions of the Larval Stage of Urophycis (Family: Phycidae) from the Northeast United States Continental Shelf

Authors: Marancik, Katrin E., Richardson, David E., and Konieczna, Małgorzata

Source: Copeia, 108(1) : 83-90

Published By: The American Society of Ichthyologists and Herpetologists

URL: https://doi.org/10.1643/CG-19-219

Copeia

Copeia 108, No. 1, 2020, 83–90

Updated Morphological Descriptions of the Larval Stage of *Urophycis* (Family: Phycidae) from the Northeast United States Continental Shelf

Katrin E. Marancik[1], David E. Richardson[2], and Małgorzata Konieczna[3]

Including early life history data in assessments can improve fisheries management by increasing our knowledge of stock structure, spawning habitat, and population trends. The identification of fish larvae to species is a necessary step in using early life history data toward this goal. Three species of hakes from the genus *Urophycis* are common on the northeast United States continental shelf: *U. chuss* or Red Hake, *U. regia* or Spotted Hake, and *U. tenuis* or White Hake. Unfortunately, identification of larval *Urophycis* has long been only possible at the genus level. Larvae of *Urophycis* ($n = 277$) collected in a subset of ethanol-preserved samples were identified genetically through sequencing of the cytochrome oxidase I gene and were used to update morphological descriptions with characters that separate these three species at the larval stage. Sequencing occurred in two stages: the first ($n = 88$) to develop a set of known-identity larvae to define species-specific traits, the second ($n = 189$) to test morphological identification based on the traits described in this study. We describe a combination of the location of dorsal and ventral pigment, head pigment, lower jaw pigment, and the timing of development of the pectoral fins to distinguish the larvae of these three species at sizes <6 mm. Using molecular techniques to improve morphological identifications is a powerful and efficient way to obtain the species-level data needed for assessments and management.

DIFFICULTY identifying larval-stage hakes of the genus *Urophycis* (*U. chuss*, *U. regia*, and *U. tenuis*) to species has limited the use of over 40 years of fisheries-independent data collected on these taxa on the northeast United States continental shelf. Larvae of *Urophycis* are among the most abundant ichthyoplankton taxa collected in this region (Walsh et al., 2015). The frequency and long duration of plankton monitoring on the northeast U.S. shelf means these data could provide important insights into the population structure of these species and an independent estimate of trends in spawning stock biomass (Richardson et al., 2009).

Larvae of *Urophycis* are morphologically similar, and previous descriptions did not produce reliable identifying characteristics at the species level for small larvae. Individual species have been described in detail based on reared larvae from known adults (*U. chuss*: Miller and Marak, 1959; *U. regia*: Barans and Barans, 1972 and Serebryakov, 1978), but comparisons among the common species relied heavily on characters that are unavailable at the small sizes common in plankton tows (<6 mm) or characters that could be misleading (i.e., the presence of pelvic filament pigment is unreliable; Methven, 1985; Comyns and Grant, 1993; Comyns and Bond, 2002; Fahay, 2007).

Genetic barcoding provides a means to identify larvae at the species level (e.g., Marancik et al., 2010). Although quite effective, genetic identification of entire surveys would be costly. However, genetic barcoding of a subset of specimens is a relatively inexpensive way to improve and test the accuracy of identifications made through morphological analysis.

The objectives of this study are to use DNA barcoding to create a set of larvae of *Urophycis* of known identity, to examine those larvae for species-specific traits, and then to test the accuracy of identifications made with those traits.

MATERIALS AND METHODS

Since the late 1970s, fish larvae have been collected through the Marine Resources Monitoring, Assessment, and Prediction (MARMAP) and Ecosystem Monitoring (EcoMon) programs of the National Oceanic and Atmospheric Administration's (NOAA) Northeast Fisheries Science Center, which survey plankton, hydrography, and water chemistry on the entire continental shelf from Cape Hatteras, North Carolina, to Cape Sable, Nova Scotia. The shelf is divided into strata based on bathymetry and latitude and sampled using a random stratified sampling design. For each survey, stations are randomly selected within strata with up to six surveys per year: January/February, March–April, May/June, August, September–October, and November. Samples are collected with oblique tows of a 61 cm diameter bongo net with 505 μm (MARMAP) or 333 μm (EcoMon) mesh, which is towed to within 5 m of the bottom or to a maximum depth of 200 m (Walsh et al., 2015). All standard samples are preserved in 5% buffered formalin and are sorted and initially identified at the Plankton Sorting and Identification Center (ZSIOP) in Szczecin, Poland. Since 2013, additional samples have been collected at each station during EcoMon surveys with a 20 cm diameter bongo with 333 μm mesh nets attached above the 61 cm bongo frame. These additional samples were preserved in 95% ethanol, sorted and identified in house, and used for genetic identification of eggs (Lewis et al., 2016) and larvae of species of *Urophycis*.

[1] Integrated Statistics, 28 Tarzwell Drive, Narragansett, Rhode Island 02882; Email: Katey.Marancik@noaa.gov. Send reprint requests to this address.
[2] NOAA Fisheries Northeast Fisheries Science Center, 28 Tarzwell Drive, Narragansett, Rhode Island 02882; Email: David.Richardson@noaa.gov.
[3] Morski Instytut Rybacki, Plankton Sorting and Identification Center (ZSIOP), Szczecin, Poland.
Submitted: 2 April 2019. Accepted: 4 December 2019. Associate Editor: M. P. Davis.
 DOI: 10.1643/CG-19-219 Published online: 20 February 2020

In addition to the EcoMon samples, a cruise in May 1993 on the NOAA ship *Albatross IV* sampled off the U.S. mid-Atlantic shelf edge, the suspected spawning ground for *Urophycis tenuis* (Fahay and Able, 1989; Lang et al., 1996). Larvae collected in these off-shelf samples had morphological differences from the shelf larvae collected in August–November, so they are suspected to be *U. tenuis*. Unfortunately, these samples were preserved in 5% buffered formalin and were not available for genetic barcoding. Therefore, these specimens were insufficient for unequivocal identification.

For genetic barcoding, eyeballs from ethanol-preserved larvae were removed in the lab, placed in a 96-well plate, and shipped to the University of Guelph Canadian Centre for DNA Barcoding. Genetic identification procedures were based on the Barcode of Life protocols amplifying a 650 bp section and a 184 bp section of the cytochrome oxidase I gene (Ivanova et al., 2012; Lewis et al., 2016).

Larvae were barcoded in two groups. The first group (n = 88) were morphologically identified to genus. After barcoding, these larvae of known identity were used to define species-specific traits for morphological identification. The second group of larvae (n = 189) were morphologically identified to species, and then these identifications were tested with barcoding to confirm validity of the traits described for separating species. Sequence data, electropherograms, and primer details for these specimens are available on the Barcode of Life Database under the completed *Urophycis* Larval ID project (UROPH). Sequence data were also submitted to GenBank (accession numbers: MK779481–MK779594). The genetically identified larvae are stored in the Plankton Archives at the National Marine Fisheries Service Narragansett Lab in Narragansett, Rhode Island.

The morphological traits described here were examined further during the re-identification of approximately 25,000 formalin-preserved larvae from the standard EcoMon sampling to examine additional variability across a much larger sample size.

RESULTS

A total of 277 *Urophycis* were used to define morphological traits for species-level identification (Table 1). Polymerase chain reaction (PCR) of the 650 bp section of the cytochrome oxidase subunit I (COI) gene resulted in a low rate of amplification, possibly due to a poor match of primers. The second round of PCR and sequencing using primers for the 184 bp section, however, produced consistent results. Of these 277 larvae, 13 (4.7%) did not sequence and 15 (5.4%) produced low quality sequences and were excluded from further analysis. This left 249 genetically identified larvae for trait examination (success rate of 90%).

Only larvae of *Urophycis chuss* and *U. regia* were identified in ethanol samples collected on the shelf during these rounds of genetic and morphological identification. Plankton sampling rarely occurs offshore of the shelf edge, and the only samples available from these deep waters were preserved in formalin and not compatible with genetic barcoding.

The traits described here were sufficient to identify larvae between 1.5–6 mm (approximately 95% of the EcoMon collections of *Urophycis*) based on examination of ethanol- and formalin-preserved larvae. While pigment was present in other regions (e.g., nape, gut), the individual-level variability was sufficient to preclude this from being useful for species-level identifications.

Table 1. Confusion matrix comparing morphological identifications to molecular results for the two rounds of barcoding. Failed refers to No sequence or Low-Quality sequences amplified during multiple rounds of barcoding. Round 1 included 88 larvae morphologically identified to genus and used to define species-level traits. Round 2 included 189 larvae morphologically identified to species and used to test identifications based on the traits described in this study.

Morphological results	Molecular results				
	U. chuss	*U. regia*	*U. tenuis*	Failed	Total
Round 1					
Urophycis spp.	80	6	0	2	88
Round 2					
U. chuss	108	1	0	17	126
U. regia	1	43	0	5	49
U. tenuis	0	0	0	0	0
Urophycis spp.	7	3	0	4	14
Total	196	53	0	28	277

***Separating* Urophycis chuss, U. regia, *and* U. tenuis *from other phycids.*—**There is one closely related lotid (*Enchelyopus cimbrius*) and seven species of Phycidae in the northwest Atlantic: *Phycis chesteri, Urophycis chuss, U. cirrata, U. earlli, U. floridana, U. regia,* and *U. tenuis*. *Enchelyopus cimbrius* is easily distinguished from larvae of *Urophycis* based on a melanophore on the ventral notochord tip and four pelvic rays versus three on larvae of *Urophycis* (Markle, 1982). *Phycis chesteri*, *U. cirrata*, *U. earlii*, and *U. floridana* rarely occur on the northeast U.S. shelf, spawn in late fall or early spring, and are not included in the following descriptions, which are based on larvae collected from May to November (Comyns and Bond, 2002; Fahay, 2007).

Urophycis chuss

Figures 1, 2A, 3A

***Genetic confirmation.*—**n = 196; 1.4–10.7 mm SL.

***Formalin-fixed larvae examined.*—**n = 18,685; 1.1–6.5 mm SL.

***Identification.*—**The earliest stages of development in *U. chuss* were described by Miller and Marak (1959) from reared eggs and larvae collected from strip-spawned adults. These recently hatched specimens have melanophores on the mid- and forebrain and multiple dorsal and ventral melanophores on the tail. Specimens at this stage were not included in our genetic analyses and are not described here, but were observed at sizes 1–1.5 mm notochord length (NL) in our preserved samples. Based on Miller and Marak's (1959) work, an abrupt change in pigmentation occurs after 24 hours post hatch.

The traits described here were observed on larvae as small as 1.1 mm NL and were common by 1.5 mm NL. The lower jaw is lightly pigmented with two small melanophores, one on either side of the jaw, usually about midway down the jaw rami. The forebrain is unpigmented until external melano-

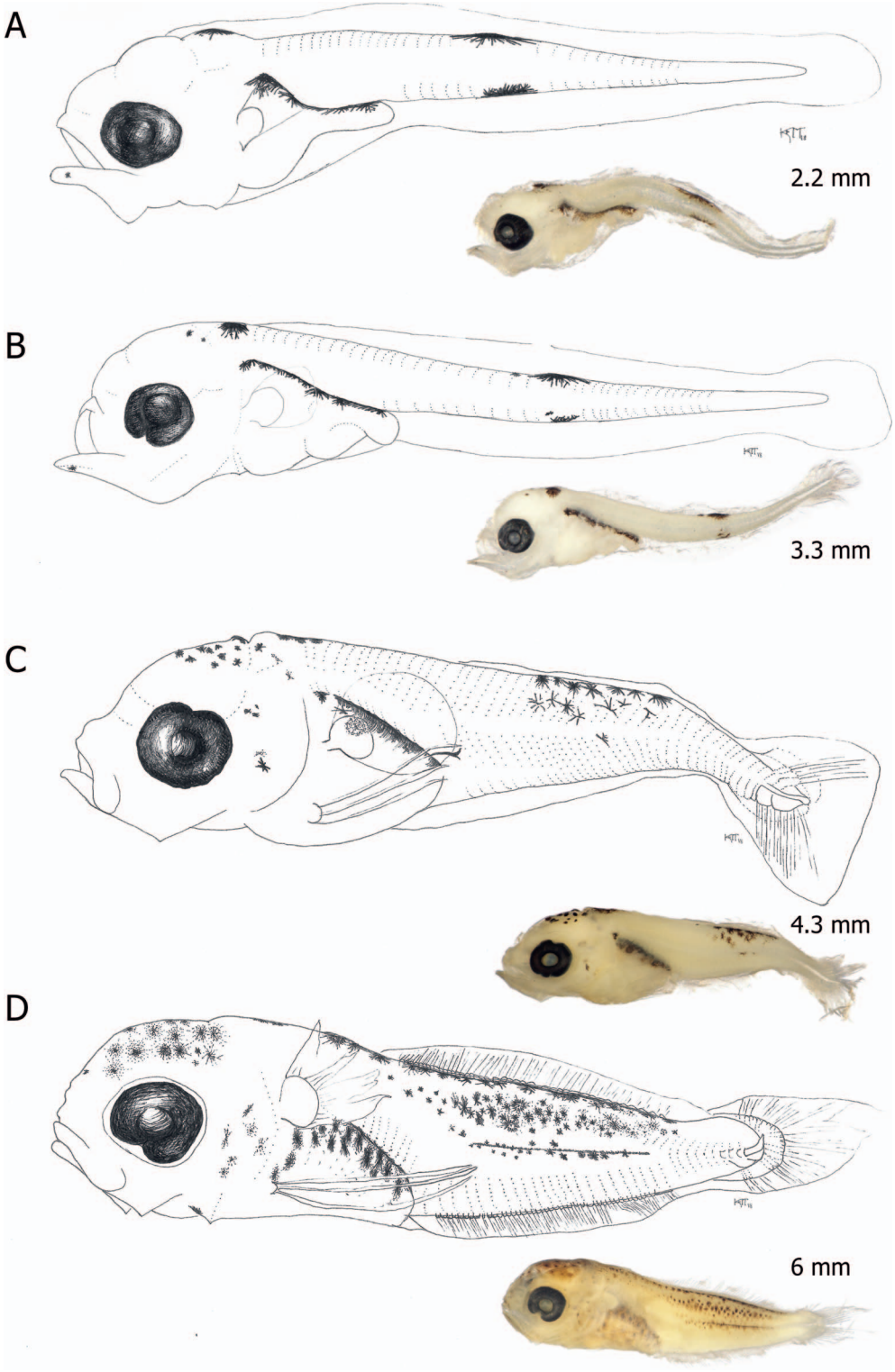

Fig. 1. Line illustrations and images of *Urophycis chuss* at (A) 2.2 mm, (B) 3.3 mm, (C) 4.3 mm, and (D) 6 mm.

phores develop late in the postflexion stage. Pelvic fins begin to form at approximately 3–3.5 mm, later than on *U. tenuis* (2–2.5 mm), and are pigmented on the tissue between the fin rays, especially on the outer one third by 4 mm. Pelvic filaments are often damaged on net-collected larvae, so this pigment is often absent. Dorsal tail pigment is approximately mid-tail in small larvae and remains postanal until postflexion (6–7 mm). Ventral tail pigment is present about mid-tail on the smallest larvae. Ventral pigment is absent by 3.5 mm and reappears by 10 mm SL. The caudal area is unpigmented until late in the postflexion stage (7–10 mm).

Urophycis regia
Figures 2B, 3B, 4

Genetic confirmation.—$n = 53$; 1.7–26.5 mm SL.

Formalin-fixed larvae examined.—$n = 3{,}252$; 1.1–7 mm SL.

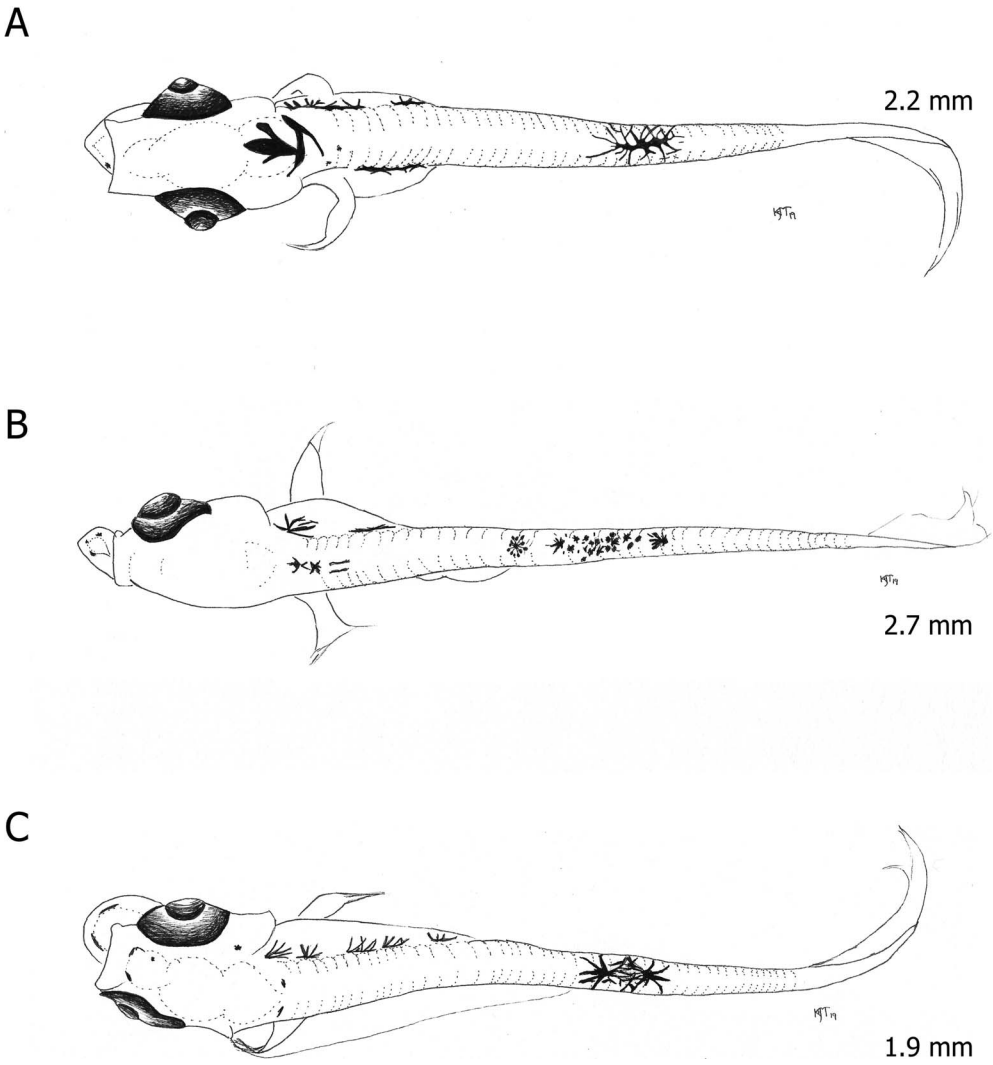

Fig. 2. Line illustrations of dorsal view showing location of head and dorsal midline pigment of (A) 2.2 mm *Urophycis chuss*, (B) 2.7 mm *Urophycis regia*, and (C) 1.9 mm *Urophycis tenuis*.

Identification.—Very recently hatched *U. regia* were illustrated by Barans and Barans (1972) and Serebryakov (1978) from lab-reared eggs and larvae collected from strip-spawned adults. These smallest and least-developed larvae appear very similar to the recently hatched *U. chuss,* with possible differences in head pigment. Specimens at this stage were not included in our analyses and are not described here, but this stage included larvae 1–1.4 mm NL in our preserved samples.

The traits described in this study were visible in larvae as small as 1.1 mm NL and common on larvae by 1.5 mm NL. The lower jaw is more heavily pigmented than *U. chuss* with 2–4 melanophores that are often elongate and present on either side of the jaw. Occasionally, forebrain pigment was present externally in smaller larvae, but was generally absent until later in the postflexion stage. The pelvic fins develop at approximately 3–3.5 mm, later than *U. tenuis* (2–2.5 mm), but may also be pigmented. Pelvic filaments are often damaged on net-collected larvae and this pigment is rarely available, so neither the presence nor absence of pigment is diagnostic. Dorsal tail pigment occurs anterior to the area over the anus (approximately above the mid-gut) and usually stretches to mid-tail. Ventral pigment is present throughout the larval stage and is often located more closely to the anus than *U. chuss* and *U. tenuis*. Pigment is absent on the caudal area until late postflexion stage (7–10 mm).

Urophycis tenuis (?)

Figures 2C, 3C, 5

Genetic confirmation.—$n = 0$.

Formalin-fixed larvae examined.—$n = 47$; 1.9–5.8 mm SL.

Identification.—The most recently hatched larvae of *U. tenuis* are undescribed and were not included in our analyses. The traits described here are based on specimens collected when and where *U. tenuis* are believed to spawn (Fahay and Able, 1989; Lang et al., 1996) and that were morphologically different from the genetically confirmed *U. chuss* and *U. regia*. The lower jaw pigment forms a line on either side of the jaw near the symphysis (jaw tip). These melanophores were larger and closer together than on *U. chuss* and *U. regia*. Internal pigment on the forebrain, between the eyes, was most visible on the dorsal view. This trait was only observed in the preflexion stage of suspected *U. tenuis*. At later stages of larval development in *U. tenuis*, the forebrain has surface pigment, which is also uncommon on the other two species,

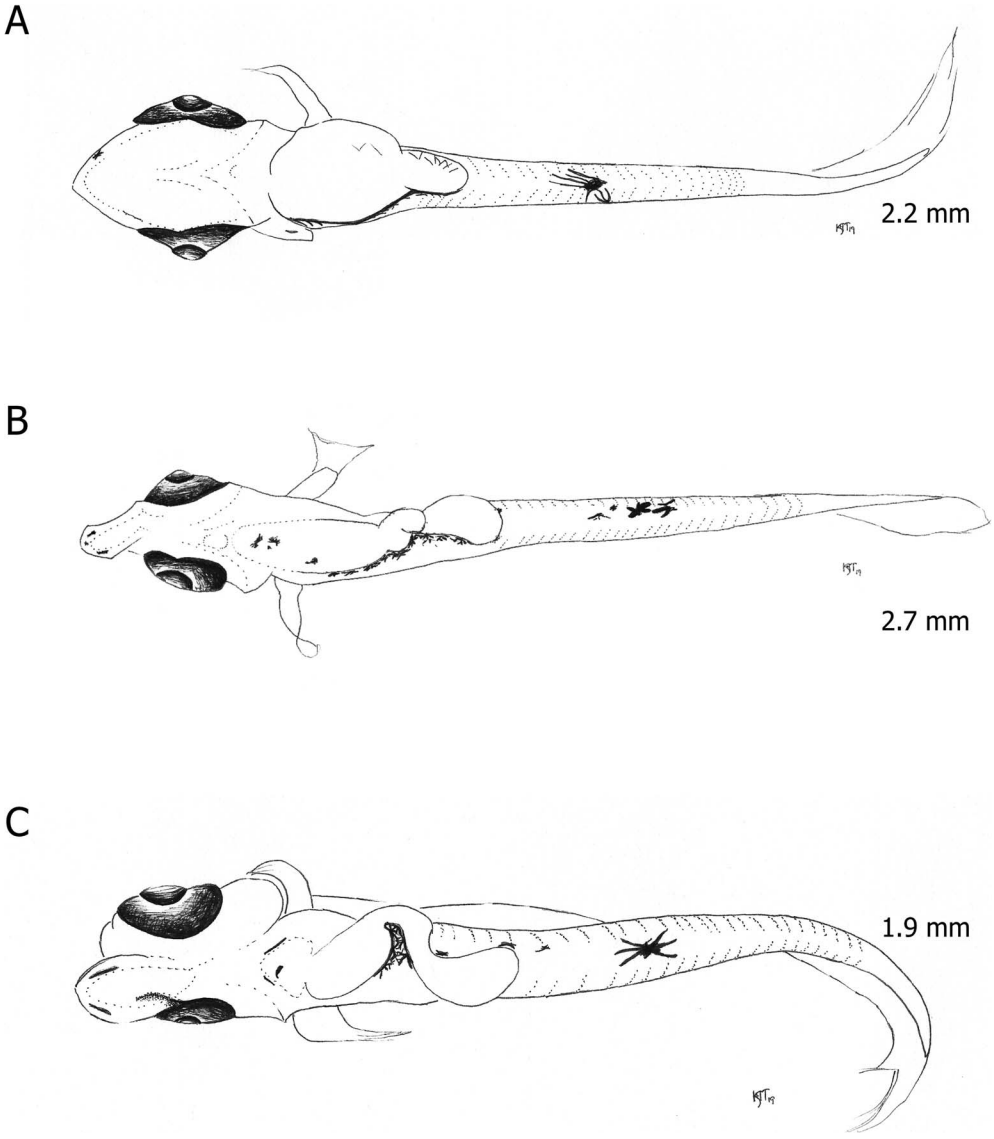

Fig. 3. Line illustrations of ventral view showing lower jaw and ventral midline pigment of (A) 2.2 mm *Urophycis chuss*, (B) 2.7 mm *Urophycis regia*, and (C) 1.9 mm *Urophycis tenuis*.

although *U. regia* do occasionally have pigment over the forebrain during the preflexion stage. Pelvic fins begin budding at 2–2.5 mm and are unpigmented until approximately 2.5 mm. Pelvic-fin development is earlier than the other two species (3–3.5 mm). The pelvic fins are fully pigmented at small sizes and pigmented heavily over half to one third of the membrane between rays in medium and larger larvae. Pigment may occur on the pelvic fins of all species, but does appear to be more extensive on *U. tenuis*. The membrane between pelvic rays is often damaged on net-collected larvae, and is not always present. Dorsal pigment occurs on the midline of the tail immediately post anus, similar to larvae of *U. chuss*. Ventral pigment is present on small larvae, but was absent in medium sized fish (approx. 3.5 mm), similar to *U. chuss*.

Urophycis spp. (>6 mm)

Table 2

Total larvae examined.—n = 3,438; 5.5–45 mm SL.

Identification.—Larvae of *Urophycis* by 6–7 mm become difficult to identify using the traits available for smaller larvae. The dorsal pigment stretches from head to caudal fin. Ventral pigment is present for all species by approximately 10 mm. Total myomere counts overlap for these three species (45–52). A combination of abdominal vertebrae counts, second dorsal-fin ray counts, caudal-fin ray counts, and gill-raker counts can be helpful to separate species at size >11 mm when these characters are fully formed (Table 2; Markle, 1982; Comyns and Grant, 1993; Comyns and Bond, 2002; Fahay, 2007).

DISCUSSION

Larvae of *Urophycis* are morphologically similar, but not identical. The differences described in this study make morphological identification to the species level both possible and cost effective. Dorsal and ventral pigmentation, development of pelvic fins, head pigment, and lower jaw pigment can separate these three species at sizes as small as 1.1 mm NL (preserved length) to the flexion stage (approximately 6 mm SL). By 11 mm, fin and gill-raker counts are useful to diagnose the species.

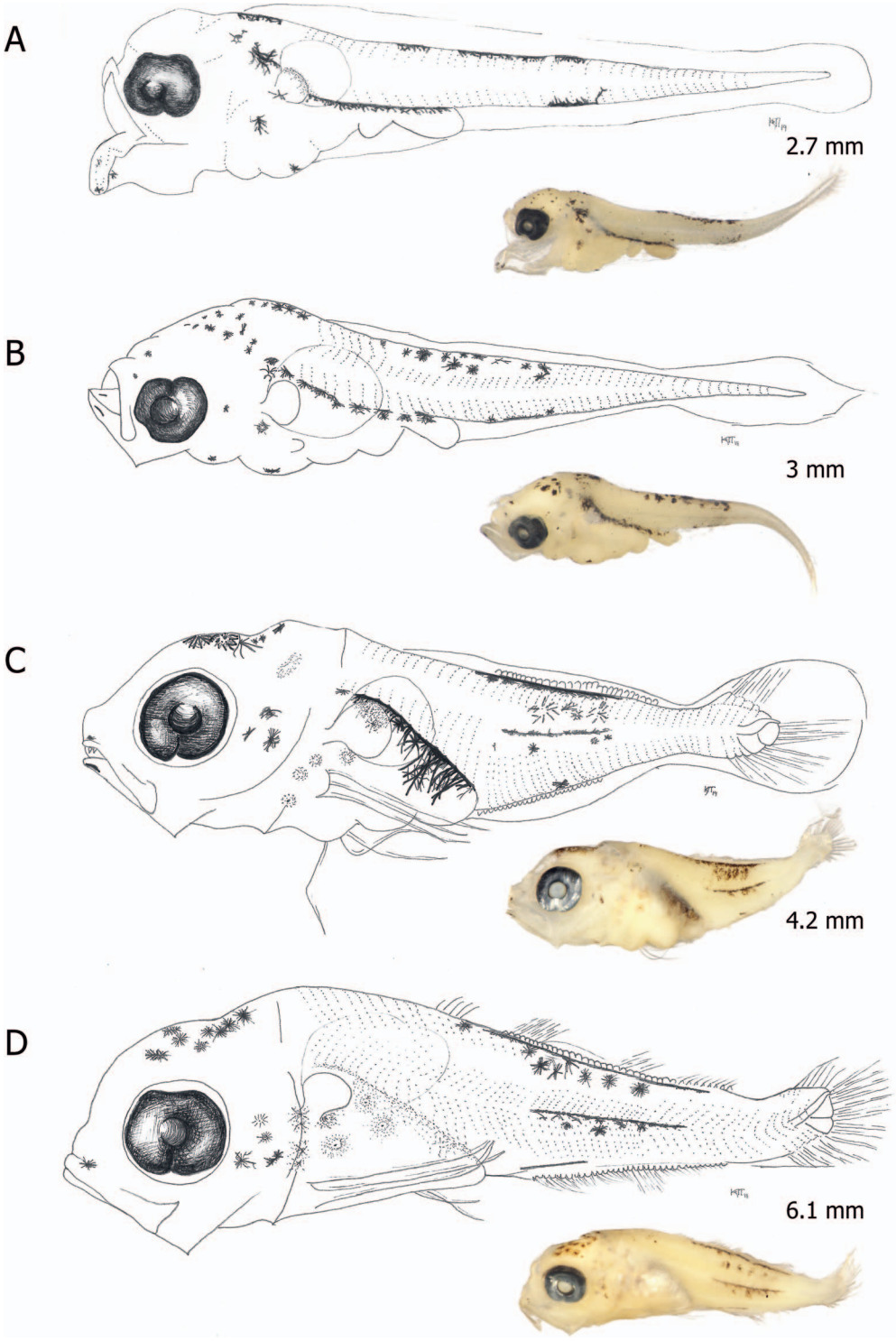

Fig. 4. Line illustrations and images of *Urophycis regia* at (A) 2.7 mm, (B) 3 mm, (C) 4.2 mm, and (D) 6.1 mm.

Identification remains difficult between 6 and 11 mm, and more work needs to be done on these sizes. Fortunately, larvae <6 mm are the most abundant in standard plankton samples (approximately 95% of EcoMon samples are <6 mm).

Although not recommended as an identification trait, these three species of *Urophycis* display separation in peak season of occurrence in the plankton with overlaps: *U. tenuis* May–June, *U. chuss* July–October, and *U. regia* September–November. These peak seasons are a slight shift from previously reported spawning seasons for these species: *U. tenuis* February–May, *U. chuss* April–November, and *U. regia* February–May and August–November (Fahay, 2007). Previous work reporting genetically identified eggs collected during EcoMon surveys largely corroborated our results, although eggs of *U. regia* and *U. tenuis* were collected in August (Lewis et al., 2016).

Independently and objectively testing identifications through DNA barcoding is a powerful tool when used in conjunction with traditional morphological techniques.

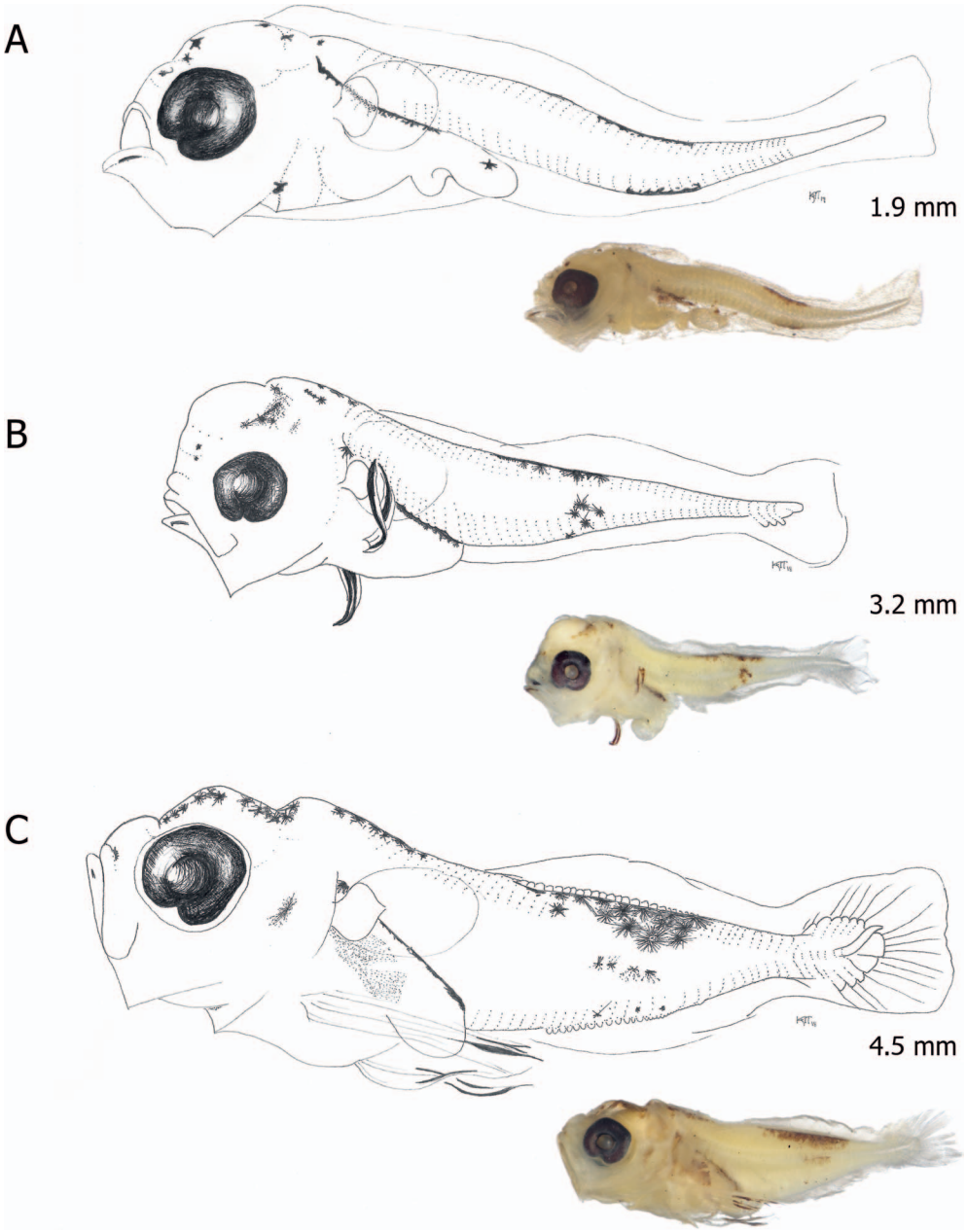

Fig. 5. Line illustrations and images of *Urophycis tenuis* at (A) 1.9 mm, (B) 3.2 mm, and (C) 4.5 mm.

Uncertainty in identifications leads to subjective choices that are often poorly documented. The solution requires both refining the characters used for identification and testing those identifications. In this study, we analyzed genetically identified larvae and then tested morphologically derived, specific identifications through barcoding. We recommend the occasional use of barcoding to test morphological identifications to reduce programmatic errors and ensure a high quality of identifications (Puncher et al., 2015). Even more, we recommend using genetically identified larvae to publish improved morphological descriptions of the larval stages of fish, removing uncertainty.

ACKNOWLEDGMENTS

A great amount of help was provided by Rebecca Konijnenberg, Harvey Walsh, Jon Hare, the crews and scientists aboard

Table 2. Summary of counts useful for separating individuals of *Urophycis* that are >11 mm. In parentheses are the modes as reported by Fahay (2007).

Species	Abdominal vertebrae	Second dorsal rays	Caudal-fin rays	Epibranchial gill rakers
U. chuss	14–17 (15)	52–64	28–34	3
U. regia	13–15 (14)	43–52	28–33	3
U. tenuis	13–17 (16)	50–62	33–40	2

NOAA ships involved in Ecosystem Monitoring Program cruises, the staff at the Canadian Centre for DNA Barcoding, and the staff at the Polish Sorting and Identification Center. This work was funded by a FY2015 NOAA Improve a Stock Assessment grant to Jonathan Hare, Katrin Marancik, Katherine Sosebee, and Michele Traver.

LITERATURE CITED

Barans, C. A., and A. C. Barans. 1972. Eggs and early larval stages of the spotted hake, *Urophycis regius*. Copeia 1972: 188–190.

Comyns, B. H., and P. J. Bond. 2002. Preliminary guide to the identification of the early life history stages of phycid fishes of the western central North Atlantic. NOAA Technical Memorandum NMFS-SEFSC-489.

Comyns, B. H., and G. C. Grant. 1993. Identification and distribution of *Urophycis* and *Phycis* (Pisces, Gadidae) larvae and pelagic juveniles in the U.S. Middle Atlantic Bight. Fishery Bulletin 91:210–223.

Fahay, M. P. 2007. Early stages of fishes in the western North Atlantic Ocean (David Strait, Southern Greenland and Flemish Cap to Cape Hatteras). Volume One: Acipenseriformes through Syngnathiformes. Northwest Atlantic Fisheries Organization, Dartmouth, NS, Canada.

Fahay, M. P., and K. W. Able. 1989. White hake, *Urophycis tenuis*, in the Gulf of Maine: spawning seasonality, habitat use, and growth in young of the year and relationships to the Scotian Shelf population. Canadian Journal of Zoology 1989:1715–1724.

Ivanova, N. V., E. L. Clare, and A. B. Borisenko. 2012. DNA barcoding in mammals. Analytical protocol, p. 153–182. *In*: DNA Barcodes: Methods in Molecular Biology. W. J. Kress and D. L. Erickson (eds.). Humana Press, Totowa, New Jersey.

Lang, K. L., F. P. Almeida, G. R. Bolz, and M. P. Fahay. 1996. The use of otolith microstructure in resolving issues of first year growth and spawning seasonality of white hake, *Urophycis tenuis*, in the Gulf of Maine-Georges Bank region. Fishery Bulletin 94:170–175.

Lewis, L. A., D. E. Richardson, E. V. Zakharov, and R. Hanner. 2016. Integrating DNA barcoding of fish eggs into ichthyoplankton monitoring programs. Fishery Bulletin 114:153–165.

Marancik, K. E., D. E. Richardson, J. Lyczkowski-Shultz, M. Konieczna, and R. K. Cowen. 2010. Evaluation of morphological characters to identify grouper (Serranidae: Epinephelini) larvae in the Gulf of Mexico using genetically identified specimens. Bulletin of Marine Science 86: 571–624.

Markle, D. F. 1982. Identification of larval and juvenile Canadian Atlantic gadoids with comments on the systematics of gadid subfamilies. Canadian Journal of Zoology 60: 3420–3438.

Methven, D. A. 1985. Identification and development of larval and juvenile *Urophycis chuss*, *U. tenuis*, and *Phycis chesteri* (Pisces, Gadidae) from the Northwest Atlantic. Journal of Northwest Atlantic Fisheries Science 6:9–20.

Miller, D., and R. R. Marak. 1959. The early larval stages of the Red Hake, *Urophycis chuss*. Copeia 1959:248–250.

Puncher, G. N., F. Alemany, H. Arrizabalaga, A. Cariani, and F. Tinti. 2015. Misidentification of bluefin tuna larvae: a call for caution and taxonomic reform. Reviews in Fish Biology and Fisheries 25:485–502.

Richardson, D. E., J. A. Hare, W. J. Overholtz, and D. L. Johnson. 2009. Development of long-term larval indices for Atlantic herring (*Clupea harengus*) on the northeast US continental shelf. ICES Journal of Marine Science 67:617–627.

Serebryakov, V. P. 1978. Development of the spotted hake, *Urophycis regius*, from the Northwestern Atlantic. Journal of Ichthyology 18:793–799.

Walsh, H. J., D. E. Richardson, K. E. Marancik, and J. A. Hare. 2015. Long-term changes in the distributions of larval and adult fish in the Northeast U.S. Shelf Ecosystem. PLoS ONE 10:e0137382.