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Age validation for goosefish (*Lophius americanus*) in the northeastern United States

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Abstract—The goosefish (*Lophius americanus*) supports a valuable commercial fishery in the northeastern United States, but annual catch limits are relatively low because of uncertainty in assumed growth rates used for stock assessment. We evaluated the accuracy of the vertebral aging protocol and explored alternative aging methods by injecting chemical markers into individual goosefish held in the laboratory and tagged and recaptured in the field. The chemical left visible marks on vertebrae, illicia, and otoliths at the time of injection, but visibility and intensity of the marks varied among these aging structures. Times after marking ranged from 185 to 860 d for the 20 fish that were analyzed. Observed and expected counts of annuli after the chemical mark indicate that growth increments on vertebrae do not represent annuli and, therefore, cannot be used to accurately determine the age of goosefish. Identification of presumed annuli after the chemical mark was not possible for otoliths because the mark was not visible in most of the samples. Identification of presumed annuli was better for illicium samples than for vertebral samples. The growth rates of the individuals recaptured in the field provide preliminary information on annual growth of goosefish.

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The goosefish (*Lophius americanus*) is an important target species in the commercial bottom fishery in the northeastern United States, with individuals caught in multiple types of fishing gears, including trawls, gill nets, and scallop dredges (Richards et al., 2008). Prior to 1980, goosefish were considered "trash fish" and discarded in favor of more desirable species, but market demand increased regionally and internationally, surpassing traditional target species, like the Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*), in price and revenue (NMFS[1]). A fisheries management plan was implemented in 1999 (Haring and Maguire, 2008), but the lack of basic life history information required a precautionary approach (NEFSC, 2010; NEFSC[2]; Richards[3]). Goosefish are not overfished; however, fishermen have had a significant reduction in their total allowable catch (e.g., allowable catch for 2017–2019 was 43% below the overfishing limit; NEFMC[4])

[1] NMFS (National Marine Fisheries Service). 2017. Fisheries of the United States 2016. NOAA, Natl. Mar. Fish. Serv., Curr. Fish. Stat. 2016, 147 p. [Available from website.]

[2] NEFSC (Northeast Fisheries Science Center). 2013. 2013 monkfish operational assessment. Northeast Fish. Sci. Cent. Ref. Doc. 13-23, 116 p. [Available from website.]

[3] Richards, R. A. 2016. 2016 monkfish operational assessment. Northeast Fish. Sci. Cent. Ref. Doc. 16-09, 109 p. [Available from website.]

[4] NEFMC (New England Fishery Management Council). 2016. Acceptable biological catch (ABC) recommendations for monkfish for fishing years for 2017–2019, 3 p. Sci. Stat. Committ., New England Fish. Manage. Counc., Newburyport, MA. [Available from website.]

as a result of the scientific uncertainty in the latest stock assessment, which was conducted in 2010 (NEFSC, 2010). A statistical catch-at-length model that assumed growth is known was used to determine the status of the goosefish fishery for the stock assessment completed in 2010 and for the operational assessment completed in 2013 (NEFSC, 2010; NEFSC[2]). The available information on age and growth from analysis of annuli on vertebrae indicates linear growth, similar growth rates for males and females, and an absence of males older than 7 years in the goosefish population, with females living up to 14 years (Richards et al., 2008). However, the vertebral aging method for goosefish had not been validated when the stock assessment was conducted, contributing to the underlying cause of the uncertainty about growth rates for this species.

The structure preferred for age determination for *Lophius* species varies, and the interpretation of growth increments on all structures of these species is challenging (Griffiths and Hecht, 1986; Maartens et al., 1999; Walmsley et al., 2005; Landa et al., 2013). European scientists used sagittal otoliths in the first aging studies of white anglerfish (*L. piscatorius*) and black anglerfish (*L. budegassa*) (e.g., Guillou, 1978; Tsimenidis and Ondrias, 1980). However, age estimation through analysis of otoliths has proven to be difficult because of confusing secondary structures (checks) and increasing opacity with age; as a result, the illicium (first spine of the dorsal fin) has become more widely used than the otolith in growth studies of both of these species (e.g., Dupouy et al., 1986; Duarte et al., 1997; Quincoces et al.[5]). The results of the 3 international aging workshops and exchanges on species of *Lophius* in Europe, during which illicia and otoliths were compared by age readers, indicate that age estimates from analysis of illicia had better repeatability, precision, and relative bias than those from analysis of otoliths (Dupouy[6]; Duarte et al.[7]; Landa[8]). The results obtained from micro-increment analysis of otoliths from white anglerfish and black anglerfish (Wright et al., 2002; La Mesa and De Rossi, 2008) and from tag-recapture and length–frequency studies (e.g., Laurenson et al., 2005; Jónsson[9]; Landa et al., 2008) have been useful for establishing better age estimation criteria and for obtaining corroborated growth patterns based on analysis of illicia from specimens caught in European waters (Landa et al., 2013; Ofstad et al., 2013).

Illicia also have been used for aging species of *Lophius* in Africa and Japan. South African scientists first used sagittal otoliths for aging devil anglerfish (*L. vomerinus*) (Griffiths and Hecht, 1986) then switched to illicia (Maartens et al., 1999; Walmsley et al., 2005). Japanese scientists have used vertebrae for aging yellow goosefish (*L. litulon*) (Yoneda et al., 1997), although in a more recent study growth rates from age estimates based on analysis of illicia and from tag-recapture experiments were similar. The age estimates from the illicia had better reader agreement than those from the vertebrae, and the illicium was determined to be the preferred structure for the yellow goosefish (Takeya et al., 2017).

Armstrong et al. (1992) developed an age estimation method for goosefish in which presumed annual growth increments are counted on baked vertebrae. A deep, coarse-textured ridge and a narrow dark band (winter ring or growth increment) interspersed by wider, uniformly textured zones were interpreted to represent 1 year of growth. Hartley (1995) compared aging structures for goosefish and concluded that the method of Armstrong et al. (1992) worked the best because winter growth increments, also referred to as *presumed annuli*, were difficult to identify on otoliths for goosefish older than 3 years and illicia provided inconsistent age estimates. On the basis of Hartley's (1995) work, the Northeast Fisheries Science Center implemented the methods described in Armstrong et al. (1992) and vertebrae became the aging structure used for goosefish in 1996. Cullen et al.[10] compared growth estimates by using aging methods based on analysis of illicia and vertebrae from large goosefish and determined that, although analysis of both structures produced similar ages, it was more difficult to detect presumed annuli on illicia than on vertebrae.

Our objective was to validate Armstrong et al.'s (1992) vertebral aging method for goosefish and to explore the suitability of alternative structures for age estimation. Our approach was to determine if growth increments on vertebrae, otoliths, and illicia are formed annually. Chemical marking was used in laboratory and field samples to establish a known timeline after marking. By using the marked samples, we attempted to identify the structure that had the most consistent and identifiable annual growth increment for age estimation.

Materials and methods

Important factors in keeping goosefish alive in captivity were determined through trial and error during the course of this study. Field protocols and laboratory

[5] Quincoces, I., M. Santurtún, and P. Lucio. 1998. Biological aspects of white anglerfish (*Lophius piscatorius*) in the Bay of Biscay (ICES Division VIIIa,b,d) in 1996–1997. ICES CM 1998/O:48, 29 p. [Available from website.]

[6] Dupouy, H. (ed.). 1997. International ageing workshop on European monkfish, 34 p. IFREMER, Lorient, France.

[7] Duarte, R., J. Landa, C. Morgado, A. Marçal, S. Warne, E. Barcala, E. Bilbao, J. Dimeet, H. Djurhuus, E. Jónsson, et al. 2005. Report of the anglerfish *Illicia*/otoliths ageing workshop; Lisbon, Portugal, 8–12 November 2004, 47 p. IPIMAR, Lisbon, Portugal. [Available from website.]

[8] Landa, J. (ed.). 2011. Report of the anglerfish (*Lophius piscatorius*) illicia and otoliths exchange 2011, 61 p. ICES Plann. Group Comm. Catch Discards Biol. Sampl. [Available from website.]

[9] Jónsson, E. 2007. Verification of anglerfish (*Lophius piscatorius*) age estimation through comparison of length modes of age read fish (illicia) to length modes of large year-classes appearing in the Icelandic stock. ICES CM 2007/K:03, 17 p. [Available from website.]

[10] Cullen, D. W., A. K. Johnson, A. Richards, and K. Lang. 2007. Comparing age and growth estimates for large monkfish (*Lophius americanus* V.) using *illicium* and vertebral aging methods. ICES CM 2007/K:24. [Available from website.]

methods were adapted throughout this study to meet the research objectives by minimizing injury and stress of fish, increasing sample size, and promoting natural growth of marked specimens.

Laboratory specimens

Live, undamaged goosefish were individually selected during commercial fishing trips on vessels outfitted with gill nets or otter trawls and during research scallop-dredge and bottom-trawl surveys (Howe, 1989; Winton et al., 2017). Specimens were collected over 6 years (2009–2015), during every month of the year, except September. Most specimens were collected from waters of southern New England, with a few specimens taken from the Gulf of Maine (Fig. 1), at depths of 45–65 m. Fish were transported to the laboratory in aerated live wells with minimal handling.

Goosefish were held separately in 15-m^3 circular tanks with sterilized fine-grain silica sand approximately 8 cm deep so that they could burrow and lie flat. The water supply was a semi-closed, recirculating seawater system consisting of 2 sand filters, 2 bag filters (50 and 25 µm), and an ultraviolet sterilizer for the incoming replacement water. Ultraviolet sterilizers were installed within the recirculating system that included a protein skimmer, biofiltration system, and degassing towers. Temperature was controlled by a heating system in the winter and a chiller in the summer. Water temperature was maintained to simulate seasonal changes in a range of 7–14°C, and the laboratory was under natural light following the seasonal cycle of light and dark hours.

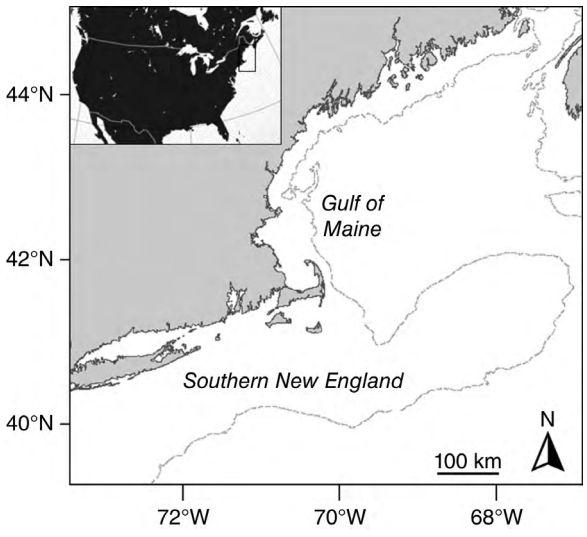

Figure 1

Map of the Gulf of Maine and southern New England, where goosefish (*Lophius americanus*) were collected as specimens for this study. Sampling occurred on commercial fishing trips and research scallop-dredge and bottom-trawl surveys between 2009 and 2015. Specimens were either transported to a laboratory or tagged and recaptured in the field.

A variety of food and feeding techniques were used throughout this study. One or 2 live fish (Cyprinodontidae; Atlantic silverside, *Menidia menidia*; or golden shiner, *Notemigonus crysoleucas*) were introduced into the tank to stimulate normal feeding behavior (Suppl. Videos 1 and 2). Dead fish (mackerel scad, *Decapterus macarellus*; Clupeidae; or Engraulidae) or longfin inshore squid (*Doryteuthis* (*Amerigo*) *pealeii*) were dangled in front of the goosefish specimens to elicit feeding strikes (Suppl. Video 3). If the first 2 methods of feeding failed, attempts were made to nudge dead prey into the corner of the mouth of the goosefish to trigger a feeding response. Feeding attempts were made every few days and increased during the summer months. Each feeding attempt was recorded, including technique and feed species used, weight of ingested food, water temperature, fish behavior, and general health of fish.

Acclimation time averaged 30 d but varied depending on the health of the fish. Behavioral indicators of acclimatization included camouflaging, burrowing into the sand, waving the illicium to attract prey, and eating. When one or more of these indicators were observed, specimens were measured and injected with oxytetracycline (MP Biomedicals[11], Irvine, CA) or fluorexon calcein (Acros Organics, Geel, Belgium) by using a 10-mL Norm-Ject Luer lock syringe (Air-Tite Products Co. Inc., Virginia Beach, VA) with a 20 G1 precision glide needle.

Initially, all injections were intramuscular and consisted of 75 mg/kg of oxytetracycline (Oliveira, 1995; Dekker[12]), but we adjusted our methods after we observed swelling, fluid-filled abscesses, and tissue necrosis at the injection site of both recaptured and laboratory specimens. Oxytetracycline was tested in 3 concentrations: 25 mg/kg, 50 mg/kg, and 75 mg/kg (McFarlane and Beamish, 1987), and the powder was mixed in a 90% saline solution until it dissolved, creating a clear yellow liquid with a pH of 1.6. As an alternative marker, fluorexon calcein was injected in 2 concentrations: 25 mg/kg and 75 mg/kg. The powder was mixed in a 90% saline solution, and approximately 1 g of sodium carbonate was added as a buffer for each gram of fluorexon calcein. The liquid became dark orange with a pH of 6.5. Injections were intraperitoneal and administered on the ventral side by pulling out the pelvic fins to create space between the skin and internal organs for insertion of the needle (Suppl. Fig. 1). Five specimens were not injected so that they could serve as controls for investigating the effects of injections.

[11] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

[12] Dekker, W. 1986. Age reading of European eels using tetracycline labelled otoliths. ICES CM 1986/M:16, 14 p. [Available from website.]

Field specimens

The specimens of goosefish that were used for field experiments were caught in the wild in gill nets of commercial vessels and tagged during 2009–2015. Selected specimens were kept aboard the fishing vessel in live wells to ascertain health before tagging. Only healthy fish (i.e., fish with no body damage and bright clear eyes, exhibiting active behavior) were chosen for this study. Following the protocols developed in the laboratory, each fish received a chemical injection. A centi-TD data storage tag (Star-Oddi, Garðabær, Iceland) was implanted under the skin on the dorsal side, and a visible, external tag was attached through the tail muscle to alert fishermen that the recaptured fish was worth $500 (Cadrin et al.[13]). Initially, pink T-bar tags (Hallprint Fish Tags, Hindmarsh Valley, Australia) were attached dorsally, but they were replaced with Petersen disc tags (Floy Tag Inc, Seattle, WA), attached through the tail muscle to increase tag retention, visibility, and recovery rate. Fish were returned to live wells for a short period of time to recover, and health was assessed again before releasing the fish.

Preparation of aging structures

When fish died in the laboratory or were recaptured in the wild, they were measured to the nearest millimeter, weighed to the nearest kilogram, and dissected. Sex and maturity stage was determined from macroscopic examination of gonads (Armstrong et al., 1992). Whenever possible, all 3 aging structures (illicia, sagittal otoliths, and vertebrae) were extracted, embedded in epoxy in silicone molds, and allowed to harden in the dark. Samples were sectioned with a double-bladed IsoMet Low Speed saw (model no. 11-1280-160, Buehler, Lake Bluff, IL) and mounted on glass slides. Protocols for each structure were as follows: the 10th vertebra was sectioned (0.3 mm) in the sagittal plane at the focus of the centrum; illicia were sectioned (0.31–0.58 mm) 0.5 cm above the basal bulb (Duarte et al., 1997); and one otolith of each pair was sectioned (0.18–0.20 mm) transversely through the nucleus. An Olympus BX51 microscope (Olympus, Tokyo, Japan) with an ultraviolet light attachment was used to view the chemical mark. A combination of ultraviolet light and transmitted light produced an image showing both the chemical mark and growth increments (Fig. 2). Images of the sections were taken with a CoolSNAP-Procf color digital camera (Media Cybernetics Inc., Rockville, MD).

For all specimens, the 8th vertebra was kept intact. For a subset of 7 fish, the second otolith was kept whole and hand ground to a thin lateral section by using sequentially finer-grit abrasive discs resulting in a ~1 mm flat disc. Prepared otoliths were then fixed to a glass slide by using a mounting adhesive. The exposed side of the otolith was polished by using a polishing cloth and MicroPolish II (0.3 µm) alumina powder (Buehler) in water. Whole vertebrae and laterally sectioned otoliths were viewed under a Nikon SMZ1500 microscope (Nikon Instruments Inc., Melville, NY) fitted with an ultraviolet-light attachment and Nikon Digital Sight DS-Fi1c camera. Images of the structures were taken with imaging software (NIS-Elements, Nikon Instruments Inc.) under reflected light to show the growth increments and under ultraviolet light with different filters to show the chemical mark. The images were then merged by using Adobe Photoshop (Adobe Inc., San Jose, CA), and opacity was adjusted to create a new image showing the exact locations of the chemical mark and the growth increments (Figs. 3 and 4).

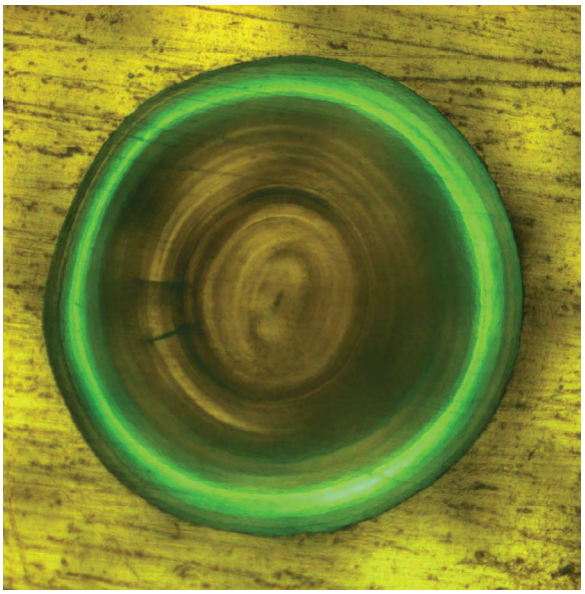

Figure 2

Image of a section of an illicium from a goosefish (*Lophius americanus*) tagged and recaptured in waters of the Gulf of Maine (fish ID 2). The illicium was sectioned (0.31–0.58 mm) 0.5 cm above the basal bulb and placed under a mixed ultraviolet and transmitted light source to show the chemical mark (25 mg/kg of fluorexon) and growth increments. This fish was tagged in October 2012 and recaptured in October 2013 after 365 d at large. Its total length was 67 cm at time of recapture.

Image analysis and validation of age estimates

Aging structures from fish that lived less than 6 months were compared to those from control fish that had no injections to determine if the chemical was incorporated into the structure. Aging structures from fish that lived more than 6 months were analyzed for mark visibility and validation of annual growth increments. Images alone cannot be used to identify presumed annuli on vertebrae because

[13] Cadrin, S. X., C. Bank, J. H. Grabowski, and G. D. Sherwood. 2017. Archival tagging and age validation in the mid-Atlantic. 2014 monkfish RSA. Project completion report, 51 p. Northeast Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Woods Hole, MA. [Available from website.]

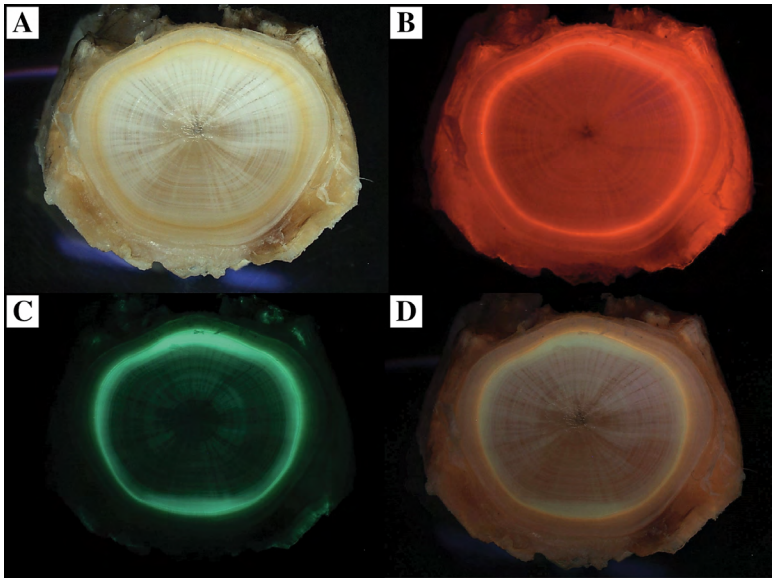

Figure 3

Images of a vertebra from a goosefish (*Lophius americanus*) (fish ID 2) under different light sources: (**A**) reflected light, (**B**) ultraviolet light with a GFP-B filter, and (**C**) ultraviolet light with a DSRed filter. (**D**) Image created by merging the images shown in the other 3 panels. This fish was caught, injected with 25 mg/kg of fluorexon, and released in waters of the Gulf of Maine in October 2012. After 365 d at large, it was recaptured in October 2013 and measured 67 cm in total length.

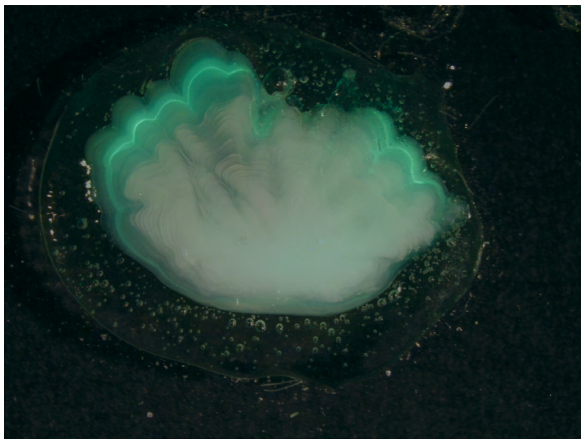

Figure 4

Image created by merging a reflected-light image with an ultraviolet-light image of a laterally sectioned otolith from a goosefish (*Lophius americanus*) caught in November 2010 in waters of southern New England and held in the laboratory (fish ID S). The mark (green line) is visible but discontinuous. This fish measured 51 cm in total length [TL] at the time of injection with 25 mg/kg of oxytetracycline. It grew 6 cm TL during 467 d in the laboratory and was 57 cm TL at time of death.

a physical ridge is associated with a growth increment (Armstrong et al., 1992). The location of the chemical mark was drawn on each 8th vertebra with a pencil, and vertebrae were baked in a drying oven at 230°C for 20–60 min (Armstrong et al., 1992). Two age readers who had experience with goosefish vertebrae viewed the 8th vertebra to identify the growth increments, to estimate the age of each fish, and to count the number of presumed annuli after the chemical mark. The readers did not know when the fish was injected, how long it lived after injection, or the size of the fish. As a condition of the Armstrong et al. (1992) protocol, readers were provided the month the fish died because aging protocols assume a birth date of 1 January.

Eleven chemically marked illicium samples from the same fish used in the vertebral validation study were analyzed independently by the senior author and by an age reader experienced with other *Lophius* species. Illicium aging consists of identifying dark (opaque) and light (hyaline) growth increments under magnification of 50–20× with transmitted light. Each dark increment is counted and assumed to represent 1 year of growth. Aging criteria followed protocols developed by Duarte et al. (1997), with modifications and improvements suggested by Landa et al. (2013) and Ofstad et al. (2013). The first presumed annulus was located on the basis of criteria developed for white anglerfish by Wright et al. (2002), who concluded that the oval structure in the center represents a benthic growth increment and that the true first presumed annulus is a clearly identifiable growth increment beyond the oval. Each growth increment was identified, the age of the fish was estimated, and the number of presumed annuli identified after the chemical mark was documented in the images.

Results

Laboratory specimens

Between 2009 and 2015, 74 goosefish were transported to the laboratory, 36 specimens were injected with a chemical marker (20 fish with oxytetracycline and 16 fish with fluorexon), 5 specimens were used as controls, and the remaining 33 goosefish did not survive acclimation. Total length [TL] of all laboratory fish ranged from 15 to 69 cm, with an average of 51 cm. Of the injected laboratory fish, 10 specimens were included in the validation analysis, with times after marking of 223–860 d (Table 1) and

Table 1

Summary information for specimens of goosefish (*Lophius americanus*) used for validation of age estimates from analysis of vertebrae and illicia. Specimens, collected in waters of southern New England and the Gulf of Maine and injected with a chemical marker during 2009–2015, were held in the laboratory or tagged and recaptured in the field. Growth is from time alive after injection. All lengths and growth rates are provided in total length. Asterisks (*) indicate fish for which readers did not identify on vertebral and illicium samples the expected number of annuli after the chemical mark. Samples from some specimens were not analyzed or not available (N/A). For some other specimens, no mark was visible on the structure.

										No. of annuli after chemical mark				
											Vertebrae		Illicia	
Fish ID	Lab/field	Injection date	Death date	No. of days alive	Length at injection (cm)	Length at death (cm)	Growth (cm)	Annual growth rate (cm)	Sex	Expected	Reader 1	Reader 2	Reader 1	Reader 2
C	Lab	10/20/15	5/30/16	223	14.9	35.5	20.6	33.7	M	1	3*	1	No mark	No mark
L	Lab	3/27/14	08/03/16	860	28.9	61.2	32.3	13.7	M	2	2	2	N/A	N/A
W	Lab	5/24/12	3/18/13	298	33.0	41.0	8.0	9.8	F	1	2*	1	No mark	No mark
F	Lab	09/08/10	10/15/12	767	40.0	58.0	18.0	8.6	M	2	2	2	2	2
G	Lab	6/14/13	9/16/15	824	43.5	51.5	8.0	3.5	M	2	1*	0*	N/A	2
J	Lab	06/06/11	07/09/12	398	48.0	50.0	2.0	1.8	F	1	0*	0*	1	1
T	Lab	06/06/11	02/02/13	606	49.0	54.0	5.0	3.0	F	2	2	0*	2	1*
S	Lab	11/15/10	2/25/12	467	51.0	57.0	6.0	4.7	M	2	1*	0*	1*	1*
M	Lab	06/06/11	02/12/12	251	57.0	60.0	3.0	4.4	F	1	1	0*	1	1
O	Lab	2/13/14	12/22/14	312	63.0	60.0	–3.0	–3.5	F	1	0*	0*	N/A	N/A
7	Field	4/16/11	10/18/11	185	59.0	63.0	4.0	7.9	F	0	0	0	0	0
12	Field	05/09/15	12/17/2015	222	66.0	67.0	1.0	1.6	F	0	0	0	N/A	N/A
1	Field	11/10/09	7/16/10	248	68.0	75.5	7.5	11.0	F	1	0*	0*	1	1
10	Field	08/01/14	7/14/2015	347	70.0	77.5	7.5	7.9	F	1	1	1	N/A	N/A
11	Field	08/02/14	7/28/2015	360	75.0	79.0	4.0	4.1	F	1	0*	0*	N/A	N/A
9	Field	7/31/2014	4/20/2015	263	78.0	82.0	4.0	5.6	F	1	0*	0*	N/A	N/A
8	Field	08/02/14	3/29/2015	239	80.0	83.0	3.0	4.6	N/A	1	1	1	N/A	N/A
2	Field	10/17/12	10/17/13	365	N/A	67.0	N/A	N/A	M	1	0*	0*	1	1
4	Field	10/17/12	9/17/13	333	N/A	71.0	N/A	N/A	M	1	0*	0*	No mark	No mark
5	Field	10/17/12	04/07/14	537	N/A	76.5	N/A	N/A	F	2	2	0*	2	2

annual growth rates of –3.5–33.7 cm TL (Table 1, Fig. 5). All fish died, except for 1 specimen (fish ID L), which was sacrificed after 860 d. Food consumption increased from June through September as temperatures increased, peaking in September (12.8°C), and then decreased sharply in October, although water temperatures remained high (13.2°C). Food consumption was lowest from March through May (Fig. 6).

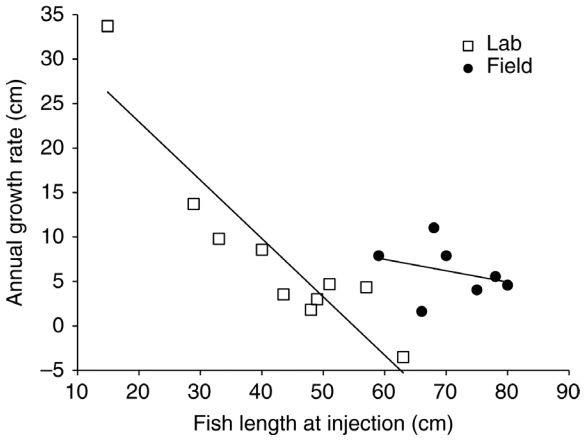

Figure 5

Relationship of annual growth rate with fish length at time of injection for goosefish (*Lophius americanus*) that were caught in waters of southern New England and the Gulf of Maine and injected with a chemical marker between 2009 and 2015. Open squares indicate growth for specimens held in the laboratory, black circles indicate growth for specimens that were tagged and recaptured in the field, and the diagonal lines indicate the trend lines. Growth and lengths are given in centimeters in total length.

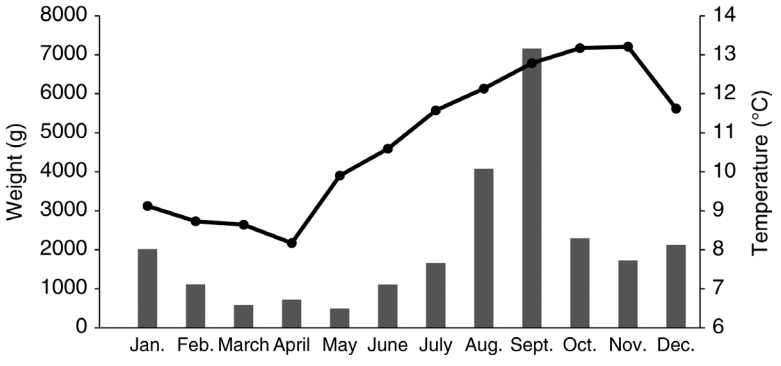

Figure 6

Monthly consumption of all prey by goosefish (*Lophius americanus*) held in the laboratory after capture in waters of southern New England and the Gulf of Maine between 2010 and 2013. Also provided are monthly average temperatures of tank water for 5 of the 10 laboratory specimens used in this study during this time frame.

Field specimens

Between 2009 and 2015, 501 goosefish were caught, injected with chemical markers, and released with data storage tags. Tagging occurred during every season, with the fewest fish released in the winter and the most fish released during the spring. A total of 169 specimens were injected with oxytetracycline, and 332 fish were injected with fluorexon. Total length at release ranged from 38 to 101 cm, with an average of 67 cm. Of the injected specimens, 38 fish were recaptured. Of the recaptured goosefish, 35 specimens had received 25 mg/kg of fluorexon, 2 fish had received 75 mg/kg of oxytetracycline, and 1 fish had received 50 mg/kg of oxytetracycline. Of those recaptured in the field, 10 specimens were included in the validation analysis, with times after marking of 185–537 d (Table 1) and annual growth rates of 1.6–11.0 cm (Table 1, Fig. 5).

Chemical marking

Fluorexon at both concentrations (25 and 75 mg/kg) produced a brighter, more visible mark than oxytetracycline, and a chemical mark was frequently visible on a vertebra or illicium without an ultraviolet light. Oxytetracycline produced a visible mark at all 3 concentrations (25, 50 and 75 mg/kg); however, 2 vertebral samples lost the intensity of the oxytetracycline mark over time and could not be used in the validation analysis. We determined that an intraperitoneal injection of 25 mg/kg of fluorexon was the most successful method for marking the aging structures (Suppl. Table).

Image analysis

Whenever possible, all 3 aging structures from laboratory and field specimens were analyzed to detect chemical marks. Some fish recaptured in the field had structures missing, and some structures were analyzed in a smaller subset. Specimens from both the field and laboratory that lived less than 6 months were grouped for analysis. These specimens did not live long enough or grow enough for a clear, distinct mark to be separate from the edge of their sampled structures. The edge of the structures from injected fish fluoresced under ultraviolet light, indicating that the chemical was incorporated into the calcified structures, whereas the edge of the structures from control fish did not. No autofluorescence was detected in otoliths from control specimens. However, the chemical mark was not seen in structures from every specimen, and the visibility and intensity of the mark varied between structures. For specimens that lived less than 6 months, the rate of detection of the chemical mark was highest (81%) for the illicia (n=31) and lowest (13%)

for the transverse-sectioned otoliths (n=30). For vertebrae, both whole (n=28) and sectioned (n=23), the chemical mark was detected in 43% of the specimens.

Mark visibility for laboratory and field specimens that lived more than 6 months improved to 91% for the whole vertebrae (n=22). The detection rate for illicia (n=21) and sectioned vertebrae (n=14) was 86%, and the detection rate for transverse-sectioned otoliths remained the lowest at 36% (n=11) (Suppl. Table). Results improved to 43% for laterally sectioned otoliths (n=7), but the mark was not continuous and was not incorporated uniformly into the otolith (Fig. 4).

Age estimation: vertebrae

Twenty-two fish lived long enough to be included in the validation analysis with vertebrae (185–860 d post-marking), but only 20 specimens were used because 2 fish (1 specimen from the laboratory and 1 specimen from the field) did not have a mark that could be detected on the whole vertebrae at the time of analysis. Reader 1 identified the expected number of presumed annuli outside the mark for 45% of vertebral samples, and reader 2 identified the expected number of presumed annuli for 40% of samples (Table 1, Fig. 7A). The most common problem for both readers was the lack of a visible winter growth increment that should have been identifiable after the chemical mark. Six fish that lived for over 1 year and 3 fish that lived through a complete winter were expected to have at least 1 winter growth increment counted, but a clear winter growth ring was not visible in vertebrae sampled from them. Therefore, age was underestimated for 9 fish that were larger than 50 cm TL. Conversely, the age of the 2 smallest fish (36 and 41 cm TL) was overestimated by reader 1, who identified additional presumed annual growth increments when only 1 increment was expected (Table 1, Suppl. Fig. 2).

Age estimation: illicia

Illicia from 11 of the same chemically marked fish were analyzed for age estimation and age validation after the chemical mark. Using the same age estimation criteria, both readers agreed on the location of most of the presumed annuli. Therefore, most of the ages estimated by both readers were similar (70% of agreement and disagreements of only ±1 year for 3 illicia), and the age estimated by both readers increased with fish length (Fig. 8). Both readers agreed on the position of the presumed first annulus (mean diameter: ~210 µm) in 80% of the illicia that were analyzed (Fig. 9). An oval nucleus was noted in almost

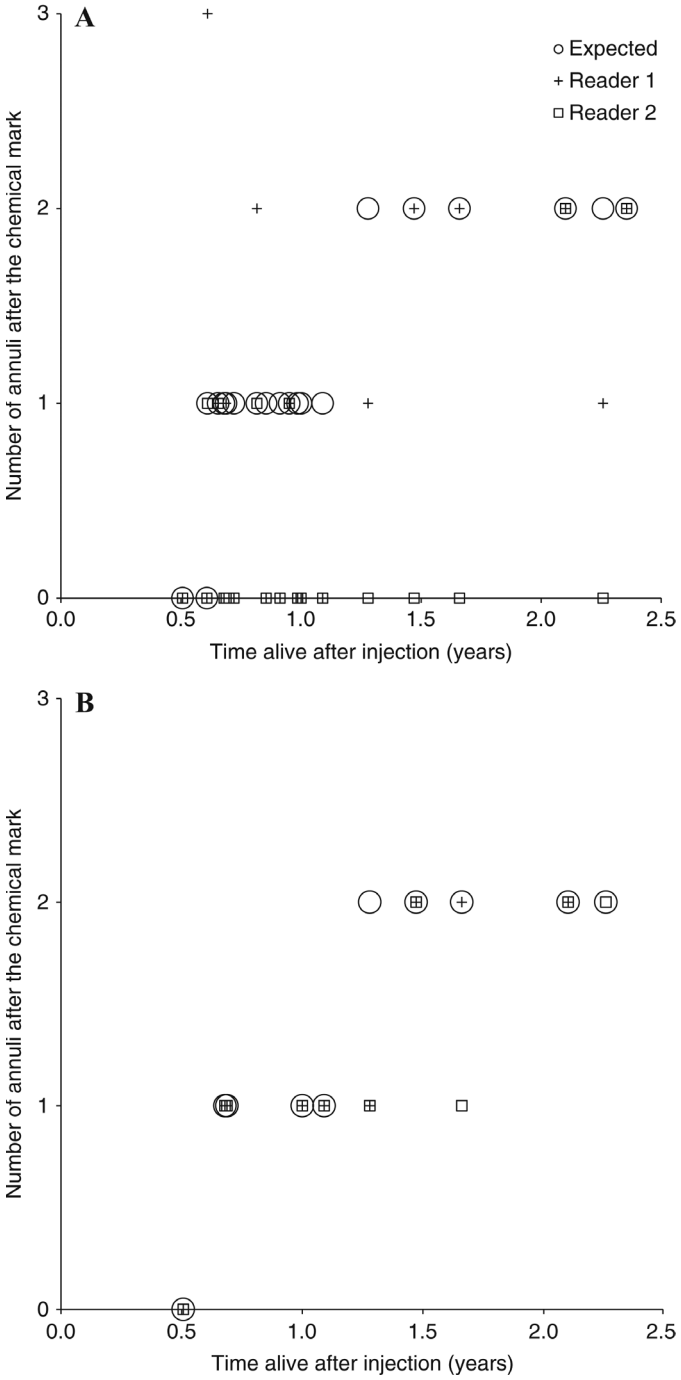

Figure 7

Relationships between the time elapsed from injection of a chemical marker to death for specimens of goosefish (*Lophius americanus*) and the numbers of annuli counted by each of 2 readers in the (**A**) vertebrae and (**B**) illicia of specimens. The number of annuli expected in that period are also included. Specimens, collected in waters of southern New England and the Gulf of Maine during 2009–2015, were injected with a chemical marker and held in the laboratory or tagged and recaptured in the field.

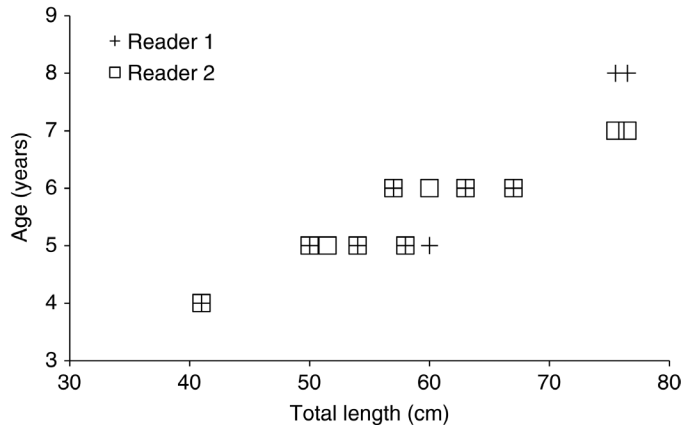

Figure 8

Relationship of estimated age, based on counts of annuli on illicia by 2 readers, and the total length of specimens of goosefish (*Lophius americanus*). Specimens were captured in waters of southern New England and the Gulf of Maine between 2009 and 2015.

all illicium samples, with a mean diameter of ~100 µm. A narrow dark increment outside the nucleus in some samples was considered to be a check, with a mean diameter of ~150 µm (Fig. 9). The chemical injections left marks in the illicia of differing intensity, from very sharp and narrow in some illicia to weaker and wider chemical marks in others (Fig. 9). The width of this mark affected the ease of interpretation.

Although the chemical mark was not clearly observed on the aging structure from 1 specimen (fish ID W), the remaining 10 fish were used for age validation. In most cases, both readers observed a number of growth increments (dark increments considered as annual) after the chemical mark that matched the number of winters elapsed (Table 1, Fig. 7B). For 3 of the fish that lived over 1 year but lacked a winter growth increment on the aged vertebra, a winter ring was seen on the analyzed illicium (Fig. 10). Therefore, reader 1 and reader 2 had an accuracy rate of 89% (9 aged illicia) and 80% (10 aged illicia), respectively (Table 1, Fig. 7B). An increasing relationship was observed between the time at large (between the chemical mark and death) and the number of presumed annuli counted by each reader in the illicia (Fig. 7B).

Discussion

Results indicate that growth increments on vertebrae do not represent annual growth and, therefore, cannot be used to accurately determine the age of goosefish. For half the fish that survived over 6 months after chemical marking, in both the laboratory and the wild, the expected number of annuli outside the chemical mark were not observed, and the difference between observed and expected number of annuli was greater than the measurement error of the technique (Table 1, Fig. 7A).

Several hypotheses explain why growth increments might not have followed an annual cycle in our study. Griffiths and Hecht (1986) and Maartens et al. (1999) hypothesized that the growth of *Lophius* species is a sporadic phenomenon rather than one of traditional seasonal growth. Armstrong et al. (1996) found a high percentage of empty stomachs for goosefish, indicating that infrequent eating could contribute to irregular growth increments. Our observations of feeding patterns in the laboratory are consistent with Armstrong's (1996) observations.

Another hypothesis to explain why annual growth increments may not form on vertebrae is the wide range of temperatures that goosefish experience during vertical movements (Rountree et al., 2008). Archival-tag data indicate that some fish in the western Gulf of Maine inhabit bottom temperatures of 6°C in the summer but vertically migrate into temperatures of 12–16°C for hours at a time before dropping back to bottom temperatures of 6°C (Bank, 2016). However, this behavior of diel vertical migration is highly variable. Therefore, the large range of temperatures experienced during a 24-h period may affect growth patterns on vertebrae.

Experimentally induced changes in behavior, resulting from the stress of tagging and injection procedure, could also disrupt a seasonal cycle of growth and alter ring formation. However, the results of various studies indicate that these effects may be minimal for other finfish species (Francis et al., 1992; Righton et al., 2006). To avoid this potential issue, we included only wild fish that were at large for 6 months or more, minimizing the effects of stress on behavior and growth. The observed annual growth rates were more variable for fish in the laboratory (–3.5–33.7 cm TL) than for those recaptured in the field (1.6–11.0 cm TL) (Fig. 5); however, our laboratory results were comparable to annual growth rates observed in a larger conventional tagging study (–2.2–31.8 cm TL; Sherwood et al.[14]). Therefore, it is unlikely that the stress of tagging fish or the rearing of fish in the laboratory influenced seasonal growth over the duration of our study. Therefore, the prominent growth increments, originally thought to represent annuli, may indicate periods of feeding, fast growth, rapid temperature changes, spawning periods, or stress, rather than annual cycles.

The failed validation of the vertebral aging method has important implications for stock assessment and management of goosefish fisheries. The pattern in this study of underestimating ages of the goosefish ≥50 cm TL and overestimating ages of small goosefish (≤41 cm) indicates

[14] Sherwood, G. D., J. H. Grabowski, and C. Brown. 2013. Tagging to assess monkfish movement: additional tagging to assess monkfish (*Lophius americanus*) movements and stock structure in the northeastern United States. 2009 Monkfish Research Set Aside Program. Final report, 30 p. Northeast Fish. Sci. Cent., Natl. Mar. Fish. Serv., NOAA, Woods Hole, MA. [Available from website.]

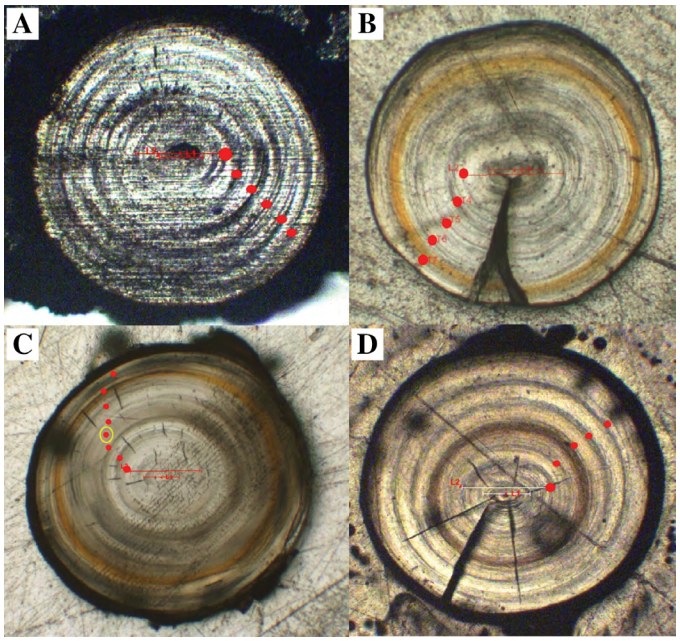

Figure 9

Images of illicia from specimens of goosefish (*Lophius americanus*) of various sizes and ages and with various times at large after tagging or times alive after injection in the laboratory. Specimens were captured in waters of southern New England and the Gulf of Maine and injected with a chemical marker during 2009–2015. The chemical mark is visible in the images as a brownish-yellow ring. Growth increments are indicated with red dots. (**A**) This specimen (fish ID 7) spent 185 d at large (April–October 2011) after an injection of 50 mg/kg of oxytetracycline and measured 63 cm in total length [TL] at death. The chemical mark is barely visible in the last wide light increment (WLI) near the edge of the illicium section. Both readers observed 6 narrow dark increments (NDIs). (**B**) This specimen (fish ID J) lived 398 d in the laboratory (June 2011–July 2012) after an injection of 25 mg/kg of fluorexon and measured 50 cm TL at death. The chemical mark is located in the penultimate WLI, and an NDI and a subsequent WDI are visible toward the edge. Both readers counted 5 NDIs. (**C**) This specimen (fish ID 5) spent 1.5 years at large (October 2012–April 2014) after an injection of 25 mg/kg of fluorexon and measured 77 cm TL at death. Both readers identified 7 NDIs, and one reader identified an additional NDI (yellow circle). Two NDIs were identified after the chemical mark toward the edge. (**D**) This specimen (fish ID F) lived for ~2 years in the laboratory (September 2010–October 2012) after an injection of 75 mg/kg of oxytetracycline and measured 58 cm TL at death. Both readers observed 5 clearly visible NDIs. Two NDIs (corresponding to 2 winters elapsed) are identified after the chemical mark toward the edge.

Chemical marking was not successful in otoliths; therefore, validation of growth increments was not possible. These results were surprising because otoliths are typically the preferred structure for age estimation in bony fish species. Otoliths are known to be metabolically inert and do not reflect physiological changes throughout the lifespan of fish (Phelps et al., 2007). Otoliths typically have limited resorption and continuous accretion of recognizable layers that result from biomineralization (calcium carbonate, mainly in the form of aragonite, is precipitated on a protein matrix of otolin; VanderKooy[15]). It is difficult to determine why the otolith did not pick up the chemical mark consistently in our study. Mohler (1997) did not have success marking bony structures in larval Atlantic salmon (*Salmo salar*) with oxytetracycline immersions, but he was successful with calcein immersions and intensity of the marks were greater for the more concentrated calcein immersions. Hernaman et al. (2000) suggested that stress may be a possible cause for the absence of a mark in otoliths from both tetracycline and calcein for a coral-dwelling goby, *Gobiodon histrio*. In our study, if the chemical was not absorbed because of stress, the mark would not be detected in all 3 of the structures we analyzed. We frequently detected marks in illicia and vertebrae but not in otoliths.

Results for accurately identifying winter growth increments after the chemical mark were better for illicium samples than for vertebral samples, but the sample size was smaller for illicia (n=9) than for vertebrae (n=20). The accuracy and agreement among readers provides a validation of this aging method and indicates the potential feasibility of using illicia for age estimation of goosefish going forward. However, the position of the first annulus has not been validated, and further studies will be required. The analysis of micro-increments in otoliths has been used satisfactorily to locate the first annulus in illicia and otoliths of both European congeners, the white anglerfish and black anglerfish (Hislop et al., 2001; Wright et al., 2002; La Mesa and De Rossi, 2008; Hernández et al., 2015), and in this study helped inform where to locate the first presumed annulus. The advances described here on the growth pattern (proving that dark increments are annuli) can help inform aging studies that use illicia for both *Lophius* species in Europe and the other 4 species of this genus for which no validation with chemical marking has been previously performed. Shifts from one aging structure (otolith or vertebra) to another, more appropriate one (mainly illicium) has taken place that the linear growth assumed in the stock assessment completed in 2010 (NEFSC, 2010; NEFSC[2]) is an artefact of aging error. In response to our results, the most recent peer review of that goosefish stock assessment concluded that the vertebral aging method is not valid and the statistical catch-at-length model should not be used as a basis for fishery management advice for this species (Richards[3]).

[15] VanderKooy, S. J. (ed.). 2009. A practical handbook for determining the age of Gulf of Mexico fishes, 2nd ed. Gulf States Mar. Fish. Comm. Publ. No. 167, 157 p. [Available from website.]

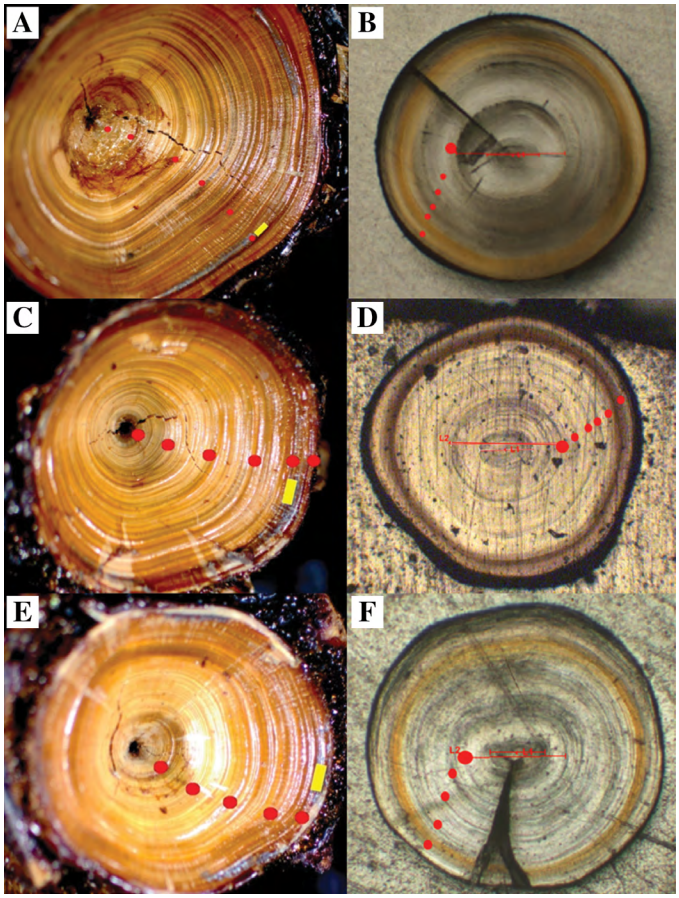

Figure 10

Images of the 8th vertebrae and illicia from 3 specimens of goosefish (*Lophius americanus*) that lived for more than 1 year and for which the expected numbers of annuli after the mark from a chemical injection were not counted on the vertebrae but were identified on the illicia. Specimens were captured in waters of southern New England and the Gulf of Maine and injected with a chemical marker during 2009–2015. Red dots indicate presumed annuli, a yellow rectangle indicates the position of the chemical mark, and red lines indicate measurements of the benthic ring and first annulus. The chemical mark is visible in each illicium as a dark or orange band. (**A**) Vertebra and (**B**) illicium of a specimen (fish ID 2) that was 67 cm in total length [TL] at death and spent 365 d at large after an injection of 25 mg/kg of fluorexon. (**C**) Vertebra and (**D**) illicium of a specimen (fish ID S) that was 57 cm TL at death and lived 467 d after an injection of 25 mg/kg of oxytetracycline. (**E**) Vertebra and (**F**) illicium of a specimen (fish ID J) that was 50 cm TL at death and lived 398 d after an injection of 25 mg/kg of fluorexon.

among studies of other *Lophius* congeners, as discussed in the "Introduction" section.

The difficulty of detecting presumed annuli in illicia from goosefish that has been reported by some authors (Hartley, 1995; Cullen et al.[10]) may be related to the specific techniques required for processing the illicium samples (e.g., recommended thickness of 0.5 mm and the precise location of the section). The wrong section thickness, for example, can make the annuli interpretation more difficult. Hartley (1995) used a section of the spine near the base of the structure and concluded that it was difficult to consistently obtain sections with prominent zones. However, that study took place before Duarte et al. (1997) described their aging protocols and techniques and advised that annual rings are best distinguished at 0.5 cm above the base of the illicium.

The maximum size obtained for goosefish (138 cm TL) (Richards et al., 2008) is intermediate with respect to those estimated for both congeners in Europe (~100 cm TL in black anglerfish and ~170–200 cm TL in white anglerfish) (Caruso, 1986; Landa et al., 2013). Annual growth rates estimated for recaptured fish in our study (about 4–8 cm TL) are comparable to those for fish of the European congers in the same size range (60–80 cm TL). Estimates are slightly lower than those of 6–8 cm TL/year and 4–10 cm TL/year for white anglerfish from tag-recapture experiments conducted in waters of the northeastern Atlantic Ocean (Landa et al., 2018, fig. 4; Ofstad et al., 2013, fig. 5B); however, black anglerfish in the Mediterranean Sea had growth rates of 4–7 cm TL/year based on length–frequency analysis for fish of similar sizes (García-Rodríguez et al., 2005; Landa and Barcala, 2017). Continuing the tag-recapture project from this study on a larger scale with either chemical or external marking, in combination with other age validation techniques that focus on juvenile fish, would help increase our understanding of the age and growth of goosefish.

In summary, the vertebral aging method for goosefish cannot be validated. Chemical marking was unsuccessful for otoliths; therefore, growth increments could not be validated. Preliminary results indicate that illicia produce a recognizable annual growth pattern, and both readers agreed on the location of most of the growth increments considered to be annual. The use of the illicium as the preferred structure for age estimation for this species should be explored further and aging protocols should be developed. Results from this study have been used to justify the rejection of the model that was employed in the last stock assessment and was based on vertebral age estimation and growth (Richards[3]). The initial growth rates estimated in this study, coupled with additional tag-recapture data, improve our understanding of the true growth of this species. With new growth estimates, and improved age estimation techniques, a validated growth pattern can be incorporated into stock assessments in the future to reduce scientific uncertainty and can lead to better management of this species.

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