PROJECT COMPLETION REPORT

Northeast Fisheries Science Center

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Project Summary

Monkfish (*Lophius americanus*) are a critical component of the commercial fisheries in the northeast. They are targeted in directed gillnet fisheries and caught as incidental catch in the groundfish and scallop fisheries, providing additional revenue for these fisheries. Despite their importance, the monkfish stock assessment is data-limited, leaving managers with limited biological information to assist them in the difficult task of determining appropriate catch limits for the commercial fisheries. However, recent research efforts funded by the NOAA Monkfish RSA Program have begun to provide insight regarding the basic biology and ecology of this important species. Reliable age determination is the cornerstone of effective age-based fisheries management, but recent assessments of monkfish in US waters points to some potential uncertainties regarding age of monkfish that may be undermining management efforts for this economically important species (NEFSC 2013).

The project goal was to reduce uncertainty in the stock assessment by providing validated age and growth information and to provide managers with accurate information on the mixing between stocks and monkfish migratory behavior. The specific objectives were to validate the age determination method for monkfish and to expand our previous tagging program by deploying archival tags for understanding habitats and movement patterns. The research addressed the top two Program Priorities:

- Priority 1: Research on monkfish life history focusing on: (a) age and growth, (b) longevity, (c) reproduction, and (d) natural mortality
- Priority 2: Stock definition, stock movements, mixing, and migration through tagging studies, DNA markers, morphological characteristics and other means, focusing on: (a) Short- and long-term movements, and (b) habitat use in relation to broad scale movements.

The research objective for program priority one was to validate the current ageing method for monkfish: The current ageing method for *Lophius Americanus* is based on the work by Armstrong et. al (1992). The age structure used is the vertebrae and an annulus (a year's growth) consists of a banding pattern driven by seasonal growth. A thick opaque band represents fast growth (summer) followed by a narrow translucent band signifying slow growth (winter). Different age structures are used for age estimation of other *Lophius* species. In Europe, the illicium (first dorsal spine) is used, whereas in South Africa, the sagittal otolith is used. Yet none of these methods had been validated.

Detailed methods, results and interpretations are described in two manuscripts drafted for publication:

Appendix 1 "<u>Monkfish Age Validation</u>" - To validate the vertebral ageing protocol and to explore alternative methods, we injected oxytetracycline or fluorexon into individual monkfish, kept them alive in the laboratory, and subjected them to a seasonal cycle of temperature, light, and feeding. Monkfish were also injected in the field with the same chemical markers as part of an on-going data storage tagging study. The chemical left a visible mark on the growth ring that was forming at the time of injection. Fish that lived six months or more after marking, from both the laboratory study and the field recaptures, were analyzed. Digital images of the vertebrae were taken with an ultraviolet light to illuminate the mark and reflected light to show the growth rings. An experienced monkfish age reader was asked to age each fish and count each annulus after the chemical mark.

Results indicate that annuli counts on vertebrae cannot be used to accurately determine the age of monkfish, and age determination using ilicia is more promising.

Appendix 2 "Otolith microchemistry analysis results for age and growth" - An otolith microchemistry analysis was conducted to aid in the interpretation of monkfish age. Specifically we analyzed a subset of monkfish otoliths for strontium/calcium ratios (Sr/Ca), a proxy for temperature, over the entire surface of sectioned and polished otoliths. The resulting maps of Sr/Ca values were overlaid on and compared to otolith cross-sectional images to increase confidence in the identification of annuli. This method was helpful for identifying the first one or two annuli. For an even smaller subset of otolith images, calcein marks from the chemical injection component of this study were visible and further aided in the identification of distal annuli. Our Sr/Ca and calcein age interpretations ranged from 2 to 8 years old for fish ranging in size from 41 to 82 cm. The resulting growth curve was curvilinear due to relatively rapid growth in the first few years and slower growth as fish age. This is in contrast to the current vertebral method for ageing monkfish which results in a linear growth curve likely due to positive age bias for mostly younger ages. Our results for asymptotic growth and the resulting Von Bertalanffy growth function parameter estimates are consistent with results from our previous tagging studies and for growth patterns in other monkfish species around the world.

Collaboration, Dissemination and Education
Many fishermen assisted in the design and execution of this project. Captains providing Research Set Aside Compensation days at sea (DAS) are listed in Table 1. In addition to the two manuscripts for publication (Appendices 1 and 2), several meetings were held with collaborative fishermen and scientific partners to develop field method and discuss results:

- June 25 2012, United Fishermen Club, New Bedford MA
- November 18 2013, Northeastern University, Nahant MA
- June 12 2014, United Fishermen Club, New Bedford MA

Project results were presented at several scientific meetings:

- "Age Validation of Monkfish" Fisheries Society of the British Isles Annual Symposium on Deep-Sea Fish Biology (July 8–11 2013, Glasgow Scotland)
- "Age Validation of Monkfish" Southern New England Chapter of the American Fisheries Society (January 29 2014, Hadley MA)
- "Age Validation of Monkfish" International Council for the Exploration of the Sea 5th International Otolith Symposium (October 20-24 2014, Mallorca Spain).

This project contributed to two graduate student research topics:

- Crista Bank's MS thesis at the University of Massachusetts Dartmouth School for Marine Science and Technology includes the monkfish age validation manuscript (Appendix 1) as well as protocols for transport and husbandry of monkfish.
- Chris Baillie, a PhD student at Northeastern University is researching tidal-based geolocation of monkfish using the data from archival tags deployed in this project, with supplementary support from a subsequent monkfish RSA award.

References

Armstrong, M., Musick, J., & Colvocoresses, J. 1992. Age, growth, and reproduction of the goosefish lophius americanus (pisces: Lophiiformes). Fishery Bulletin, 90(2), 217-230.

Reference Document 05-04. http://www.nefsc.noaa.gov/nefsc/publications/crd/crd0504/

NEFSC (Northeast Fisheries Science Center). 2010. 50th Northeast Regional Stock Assessment Workshop (50th SAW) Assessment Report. US Dept Commer, Northeast Fish Sci Cent Ref Doc. 10-09.

NEFSC (Northeast Fisheries Science Center). 2013. 2013 monkfish operational assessment. US Dept Commer, Northeast Fish Sci Cent Ref Doc. 13-23.

Table 1. Vessel names and Captains who were allocated the 129 RSA DAS.

	Captain	Vessel Name	2012 ALLOCATION
1	Eric Moniz	FV American Dream	15
2	Ian Parente	FV Argo, FV Odyssey	12
3	Dan Nerona	FV Sophia Gale	5
4	Bill McCann	FV Shamrock, FV Pilgrim	12
5	Mike Kitchen	FV Sherry Ann	5
6	Richard Walz	FV Finast Kind II, FV Ami Elizabeth	5
7	Charlie Borden	FV Drake	5
8	Ed Smith	FV Claudia Marie	5
9	Gary Hall	FV Miss Maura	10
10	Ted Platz	FV Gertrude H, FV Last Fling, FV Louise	12
11	Tim Froelich	FV Miss Independence, FV Liberty	10
12	Rich LaRocca	FV Double Vision,FV Doubled Vision	14
13	Todd Sutton	FV Sweet Misery, FV Redemption	9
14	Scott Dudley	FV Atlantic Pearl, FV Laura Peggy	10
		TOTAL DAS	**129**

Appendix 1. MONKFISH AGE VALIDATION

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DRAFT manuscript

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ABSTRACT

MONKFISH AGE VALIDATION

Monkfish, *Lophius americanus*, support an important commercial fishery in the Northeastern United States. Despite healthy stock status, annual catch limits are relatively low, largely because of scientific uncertainty in stock assessment. The age estimation method for monkfish has not been validated, contributing uncertainty in the assessment. Growth rings are assumed to follow a seasonal pattern. To validate the vertebral ageing protocol and to explore alternative methods, we injected oxytetracycline or fluorexon into individual monkfish, kept them alive in the laboratory, and subjected them to a seasonal cycle of temperature, light, and feeding. Monkfish were also injected in the field with the same chemical markers as part of an on-going data storage tagging study. The chemical left a visible mark on the growth ring that was forming at the time of injection. Fish that lived six months or more after marking, from both the laboratory study and the field recaptures, were analyzed. Digital images of the vertebrae, otoliths and illicia were taken with an ultraviolet light to illuminate the mark and reflected light to show the growth rings. An experienced monkfish age reader was asked to age each fish and count each annulus after the chemical mark. Results indicate that annuli counts on vertebrae cannot be used to accurately determine the age of monkfish, and age determination using ilicia is more promising.

Introduction

Monkfish, *Lophius americanus* (Valenciennes, 1837) are an important component of the commercial fishery in the Northeastern United States and are caught in different gear types including trawl nets, gillnet, and scallop dredge (Richards et al., 2008). Prior to 1980 monkfish were considered "trash fish" and were discarded for more desirable species. However, the demand for monkfish increased locally and internationally, surpassing cod and haddock in market value. A fisheries management plan was implemented in 1999 (Haring and Maguire, 2008), but the lack of basic biology and life history of the species has necessitated a precautionary approach to management (NEFSC 2013). Monkfish are not overfished, but annual catch limits are relatively low because of scientific uncertainty in the stock assessment. Information on age and growth suggests linear growth, the same growth rates for males and females, and an absence of males in the population over age 7 whereas the females live up to 12 years (Richards et al. 2008). However, the age estimation method for monkfish has not been validated, contributing to the underlying cause of this uncertainty.

The preferred age determination structure for *Lophius sp.* varies by region, and three calcified structures have been used. Europeans use the illicium (first dorsal spine) for *L. piscatorius* and *L. budegassa* (Duarte et al. 1997), South Africans use the sagitta otoliths for *L. upsicephalus* (Griffiths and Hecht 1986), and the Japanese use the vertebrae for *L. litulon* (Yoneda et al. 1997). Interpretation of growth rings is challenging for all structures (Griffiths and Hecht 1986; Maartens et al. 1999). Age reader agreement, accuracy and precision have been compared using illicia and sagittal otoliths (Dupouy 1991; Peronne et al. 1992; Maartens 1999; Wright et al. 2002; Woodroffe et al. 2003, Duarte et al. 2005), justifying the use of illicia by several European countries (Landa et al. 2008, Landa et al. 2013, Ofstad, 2013). Cullen (2007) compared growth estimates using illicia and vertebral ageing methods and determined that it was more difficult to detect annuli on illicia than vertebrae (Cullen 2007). Armstrong et al. (1992) developed an age estimation method for *Lophius americanus* in which growth rings were counted on the vertebra and followed a seasonal pattern: a broad, opaque band, (formed during the summer months of fast growth), combined with a narrow, translucent band and step, (formed during the winter months of slow growth), indicated one year's growth or annulus.

Our objective was to validate the vertebral age determination protocol for L. americanus and to explore alternative methods. Our approach was to validate annual growth patterns of vertebrae, otoliths and illicia using chemical marking in laboratory and field experiments, and to test multiple ageing methods through image analysis. We attempted to identify the structure that exhibits the most consistent annual banding pattern for age determination.

Methods

Live, undamaged monkfish were individually selected during commercial fishing trips on gillnet or otter trawl vessels, and during research trawl surveys. Collections occurred over a span of six years (2008 through 2014) during every month of the year except September. Most samples were from Southern New England waters with a few samples from Cape Cod Bay, and water depth ranged from 45 – 65 meters. Fish were transported to a sea water laboratory in aerated live wells and were slowly acclimated to the water temperature in the laboratory. During all fish transfers in the laboratory or in the field, specimens were either held in a plastic sling or moved directly from one container to another without handling.

Monkfish were held separately in 15 m^3 circular tanks with fine, sterilized silica sand, approximately 8 cm deep so they could burrow and lie flat. The water supply was a semi-closed, re-circulating sea water system consisting of two sand filters, two bag filters (50 um and 25 um), and an ultraviolet sterilizer for the incoming replacement water. Additional ultraviolet sterilizers were installed within the re-circulating system along with a protein skimmer, bio-filtration system, and degassing towers. The temperature was controlled by a heating system in the winter and a chiller in the summer. The water temperature simulated seasonal changes ranging from 7˚ C to 14˚ C, and the lab was subjected to natural light following the seasonal cycle of light and dark hours.

A variety of food and different feeding techniques were used throughout the study. One or two live fish (killifish - Cyprinodontidae, *Menidia menidia*, and *Notemigonus crysoleucas*) were introduced into the tank to promote normal feeding behavior. Dead fish (*Decapterus macarellus*, Clupeidae, Engraulidae) or squid (*Loligo pealeii*) were dangled in front of the monkfish specimens to stimulate feeding strikes. If the first two methods failed, attempts were made to nudge dead fish into the corner of monkfish mouths to trigger a feeding response. Feeding attempts occurred every few days and increased during the summer months. Each feeding attempt was recorded, including technique and species used, weight of ingested food, water temperature, fish behavior and general health.

Acclimation time averaged 30 days but varied depended on the health of the fish. Behavioral indicators of acclimation included camouflaging, burrowing into the sand, waving the illicium to attract prey, and eating. When one or more of these acclimation indicators were observed, monkfish specimens were measured and injected with oxytetracycline or fluorexon using a 10 ml Luer Lock Norm-Ject syringe with a 20 G1 precision glide needle. Injections were either intramuscular or intraperitoneal. Intramuscular injections were in the dorsal side of the tail muscle and intraperitoneal injections were ventral, and involved pulling out the pelvic fins to create space between the skin and internal organs for insertion of the needle (Figure 1).

Oxytetracycline was injected in three concentrations: 25 mg Kg^{-1}, 50 mg Kg^{-1}, and 75 mg Kg^{-1} (McFarlane and Beamish 1987), and the powder was mixed in a 0.9 concentrated saline solution until it dissolved creating a clear yellow liquid with a pH of 1.6. Fluorexon was injected in two concentrations: 25 mg Kg^{-1} and 75 mg Kg^{-1}. The powder was mixed in a 0.9 concentrated saline solution, and approximately 1 g of sodium carbonate was added to buffer the solution for each gram of fluorexon. The liquid became dark orange with a pH of 6.5. Some monkfish specimens were not injected to serve as controls for investigating effects of injections.

Field experiments used monkfish specimens that were caught in the wild during dedicated tagging trips and during regular fishing operations on commercial gillnet vessels. Only healthy fish, with no body damage, with bright clear eyes, and actively swimming, were chosen for the study and kept aboard the fishing vessel in live wells. Following the injection protocols developed in the laboratory, each fish received an injection of one of the chemical markers.

A Star-Oddi data storage tag was implanted under the skin of injected monkfish released back into the wild, and an external visible tag was attached alerting fishermen the recaptured fish was worth $500. During the beginning of the study, we experienced relatively low recapture rate because of poor tag visibility. The external pink T- bar tags were only visible on the dorsal side, and they often were fouled with algae (Figure 2), and shedding rate was high (18.6 %, Sherwood et al. 2009). We remedied that problem with larger external floy tags secured through the tail muscle. We revised our tagging protocols and adopted methods developed by Ofstad (2013), who had high tag retention rates for fish at large for several hundred days. The change in tagging protocols (Figure 3) increased tag retention and recovery rates. For detailed tagging procedures see Grabowski et al. (2014).

When fish died in the laboratory or were returned from the field, they were measured, weighed and dissected. All three types of age structures (illicium, otoliths, and vertebrae #8 and #10) were extracted. Sex and maturity stage was determined from macroscopic examination of gonads. Each of the three structures was embedded in epoxy (5 parts expoy:1 part hardener; West Systems©), and allowed to harden in the dark in silicon molds. A transverse section of one otolith through the nucleus and a transverse section of the illicium 0.5 cm above the basal bulb (Duarte et al. 1997) were cut with a double-bladed isomet saw (Buehler©) and mounted on glass slides. Another otolith was polished whole for image analysis. A sagittal section (0.3 mm) centered on the focus of the vertebral centra was cut and mounted using the same method. An Olympus BX51 microscope with an ultraviolet light attachment was used to detect the presence of the chemical mark and images of the sections were taken using the Cool SNAP Pro color digital camera.

The #8 vertebra was kept intact and viewed under a Nikon SMZ 1500 microscope fitted with an ultraviolet light attachment and Nikon digital sight ds-fi1c camera. Images of the intact, whole vertebra were taken with imaging software NIS-elements under reflected light to show the growth rings, and under ultraviolet light to show the chemical mark. Two images were merged using Adobe Photoshop, and the opacity was adjusted to create a third image showing the chemical mark and the growth rings together (Figure 4).

Annuli counts on monkfish vertebrae cannot be identified from an image, because a physical ridge is associated with an annulus. The location of the chemical mark was drawn on each vertebra with a pencil, and vertebrae were baked in a drying oven at 230°C between 20 - 60 minutes. Following the protocols developed by Armstrong et al. (1997), an age reader with monkfish vertebral ageing experience was asked to 1) count annuli to estimate the age of each fish, and 2) indicate how many annuli were visible after the mark. The reader did not know when the fish was injected, how long it lived after injection, or the size of the fish. The reader was provided the month that the fish died, because ageing protocols follow the assumption of a January 1 birthdate. If a fish dies between January and June, another year is added to the age of the fish. This protocol assumes that the winter mark is being formed and is not visible. Fish that die between July and December are assigned an age based on just the number of visible annuli.

Illicia from the same eleven fish were aged and the number of annuli after the mark were counted by an inexperienced age reader. The protocols developed by Duarte et al. (2002) were followed along, with recent modifications suggested by Landa et al. (2013), including the use of thicker sections of illicia (0.5 mm), less magnification (40x), and adjusting the light and focus on the microscope to better identify the well-defined growth bands (annuli). The first annulus was counted based on the criteria developed by Wright et al. (2002), who concluded that the oval structure in the center represents a benthic ring, and the true first annulus is the first identifiable ring beyond the oval (Figure 5).

We explored changes in behavior that might resulting from stress of the tagging and injection procedure could disrupt a seasonal cycle of growth and alter ring formation. The frequency of off bottom movements of injected fish released into the wild with data storage tags were compared to the frequency of off bottom movements of non-injected fish released into the wild.

Results

Short-soak gill net sets during commercial fishing operations were the most successful for obtaining healthy specimens with minimal gear damage. If monkfish had visible injuries, they did not survive in the lab. Over the course of this study, 72 monkfish were transported to the sea water lab, 34 were injected with a chemical marker, and five were controls. Size ranged from 29 cm to 69 cm, with and average total length of 51 cm. Fourteen monkfish were injected with fluorexon, and twenty monkfish were injected with oxytetracycline. Ten fish lived for six months or more, and six were used in this study. Two fish are currently alive, one fish did not show a clear mark on the vertebra at time of analysis, and one specimen died after analysis had been completed. Growth for the six laboratory fish used in the study ranged from 2 cm to 18 cm (Table 1). Important factors in keeping monkfish alive in captivity were learned through trial and error. Keeping each monkfish separate from others reduced stress, sterilized sand decreased ventral abrasions, and degassing towers on individual tanks, with additional ultraviolet sterilizers within the system helped to increase survival.

Some fish preferred live fish and would also actively strike at dead fish. However, most fish would ingest food only if force fed. Some fish used their esca to attract food, but not consistently, and other specimens did not used their esca to feed. Feeding behavior and frequency of eating in the laboratory varied widely. Some fish ceased feeding when there was no change in temperature or any noticeable change in laboratory conditions, but would resume feeding weeks later. Stimulating monkfish to eat was the most common challenge. However, one specimen apparently overfed and required gastric lavage, but the specimen survived and feeding resumed with a more conservative schedule. Despite the own unique feeding habits, food consumption generally increased as temperatures increased in the laboratory peaking in September (12.8°C) then consumption decreased in October and November, even though water temperatures remained high (13.2°C) (Figure 6).

An intraperitoneal injection of 25 mg $Kg^{-1}$of fluorexon was the more successful method for marking age structures. Oxytetracycline produced a visible mark at all three concentration levels, 25, 50 and 75 mg Kg^{-1}, but intramuscular injections caused swelling, a fluid filled abscess, and tissue necrosis at the injection site (Figure 7). The fluorexon mark was visible at both 25 and 75 mg Kg^{-1}, and produced a more visible mark than oxytetracycline. Oxytetracycline marks lost intensity over time, but flourexon did not.

Between 2009 and 2013, 254 monkfish were injected with chemical markers and released with data storage tags, and 13 fish were recaptured (5% recapture rate). Ten fish received 25 mg $Kg^{-1}$of fluorexon, two 75 mg Kg^{-1} oxytetracycline, and one 50 mg Kg^{-1} oxytetracycline. Six fish were at large for six months or more, and five fish were used in this study, because one fish did not show a clear mark on the vertebra at the time of analysis. Three fish shed their data storage tag with the unique identification number, so their growth is unknown. Growth of the other two fish was 4 cm and 7.5 cm (Table 2).

All three age structures from laboratory specimens (n=32) fish and field recaptures (n=13) were analyzed to detect chemical marks. Most specimens from the field and the laboratory, did not live long enough or grow enough to show a clear, distinct mark separate from the edge. Under ultraviolet light the edge of the age structures from the control fish did not fluoresce, but the edge fluoresced for injected fish (n=25) indicating both chemicals were being incorporated into the calcified structures (Figure 8). The chemical mark was not seen in every sample, and the visibility of the mark varied between structures. Illicia (94%, n=18) showed the mark more clearly and frequently (94%, n=18) than vertebrae (89%, n=19) for both laboratory and field recaptured fish that lived 90 days or more. Many otoliths showed no mark at all (39%, n=18), even though the illicium and vertebra from the same specimen showed a distinct mark. Although a mark was not identifiable in the sectioned otolith images, the mark became clearly visible in the image of the polished whole otolith for three of the five samples. However, the mark was not continuous throughout the otolith indicating the chemical was not incorporated uniformly into the structure (Figure 9). The other two whole polished otoliths showed no mark (60%, n=5).

The age reader correctly identified the expected number of annuli after the mark for five out of eleven samples (45% accuracy). Five samples, four which were from fish that lived over a year, were expected to have a winter ring, but a clear winter ring was not seen. Therefore, age was underestimated for five fish that were larger than 50cm (Table 3). Conversely, age of a relatively small fish (41 cm) was overestimated, because one annulus was counted and the edge was counted as a second "annulus" based on the month of the sample.

Illicia from the same eleven samples were inspected to determine if a recognizable winter ring was visible after the mark. Nine samples were analyzed, because one illicium did not show a mark (Fish ID W), and one illicium was missing (Fish ID 4). For eight of the nine samples, the correct number of annuli were counted after the mark for (Table 4). Three specimens that lived more than one year after injection had a clear annulus in the illicium after the mark, but no clear annulus on the vertebrae after the mark. Age was underestimated for one sample (Fish ID 1) that was injected in November and died in July, but the expected winter ring was not visible on the illicium or the vertebra. Three fish that lived over a year, and had a missed annulus on the vertebra, did show a winter ring on the illicia (Figure 10). The one fish that was over aged (Fish ID W) with the vertebral method, did not show a mark on the illicium and the sample had to be omitted.

Injected fish (n=29) exhibited off bottom migrations 39% of the time, as compared to 18% of the time for non-injected fish (n=6). Four fish were released in off-shore waters (100 – 280 meters) and migrated inshore (50 meters) in a short time span (30-50 days). Three fish (one injected, two not injected) exhibited one or two off bottom migrations as they moved inshore. The fourth fish (not injected) exhibited 15 off bottom migrations in the same time frame until it reached inshore waters, and then continued sporadic off bottom movements throughout the remainder of its time at large. Another example of different off bottom behavior between two injected fish of similar size (72 cm, 78.5 cm), both released in the Western Gulf of Maine. One fish, recaptured 15 days later moved 48 nautical miles northwest of the release location, showing 16 off bottom movements during that short time. The other fish, released the next day showed 2 off bottom movements over a span of 76 days and was recaptured a few miles from its release location.

Discussion

Results indicate annuli counts on vertebrae cannot be used to accurately determine the age of monkfish. The failed validation of the vertebral ageing method has important implications for stock assessment and management on monkfish fisheries. The pattern of underestimating age of large monkfish and overestimating age of young monkfish suggests that the apparently linear growth assumed in the statistical catch at length assessment (NEFSC 2013) may be misleading.

The vertebral ageing protocol includes the addition of an extra year to the observed number of annuli on a vertebrae to account for the formation on an annulus on the edge of fish sampled in the first six months of the year (January-June). However, this protocol only slightly influenced our results. Fish W that was over aged by a year would have had a correct annulus count since it lived through one winter.

There are a few hypotheses to explain why an annulus may not be apparent. Maartens (1999) and Griffiths & Hecht (1986) hypothesized that the numerous, narrow opaque and translucent rings indicate the annual growth of the *Lophius* species is a multiple, sporadic phenomenon rather than one of traditional seasonal growth. Therefore, the prominent rings we counted as annuli could indicate spawning periods or periods of fast growth that are not influenced by temperature and season but rather by food availability.

Our results suggest that the otolith is not growing uniformly and is not reliable for counting annual growth rings. The frequent absence of a mark in the otolith was surprising, because the otolith is typically the structure of choice for age determination. It appears that monkfish otoliths do not grow uniformly in all directions. Other chemical validation studies have reported similar results.

Although the illicia ageing results are based on limited sample size, this structure showed the most promise for having identifiable annual growth rings. However determining the first annulus is critically important. Despite the revised protocol (Landa et al. 2013), recognizing "true" versus "false" annuli remains difficult. One sample had an apparent annulus after the chemical mark on the illicium, but the annulus was not apparent in the vertebra (Figure 10). Six out of the nine samples aged with both methods had the same age. For, the three samples that did not have the same age, the illicia read fish were aged one year younger. Therefore, similar to vertebrae, illicia may also not having consistent, recognizable annual rings that can be counted reliably. To determine if illicia age readings are reliable, the structures used in this study need to be read by another inexperienced reader for comparison, and then by an experienced reader to help guide new protocols. Additional samples should be used to validate the illicia ageing method to determine if the new method is reliable.

Maintaining monkfish in captivity was a major challenge. Woodroffe et al. (2003) reported that previous validation studies were not been successful because of the difficulty of handling and maintaining anglerfish. Hoshino et al. (2006) had limited success developing an adult cultivation system for *Lophius litulon*. They found that anglerfish are prone to injury, and only fish with few injuries should be selected for long term breeding. Our results suggest that obtaining healthy, undamaged fish was a necessity to survival in captivity. Therefore, collaborating closely with a few fishermen who took the time to save fresh, undamaged fish and transport them carefully to shore was an important factor for survival of specimens.

Improvements in laboratory conditions also helped to increase the longevity of monkfish in captivity. Monkfish inhabit areas throughout a large temperature range, 0˚C - 21˚C, but Bigelow and Schroeder (1953) and Richards et al. (2008) collected 90% of their monkfish samples in 4.5˚C – 13.0˚C. Temperatures near the extremes of the reported range of monkfish or rapid temperature shifts in the laboratory caused stress and sometimes death. Surprisingly, data from archival tags document that monkfish in the wild can adjust to extreme temperature shifts during a short time frame. Off bottom excursions that range between 50 and 80 meters involve passing through a temperature shift from 5 to 13 °C in a few hours. Such tolerance to fast temperature changes was not observed in the laboratory. A large capacity heater and chiller, along with an emergency monitoring device for changes in temperature and water level proved to be necessary investments. However, despite improvements from the initial capture and handling techniques and continual improvements made in the laboratory, only six fish survived long enough to be included in this study.

Monkfish are reported to eat anything (Bigelow and Schroeder, 1953). Documented accounts from fishermen and researchers describe pulling strange debris (Connolly, 1920), birds

(Perry et al. 2013), and all types of fish (Bigelow and Schroeder, 1953), including their own species, (Johnson et al. 2008) from the guts of monkfish. Contrary to these field observations, monkfish in the laboratory would not eat a variety of prey species in various stages of freshness (live, freshly dead, chilled not frozen, and frozen). Furthermore, the digestion process often ceased during our holding experiments. One monkfish rejected food for 63 days, then started accepting force fed squid. Over the next month, the specimen regurgitatedeach squid undigested. Laboratory observations may not represent natural feeding, but fishery observations may also indicate unnatural behavior (i.e., net feeding).

Although injected fish exhibited off bottom migrations more frequently than non-injected fish, there are other variables that can influence off bottom migrations. There is too much variability in off bottom behavior to attribute it to stress from the injection and tagging procedure. Therefore we can't determine if the annual growth pattern was disrupted due to stress.

The temperature from data storage tags tell us that fish in the Western Gulf of Maine stay in bottom temperatures of 6° C in the summer, but they vertically migrate into temperatures of 12 – 14° C for a few hours at a time before dropping back to 6° C. If we assume a seasonal cycle of growth does the large range of temperatures they experience during a season effect the growth pattern?

Our results indicate an intraperitoneal injection of 25 mg Kg^{-1} of fluorexon is the preferred method to mark the age structures of monkfish. We recommend an intraperitoneal injection, becasue both chemicals caused a fluid filled abscess in the tail muscle after injection, and oxytetracycline caused additional tissue necrosis (Figure 7). The acidity of oxytetracycline may explain why the intramuscular injections caused tissue necrosis. Oxytetracycline has a pH of 1.6 and comes out of solution if buffered with sodium bicarbonate. Fluorexon has a pH of 6.5, and sodium bicarbonate is required to buffer the solution. Bush et al. (1996) reported that injecting high volumes (100 mg/ml) of oxytetracycline solution into muscle tissue produced tissue necrosis. There are also advantages to using fluorexon in the field, because it can be mixed beforehand and used throughout the day without coming out of solution and it does not have to be kept cold like oxytetracycline. Another advantage of fluorexon is the intensity of the mark. The fluorexon mark is more visible and it does not lose its intensity from repeat illumination like oxytetracycline. Two fish from this study, both injected with oxytetracycline, did not have a readable mark on the vertebrae when examined a second time for validation. Gelsleichter et al. (1997) and Monaghan (1993) also concluded that calcein is the better marker due to the intensity of the mark. Monaghan (1993) also noted that oxytetracycline appeared detrimental to the health of the fish, because fish treated with oxytetracycline became lethargic and stopped eating, whereas fish treated with calcein did not change behavior. During our study, injections from both chemicals caused alterations in feeding behavior. One disadvantage of fluorexon is that it is much more expensive than oxytetracycline.

In summary, monkfish age structures can be marked with oxytetracycline and flourexon at low doses. Flourexon left a more intense mark and did not disappear or lose its intensity over time like oxytetracycline. Illicia and vertebrae show a strong mark more consistently than the otolith. The vertebral ageing method cannot be validated, because age of large fish was underestimated, and age of a small fish was overestimated. Otoliths cannot be used to count annual growth rings, because the chemical was not detected uniformly throughout the structure. Preliminary results indicate illicia produce a recognizable annual growth ring. Illicia ageing protocols should be developed, and more marked samples from injected specimens should be used to validate illicia ageing method.

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Table 1. Monkfish that survived six months or more in the laboratory after injection with oxytetracycline (OTC) or fluorexon (FL). Length indicates measurement at time of injection.

FISH IDENTIFICATION	INJECTION DATE	CHEMICAL & AMOUNT (mg Kg^{-1})	LENGTH (cm)	GROWTH (cm)	SURVIVAL TIME (days)
F Male	9/8/2010	75 OTC	40	18	767
S Male	11/15/2010	25 OTC	51	6	467
T Female	6/6/2011	50 OTC	49	5	606
J Female	6/6/2011	25 FL	48	2	398
M Female	6/6/2011	25 FL	57	3	251
W Female	5/24/2012	25 FL	33	8	298

Table 2. Monkfish recaptured after six months or more in the field after injection with oxytetracycline (OTC) or fluorexon (FL). Length indicates measurement at time of recapture.

FISH IDENTIFICATION	INJECTION DATE	CHEMICAL & AMOUNT (mg Kg^{-1})	LENGTH (cm)	GROWTH (cm)	SURVIVAL TIME (days)
7 Female	4/16/2011	50 OTC	63	4	185
1 Female	11/10/2009	75 OTC	75.5	7.5	248
4 Male	10/17/2012	25 FL	71		333
2 Male	10/17/2012	25 FL	67		365
5 Female	10/17/2012	25 FL	76.5		537

Table 3. Eleven monkfish samples used in the Vertebral Validation study. Red rows indicate fish that did not have the correct number of annuli counted after the chemical mark. The green color code in the "Month of death" column indicates which fish had an extra "annulus" added to its age.

FISH ID	Injection Date	Days Alive	Month of Death	Size at Injection	Size at Death	Growth cm	Gender	# Annuli after the mark	Expected Annuli	Lab / Field
2	Oct	365	Oct		67		M	0	1	Field
F	Sept	767	Oct	40	58	18	M	2	2	Lab
J	June	398	Jul	48	50	2	F	0	1	Lab
S	Nov	467	Feb	51	57	6	M	1	2	Lab
T	June	606	Feb	49	54	5	F	2	2	Lab
4	Oct	333	Sep		71		M	0	1	Field
5	Oct	537	Apr		76.5		F	2	2	Field
W	May	298	Mar	33	41	8	F	2	1	Lab
7	April	185	Oct	59	63	4	F	0	0	Field
M	June	251	Feb	57	60	3	F	1	1	Lab
1	Nov	248	Jul	68	75.5	7.5	F	0	1	Field

Table 4. Eleven monkfish samples used to test illicia ageing methods. Red row indicates fish that did not have the expected winter annulus counted.

FISH ID	Injection Date	Days Alive	Month of Death	Size at Injection	Size at Death	Growth cm	Gender	# Annuli after the mark	Expected Annuli	Lab / Field
2	Oct	365	Oct		67		M	1	1	Field
F	Sept	767	Oct	40	58	18	M	2	2	Lab
J	June	398	Jul	48	50	2	F	1	1	Lab
S	Nov	467	Feb	51	57	6	M	1	1	Lab
T	June	606	Feb	49	54	5	F	1	1	Lab
4	Oct	333	Sep		71		M	NO ILLICIUM		Field
5	Oct	537	Apr		76.5		F	1	1	Field
W	May	298	Mar	33	41	8	F	NO VISIBLE MARK		Lab
7	April	185	Oct	59	63	4	F	0	0	Field
M	June	251	Feb	57	60	3	F	0	0	Lab
1	Nov	248	Jul	68	75.5	7.5	F	0	1	Field

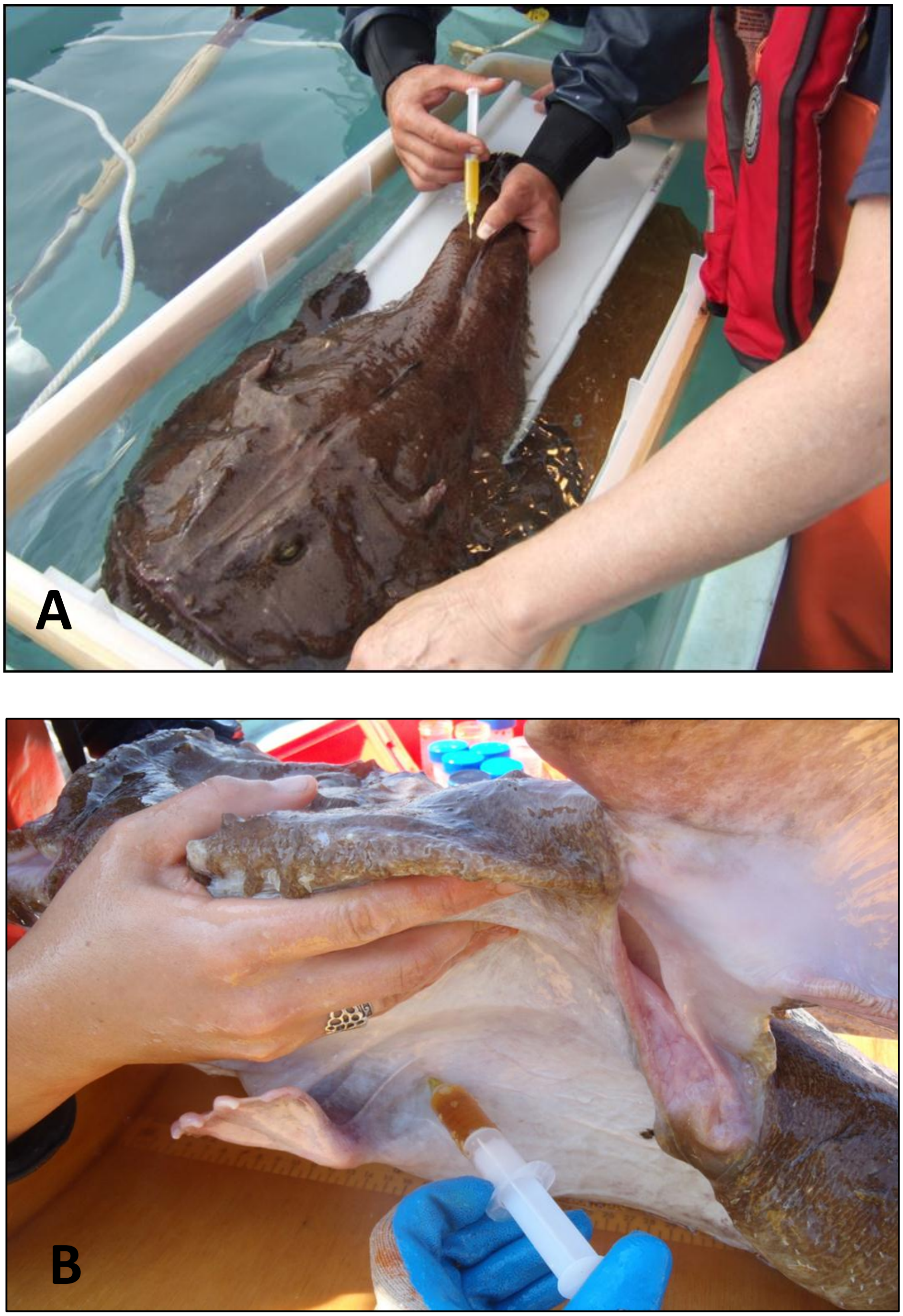


Figure 1. A) Monkfish receiving an intramuscular injection of oxytetracycline

B) Monkfish receiving an intraperitoneal injection of fluorexon

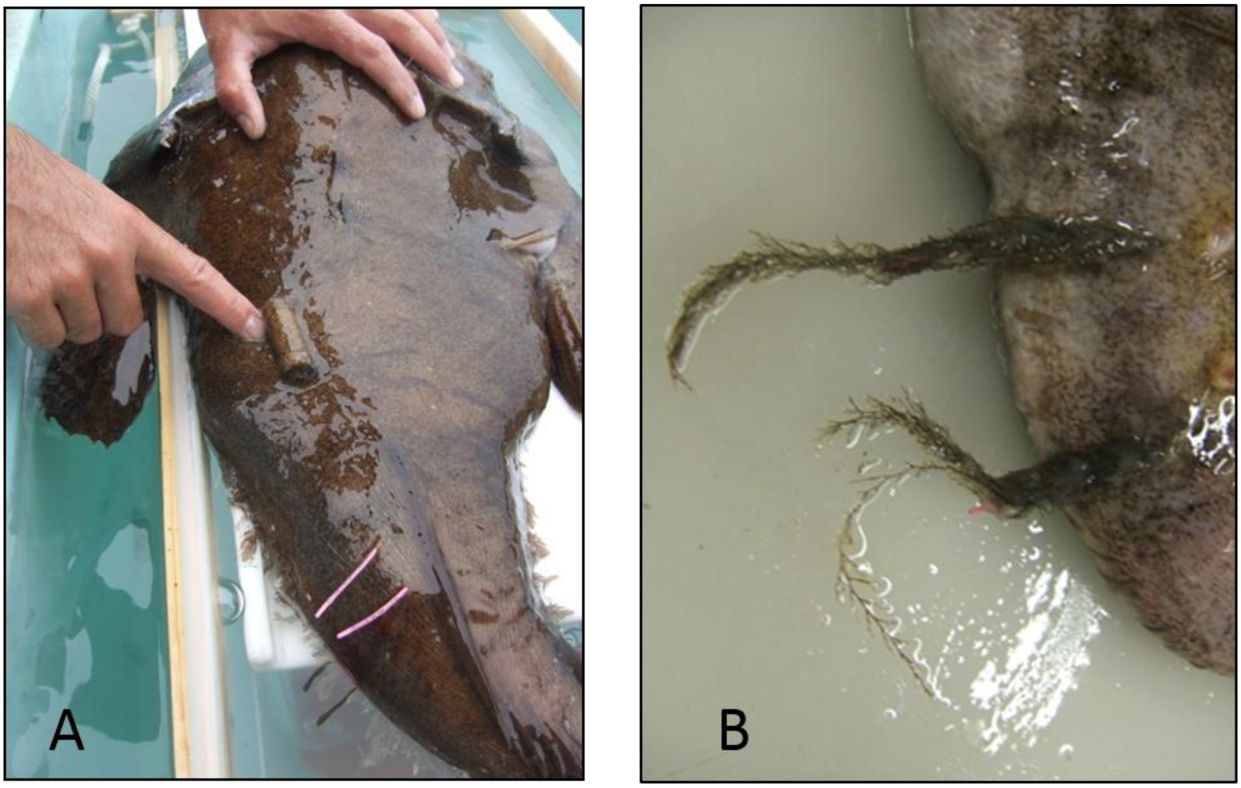
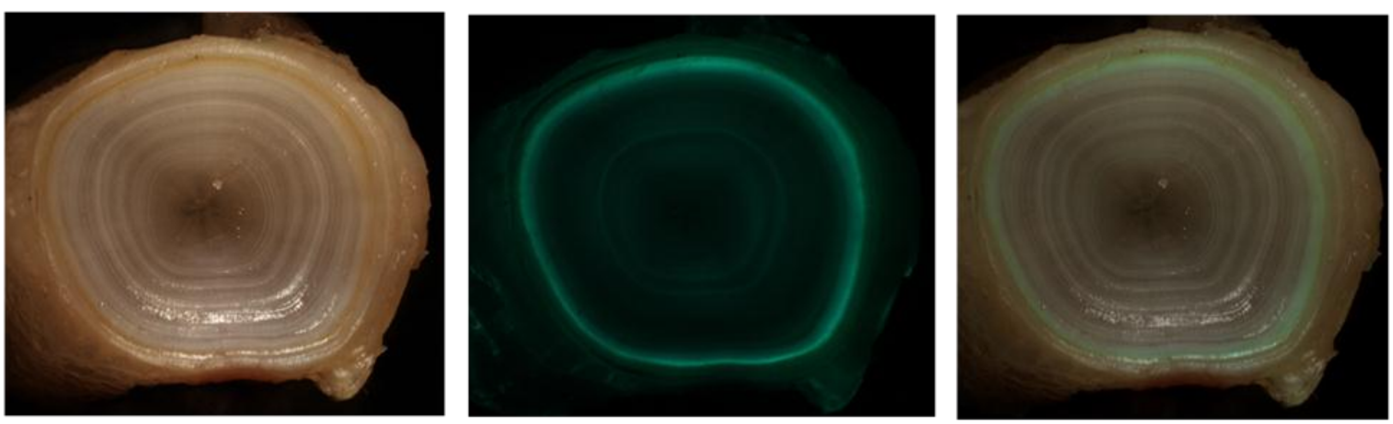


Figure 2. A) Monkfish with a Star-Oddi data storage tag implanted under the skin (where finger is pointing), and two external pink T-bar tags visible on dorsal side. B) Pink T-bar tags fouled with algae after Fish ID 7 was recaptured 185 days later.

Figure 3. Monkfish with new more visible external pink floy tags, and new implantation technique for data storage tags.



a) Vertebra under reflected light | b) Vertebra under UV light | c) Vertebra images merged

Figure 4. Vertebra from a recaptured monkfish, 119 days at large after a 25 mg/kg injection of fluorexon. 3 cm growth, gender unknown,

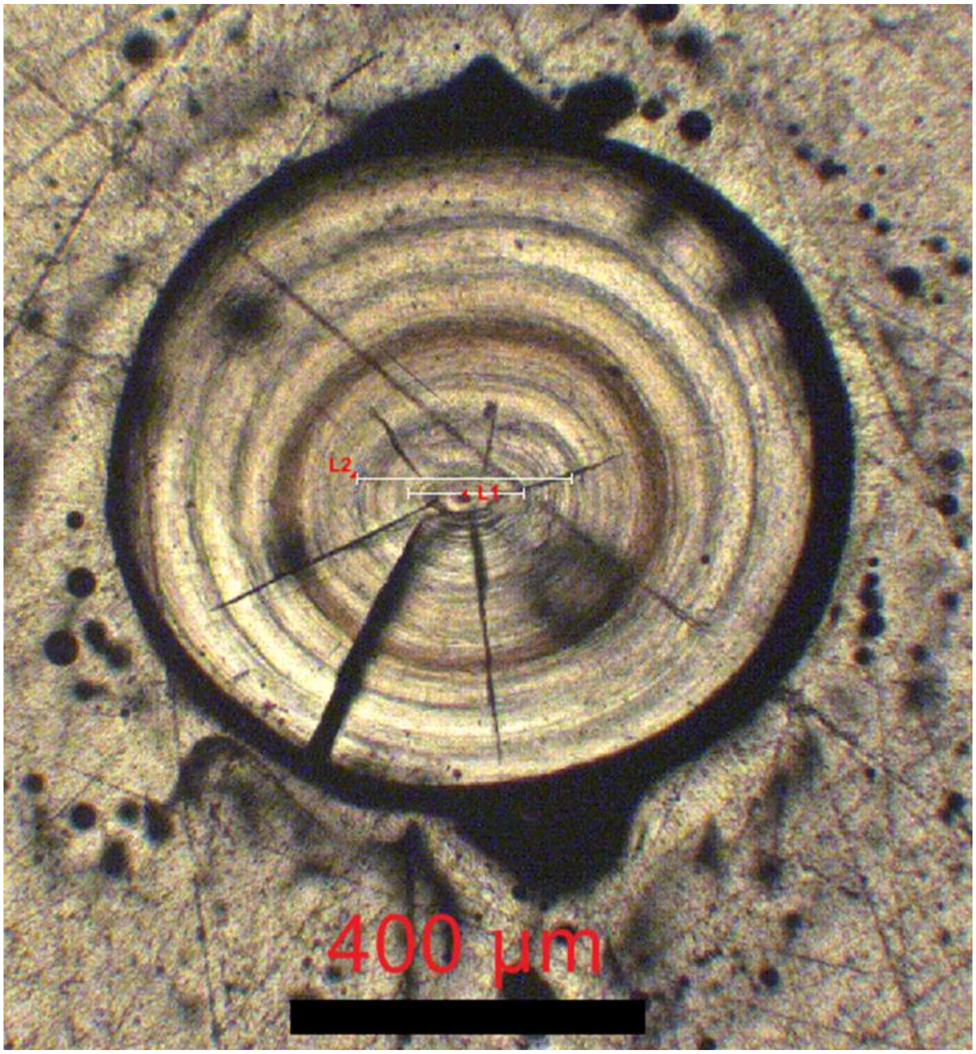


Figure 5. Illicium from Fish ID F: The short white line indicates benthic ring, long white line indicates first annulus.

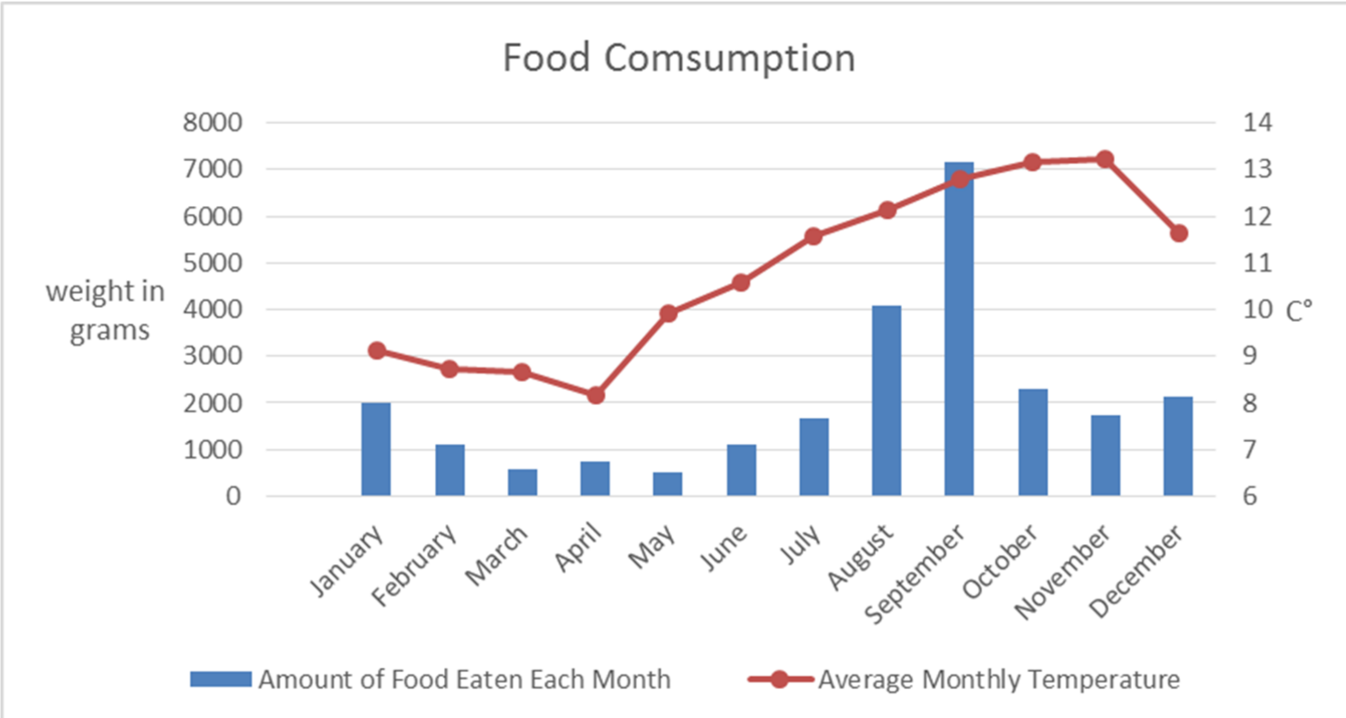


Figure 6. Combined weight each month of ingested food from five of the six laboratory fish used in this study.

Figure 7. Tissue necrosis and discoloration in tail muscle 248 days after a 75 mg Kg^{-1} injection of oxytetracycline.

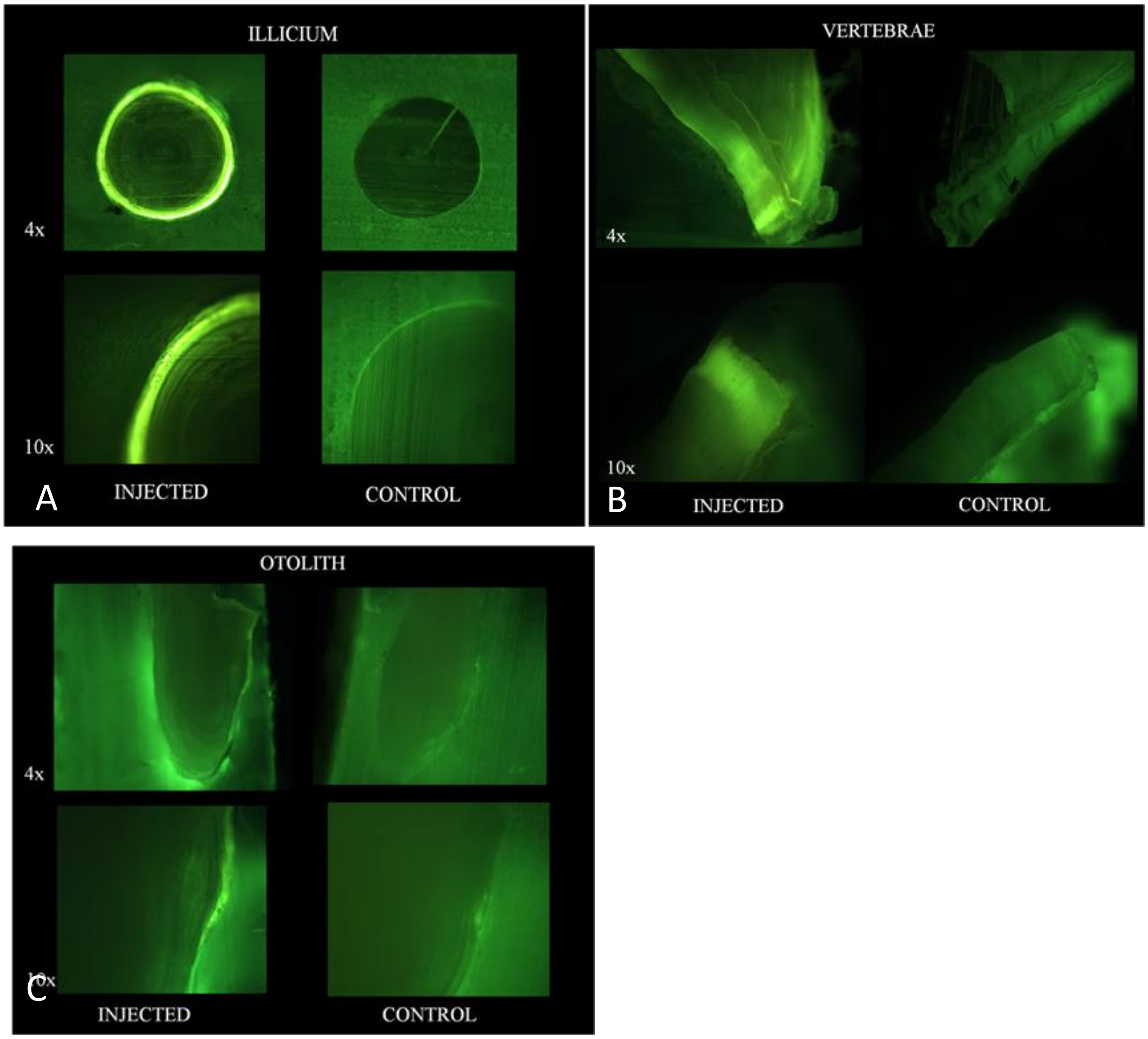


Figure 8. Age structures from a recaptured monkfish, 213 days at large, 2.5 cm growth, male. Images on the left from the 75 mg Kg^{-1} oxytetracycline injected fish, and a control fish - no injection - on the right. A) Illicium B) Section of vertebra centra C) Otolith

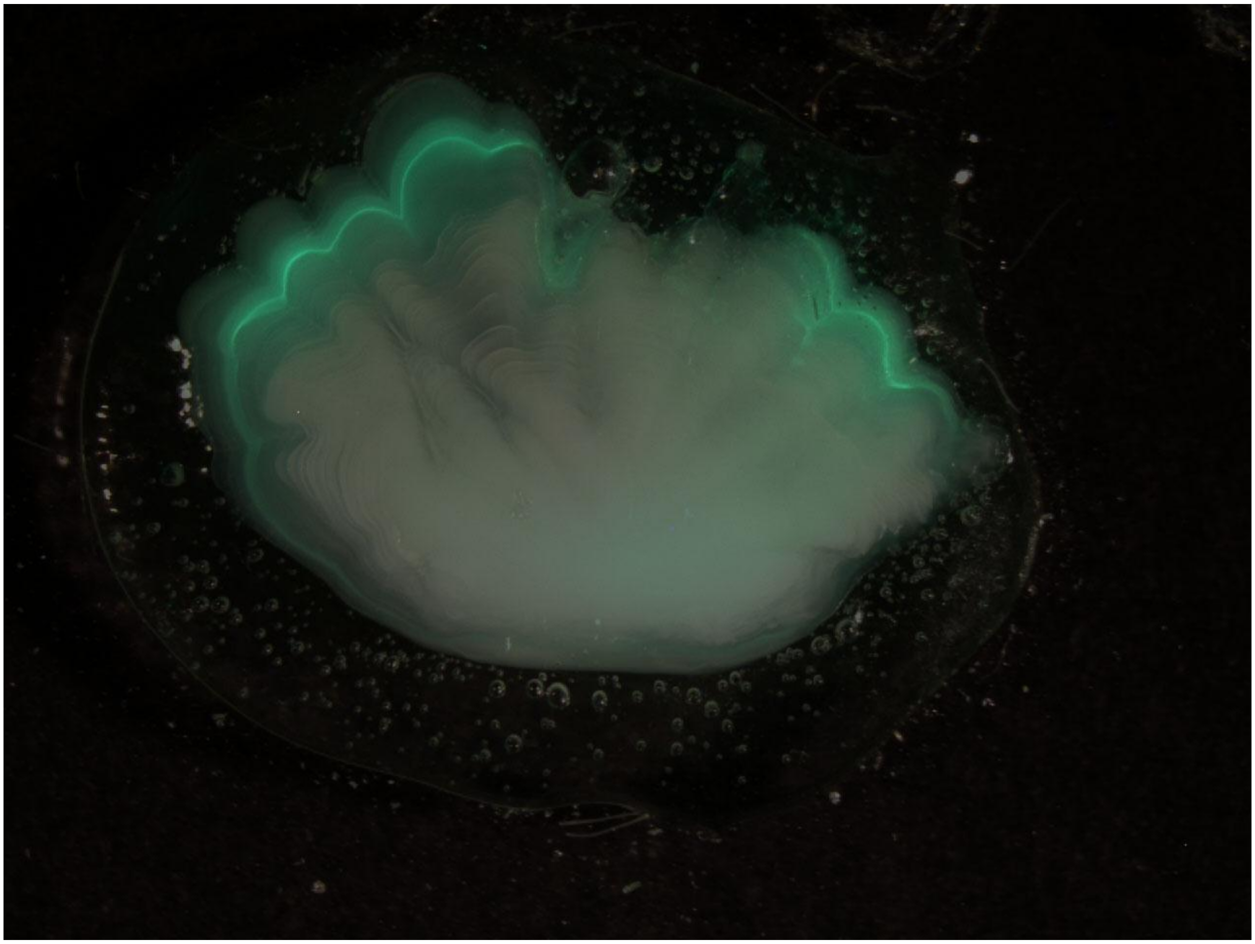


Figure 9. Polished whole otolith from Fish ID S. Mark is visible but not continuous.

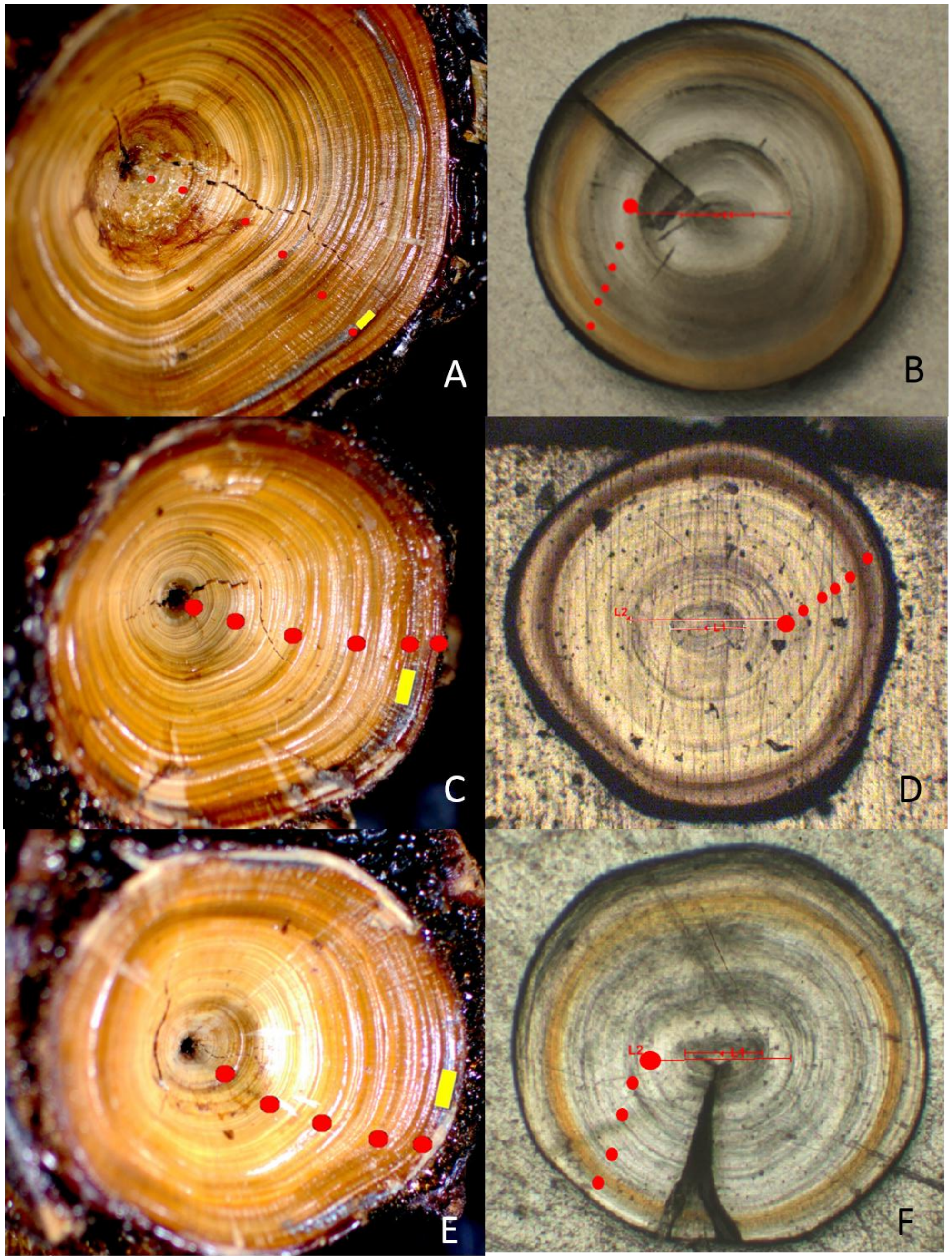


Figure 10. Vertebrae and illicia samples from three fish that lived for over a year.

Top row: A) vertebra from Fish ID 2. Red dots indicate annuli, yellow rectangle indicates where chemical mark is. B) Illicium from same fish, red dots indicate annuli, chemical mark is visible.

Middle row: C) Vertebra from Fish ID S. D) Illicium from same fish.

Bottom row: E) Vertebra from Fish ID J. F) Illicium from same fish.

Otolith microchemistry analysis results for age and growth

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EXECUTIVE SUMMARY

Reliable age determination is the cornerstone of effective age-based fisheries management. Recent assessments of monkfish (*Lophius americanus*) in US waters points to some potential uncertainties regarding age of monkfish that may be undermining management efforts for this economically important species. Here, we outline results of an otolith microchemistry analysis to aid in the interpretation of monkfish age. Specifically we analyzed a subset of monkfish otoliths (N = 8) for strontium/calcium ratios (Sr/Ca), a proxy for temperature, over the entire surface of sectioned and polished otoliths. The resulting 2D surface (heat) maps of Sr/Ca values were overlaid on and compared to otolith cross-sectional images to increase confidence in the identification of annuli. This was particularly helpful for identifying the first one or two annuli. For an even smaller subset of otolith images (N= 3), calcein marks from the chemical injection component of this study were visible and further aided in the identification of distal annuli. Our Sr/Ca and calcein age interpretations ranged from 2 to 8 years old for fish ranging in size from 41 to 82 cm. The resulting growth curve was curvilinear due to relatively rapid growth in the first few years and slower growth as fish age. This is in contrast to the current vertebral method for ageing monkfish which results in a linear growth curve likely due to positive age bias for mostly younger ages. Our results for asymptotic growth and the resulting Von Bertalanffy growth function parameter estimates are consistent with results from our previous tagging studies (Sherwood et al. *in prep*) and for growth patterns in other monkfish species around the world.

1. INTRODUCTION

Monkfish (or goosefish, *Lophius americanus*) has been the highest valued finfish in the northeastern United States since the mid-1990s following the decline of traditional groundfish species (e.g., Atlantic cod, *Gadus morhua*, and haddock, *Melanogrammus aeglefinus*) and the rapid development of the monkfish fishery (Richards et al., 2008). Despite its economic importance to the U.S. east coast fishery, the life history of monkfish is poorly understood when compared to other commercially important species in the region. This lack of scientific attention means that very little is known about monkfish life-history parameters in U.S. waters including what they eat, where they move and how fast they grow. A minimal understanding of monkfish life-history parameters, including an uncertainty with age determinations, may be undermining effective fisheries management of this valuable resource (Richards et al., 2008).

Age for US monkfish is currently assessed by examining banding patterns (annulus formation) in vertebrae (Richards et al. 2008) based on work by Armstrong et al. (1992). Most recent estimates show linear growth (9.9 cm yr^{-1}) in U.S. monkfish with no difference between sexes and management areas (i.e., the southern fishery management area or SFMA, and the northern fishery management area or NFMA; these are south and north, respectively, of the 41st parallel with all points west of 70 degrees longitude and south of Cape Cod in the SFMA). Conversely, European monkfish (*L. piscatorius*) age is typically assessed using illicia (the first dorsal fin ray; Duarte et al. 1997, Duarte et al. 2005, Laurenson et al. 2005). In both cases, cross sections are prepared and annuli (yearly rings) are counted. Otoliths, the age structure of choice for many other species, have also been used in European monkfish (Woodroffe et al. 2003, Laurenson et al. 2005) as well as South African monkfish (*L. upsicephalus*, Griffiths and Hecht 1986), but are often considered to be less reliable (Peronnet et al. 1992) due to the presence of confusing banding patterns that include many secondary characteristics (checks or pseudoannuli) and a wider than normal opaque zone (Tsimenidis and Ondrias 1980, Griffiths and Hecht 1986, Crozier 1989, Woodroffe et al. 2003).

In terms of validating banding patterns and recent annulus formation in age structures, a previous tagging study of the European monkfish, suggested the use of an antibiotic (e.g., oxytetracycline or OTC) to validate age determination (Laurenson et al. 2005). This method can provide information on most recent annulus formation. No such validation has been conducted for U.S. monkfish stocks and indeed the Monkfish Assessment Report for 2007 (NEFSC Ref. Doc. 07-21) stated that "*There are some concerns with the ageing results. An ageing validation study should be undertaken to confirm the accuracy of catch at age estimates. Direct validation studies (e.g., oxytetracycline marking) have not been done*". Some of the concerns should include skepticism over findings of linear growth and no difference between female and male monkfish growth rates (Richards et al. 2008), particularly since male monkfish virtually disappear from the population at around 60-70 cm (Richards et al. 2008), whereas females routinely exceed 100 cm (Johnson et al. 2008). Conversely, growth in European monkfish, as in countless other non-related species, typically asymptotes (Duarte et al. 1997, Maartens et al. 1999, Landa et al. 2001, Fariña et al. 2008), and sexual dimorphism in growth has also been observed in other monkfish species (i.e., smaller size-at-age in males; Landa et al. 2001).

To address the recommendation of the Monkfish Assessment Report for 2007 (NEFSC Ref. Doc. 07-21), we conducted an age validation study of monkfish using a variety of complementary techniques. The main body of this report, to which this text is an addendum, outlines the results of multiple years of chemically tagging monkfish both in the laboratory and in the field as part of our ongoing data storage tag (DST) studies. These chemical marking efforts have provided excellent information on the process of annulus formation for the time at large or time in captivity (up to and in some cases exceeding 2 years). In order to understand annulus formation over the life of the fish we also made use of natural chemical tags or markers. A number of naturally occurring isotopes and heavy metals have been observed to track seasonal temperature fluctuations in fish otoliths (Gauldie et al. 1995, Townsend et al. 1995, Dufour et al. 2007, Weidel et al. 2007). One of these natural tags, strontium (Sr), can be measured at very high resolution (~ 1,000 samples cm^{-1}) via laser ablation inductively coupled mass spectrometry (LA-ICPMS) so that seasonal variations in Sr, and hence temperature, can be interpreted as a function of annulus formation on a micro scale. The advantage of the Sr technique is that annulus formation over the entire life of the fish can be examined as opposed to annulus formation at the edge (i.e., as in the case for chemical injections). In our previous report on this subject (Sherwood et al. 2012) we presented results to suggest that analyzing Sr over the entire surface of a prepared otolith (i.e., via multiple sample transects) was more powerful for age interpretations than conducting single transects (one otolith was previously analyzed for multiple transects). The purpose of this component of our study was to conduct multiple transects on a larger number of samples to aid in the age interpretation of a larger number of monkfish. In this case, we completed analyses for an additional 7 otoliths bringing the sample size of Sr aged monkfish to 8. We also used the resulting age and size data to construct a growth curve to compare to growth as derived by vertebral estimates of age as well as growth estimated from other sources (e.g., tagging; Sherwood et al. *in prep*).

2. METHODS

Monkfish otolith samples were made available from a variety of sources related to our ongoing Monkfish RSA activities. One sample, previously reported in another study (Sherwood et al. 2012) was collected as part of our first RSA project (Sherwood et al. 2008). Four samples came from fish held in the laboratory for chemical injection studies (the other component of this study). Finally, 3 samples came from recaptures as part of our ongoing DST studies.

Monkfish otoliths were chosen for strontium ageing rather than vertebrae (standard for NMFS age assessment), because previous studies examining the relationship between temperature and strontium concentrations have focused on otoliths (Gauldie et al. 1995, Townsend et al. 1995). Also, otoliths, along with illicia (first dorsal ray) are routinely used for ageing in European monkfish, *L. piscatorius* (e.g., Woodroffe et al. 2003). Monkfish otoliths can be prepared in a variety of ways for age reading including transverse and diagonal sections (using a diamond saw) and hand ground lateral sections. We chose the latter method since others have found that transverse and diagonal sections can be difficult to interpret (Maartens et al 1999). This may be because monkfish otoliths accrete along multiple “lobes” (Figure 1) and sections may sample more than one lobe, or in between lobes, thus obfuscating banding patterns. Monkfish otoliths were hand ground along the sagittal plane to thin lateral sections (Figure 1) using sequentially finer (180, 320, 400, 600, 1000) grit abrasive discs (Buehler©). Grinding was done on both the proximal and distal surface to the primordium resulting in a ~ 1 mm flat “disc”. Prepared otoliths were then fixed to a glass slide using Crystalbond™ mounting adhesive. The exposed side of the otolith was polished using a polishing cloth and MicroPolish II (0.3 micron) alumina power (Buehler©) in water

Prepared monkfish otoliths were analyzed for metal concentrations by laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) at the Bruneau Centre for Research and Innovation, Memorial University, St. John’s, NL Canada (Sample H) and by LA-ICPM at Woods Hole Oceanographic Institution, Department of Marine Chemistry and Geochemistry, Woods Hole, MA (all other samples). For Woods Hole samples, prior to ablation, a preablation pass was made to remove impurities on the surface of the otolith. Laser settings for power, laser fire rate and speed for the preablation pass were 10%, 10 Hz and 25μm/sec, respectively. Laser spot size was 50μm. No data was collected during preablation. For the data collection ablation pass, settings were 75% power, 10 Hz, 25μm/sec and 35μm spot size. The following metals were analyzed by ICPMS at Woods Hole: Mg25, Ca48, Mn55, Cu63, Zn68, Sr86, Sr87, Sr88, Cd114, Ba137, and Ba138. Ablation tracks were run parallel to one another from the top to bottom of each otolith with a spacing of 210 μm. Five of the otoliths analyzed at Woods Hole were sampled entirely; the number of transects per otolith ranged from 21 to 28 and samples from 3,610 to 4,340. Due to time constraints, two of the otoliths analyzed at Woods Hole were sampled only through the center so that transect number and sample size ranged from 5 to 6 and 869 to 1,396, respectively. For the Memorial University sample, ablation tracks were run from the primordium to the edge in a radial pattern (22 transects and 6,390 samples). Laser fire rate was 10 Hz and speed was 10 μm. Metals analyzed at the Memorial facility included Li7, Mg24, Mg25, Ca43, Mn55, Cu63, Zn66, Sr88, Cd111, Ba137, Ba138 and Pb208. Blanks and standards (MACS-3) were run every 3-4 samples to account and correct for any drift in detection.

In addition to otolith samples, 4 vertebrae samples were also analyzed for Sr/Ca values. Thin sections of vertebrae from the chemical marking portion of this study were mounted on slides and ablated along the longest exterior axis. Only one ablation transect per sample was performed. Sample sizes for each ablation transect ranged from 224 to 437.

For all data, Sr88 was standardized to Ca concentrations, Ca48 for Woods Hole samples and Ca43 for Memorial samples. Thus, we report Sr/Ca ratios. The ratios for Memorial University data was unitless (counts per count). The ratios for Woods Hole data were converted to absolute values (□g Sr/g Ca). To plot the data in two dimensions we digitized images of ablation tracks and interpolated x/y coordinates along each track so that each sample for each transect had a unique x/y coordinate. Contour maps of Sr/Ca values were then created in Surfer® 13.0.383 (Golden Software LLC) from grid files by kriging. Resulting "heat" maps show Sr/Ca values with red (warm) for low values and blue (cool) for high values. The strong relationship between otolith Sr/Ca and temperature for monkfish was shown in Sherwood et al. (2012).

Images of prepared otoliths were compared to heat maps by aligning the two and by superimposing the heat map on the otolith image so that both visual annuli (i.e., opaque and translucent zones) and temperature for each zone were visible in one image. For three of the images, a calcein mark was available and evident and could also be used in interpretation of the distal annuli. Otoliths were aged by counting annuli (translucent + opaque zones = 1 annulus and cold + warm rings = 1 annulus). A conventional birthdate of January 1 was used so that annuli began on a translucent/cold zone. In reality, monkfish are likely born sometime in the late fall (Richards et al. 2008) so that a January 1 birthdate would be passed at 1-2 months old. This early annulus was included in the first full annulus so as not to artificially inflate the age of monkfish. Because this ageing technique is experimental no independent reader verifications were performed. Rather, to validate our results, we compared the growth curve generated by the Sr/Ca aided age estimates with a growth curve generated from a completely independent method using tagging data (Sherwood et al. *in prep*). In this case, growth was approximated by fitting a Von Bertalanffy growth curve to the limited data set.

3. RESULTS AND DISCUSSION

Images of all prepared otoliths, Sr/Ca heat maps and image overlays are shown in Figure 2 A-H. In many cases annuli were clearly visible on the heat maps alone, some more prominent than others. In cases where annuli from the heat map were more ambiguous, the heat map overlain on the otolith image resulted in higher confidence of interpretations. Additionally, the calcein mark visible in samples B, C and E helped to identify the last 1-1.5 years of otolith accretion for those samples. See Table 1 for length of time alive following chemical marking for these samples. In general, the results of the calcein marking and the Sr/Ca analysis revealed that annuli are rather large/wide for the first year or two and quite narrow for the most recent bands. Age estimates from examining Sr/Ca trends on the vertebral samples agreed perfectly with the age estimates from the otoliths (Table 1, Figure 3).

Age results for the samples analyzed for Sr/Ca (N = 8) were plotted against length to produce growth curves (Figure 4). Age estimates ranged from 2 to 8 years old. The resulting growth curve was curvilinear or assymptotic reflecting rapid early growth and slower growth at older ages. This growth curve was very similar (Table 2) to a modelled growth curve using tagging data which also showed assymptotic growth (Sherwood et al. *in prep*). The Sr/Ca and tagging data-derived growth curves deviated substantially from the linear growth curve generated by vertebral-based age estimates and currently used in the assessment (Richards et al. 2008).

It would appear that the visual method for interpreting vertebral annuli overestimates age for the majority of monkfish. In our limited dataset, the discrepancy ranged from 1 to 3 years (positive bias). Examining the different growth curves (Figure 4), the biggest difference in age interpretations are likely to occur for monkfish between the ages of 2 and 7 where the curves diverge the most. The age estimates appear to converge around 8-10 years old. If the Sr/Ca age estimates are accurate, initial growth in monkfish is much more rapid than is currently recognized. This would mean that the species reaches sexual maturity and recruits to the fishery at a much younger age. It is beyond the scope of this study to examine what this means for the assessment. However, if the situation is similar to that reported by Bertignac and Pontual (2007) for European hake (*Merluccius merluccius*), positive age bias in the assessment would mean that biomass and abundance are currently overestimated, fishing mortality is underestimated and productivity (yield per recruit) is underestimated.

All ageing studies carry an element of subjectivity. Using multiple lines of evidence this study attempted to reduce this potential age bias by reducing the reliance on visual inspection of banding patterns alone. By examining physical banding patterns (i.e., opaque/translucent zones) along with Sr/Ca banding patterns, that further carry information on seasonal cycles in temperature, and calcein marks (an absolute indication of recent annulus formation), it is likely that the age estimates in this study represent the closest estimate of true age available for this poorly understood fish species. The fact that the Sr/Ca age estimates for both otoliths and vertebrae agreed and that the growth curve derived from these agreed with the growth curve modelled using tagging data suggests that the assymptotic growth curve is the correct growth pattern to apply. Further work should be done on how to incorporate this new information, mostly the finding that the first annulus is larger than recognized and therefore monkfish are 1-3 years younger than assumed, into the monkfish assessment.

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Table 1. Sample summary: study refers to what study the otolith sample originated ('chem' for chemical injection studies, 'DST' for data storage tag studies and 'diet' for original RSA diet study); lab refers to laboratory where LA-ICPMS analysis was conducted; transects is the number of laser ablation tracks that were completed; samples is the number of individual Sr/Ca measurements made for each otolith over all transects; length is total length (cm); $Age_{Sr/Ca(oto)}$ and $Age_{Sr/Ca(vert)}$ are age estimates from the Sr/Ca method for otoliths and vertebrae, respectively; Age_{vert} is the age estimate from reading vertebrae; and calcein mark refers to whether the calcein mark from the chemical injection portion of this study was available and visible.

Sample ID	Sample name	Study	lab	transects	samples	Length	Sex	Days alive*	$Age_{Sr/Ca(oto)}$	$Age_{Sr/Ca(vert)}$	Age_{vert}	Calcein mark
A	Baby F	chem	WH	21	3939	58	M	767	3	3	5	no
B	JJ	chem	WH	5	869	50	F	398	4	NA	5	yes
C	Single	chem	WH	26	3610	57	M	467	3	3	6	yes
D	WS	chem	WH	6	1396	41	F	298	2	2	5	no
E	DST 537	DST	WH	27	4340	76.5	F	537	6	6	9	yes
F	DST 7240	DST	WH	27	3659	82	F	263	8	NA	NA	no
G	DST 7408	DST	WH	28	4020	77.5	F	347	5	NA	NA	no
H	Monk 31	diet	MU	22	6390	50	M	NA	3	NA	NA	no

Table 2. Von Bertalanffy growth function parameters for ages estimated by the Sr/Ca method and from another where growth curves were modelled using lengths and growth increments from tag returns (Sherwood et al. *in prep*).

Data source	L_{inf}	K	t_o
Sr/Ca	97.1	0.24	-0.38
Tagging	108.3	0.25	-0.04

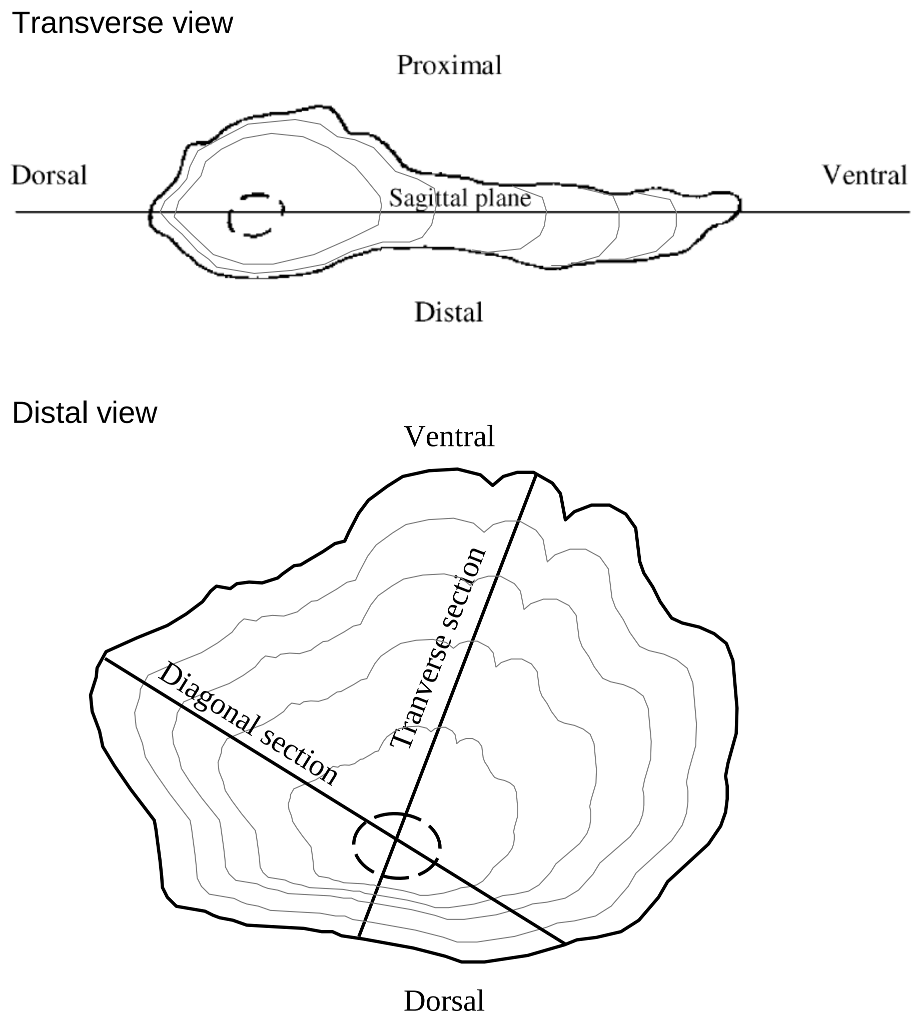

Figure 1. Illustration of different otolith sectioning techniques. Grinding down from the proximal and distal sides (parallel to the sagittal plane) creates a flat disk-like section which brings into view all rings. Sectioning the otolith perpendicular to the sagittal plane (either transverse or diagonal section) brings into view rings only along that section. Top transverse view modified from Woodroffe et al. (2003).

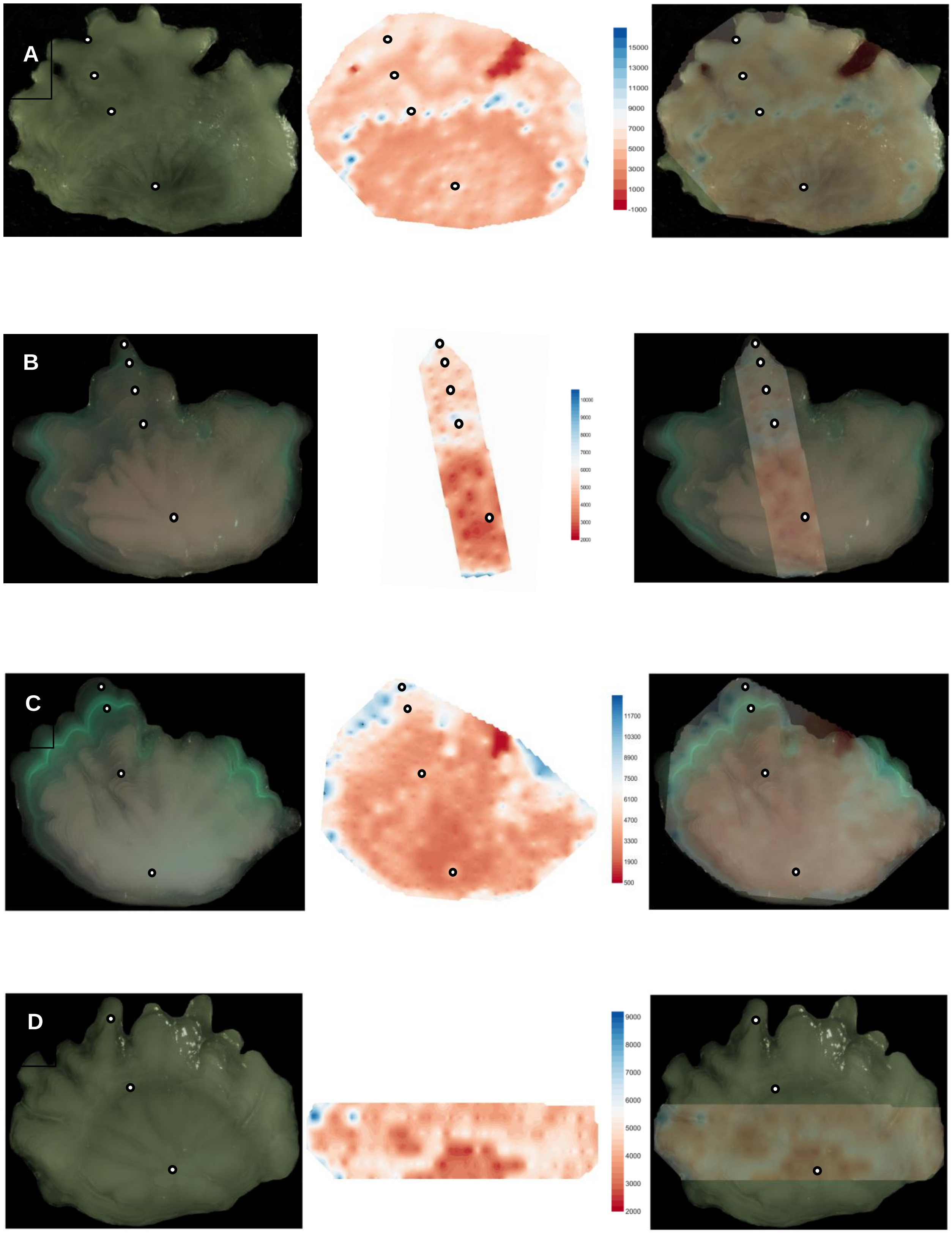
A
15000
13000
11000
9000
7000
5000
3000
1000
-1000
B
10000
9000
8000
7000
6000
5000
4000
3000
2000
C
11700
10300
8900
7500
6100
4700
3300
1900
500
D
9000
8000
7000
6000
5000
4000
3000
2000

Figure 2A-D. Otolith images, Sr/Ca contour (heat) maps and overlays of the two. Annuli are bracketed by white circles; first annulus (age 0) is bracketed by 1st (center most) and 2nd circle, 2nd annulus (age 1) by 2nd and 3rd circle, and so on. Last counted annulus extends beyond last circle.

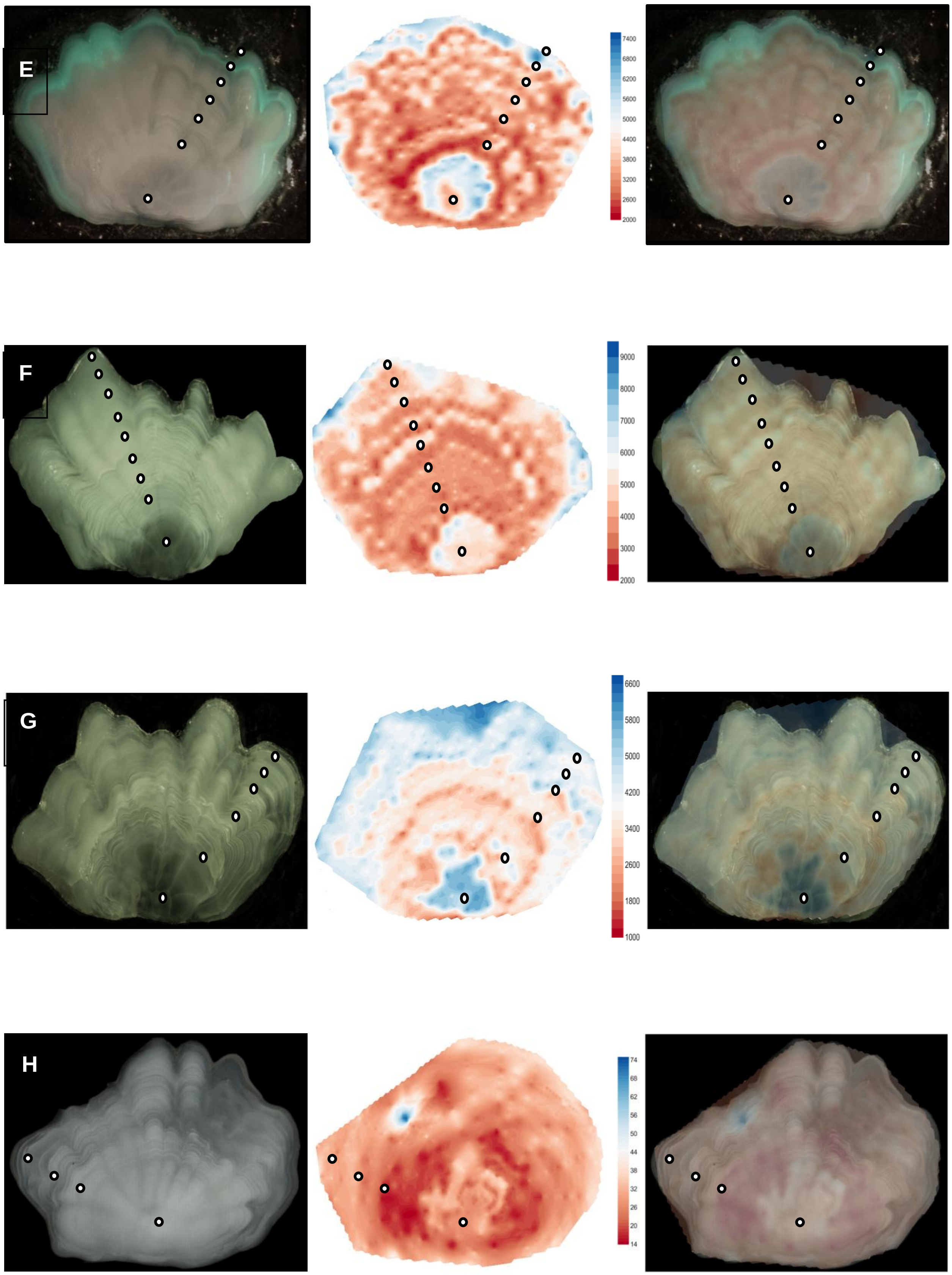
E
7400
6800
6200
5600
5000
4400
3800
3200
2600
2000
F
9000
8000
7000
6000
5000
4000
3000
2000
G
6600
5800
5000
4200
3400
2600
1800
1000
H
74
68
62
56
50
44
38
32
26
20
14

Figure 2E-H. Otolith images, Sr/Ca contour (heat) maps and overlays of the two. Annuli are bracketed by white circles; first annulus (age 0) is bracketed by 1^{st} (center most) and 2^{nd} circle, 2^{nd} annulus (age 1) by 2^{nd} and 3^{rd} circle, and so on. Last counted annulus extends beyond last counted circle.

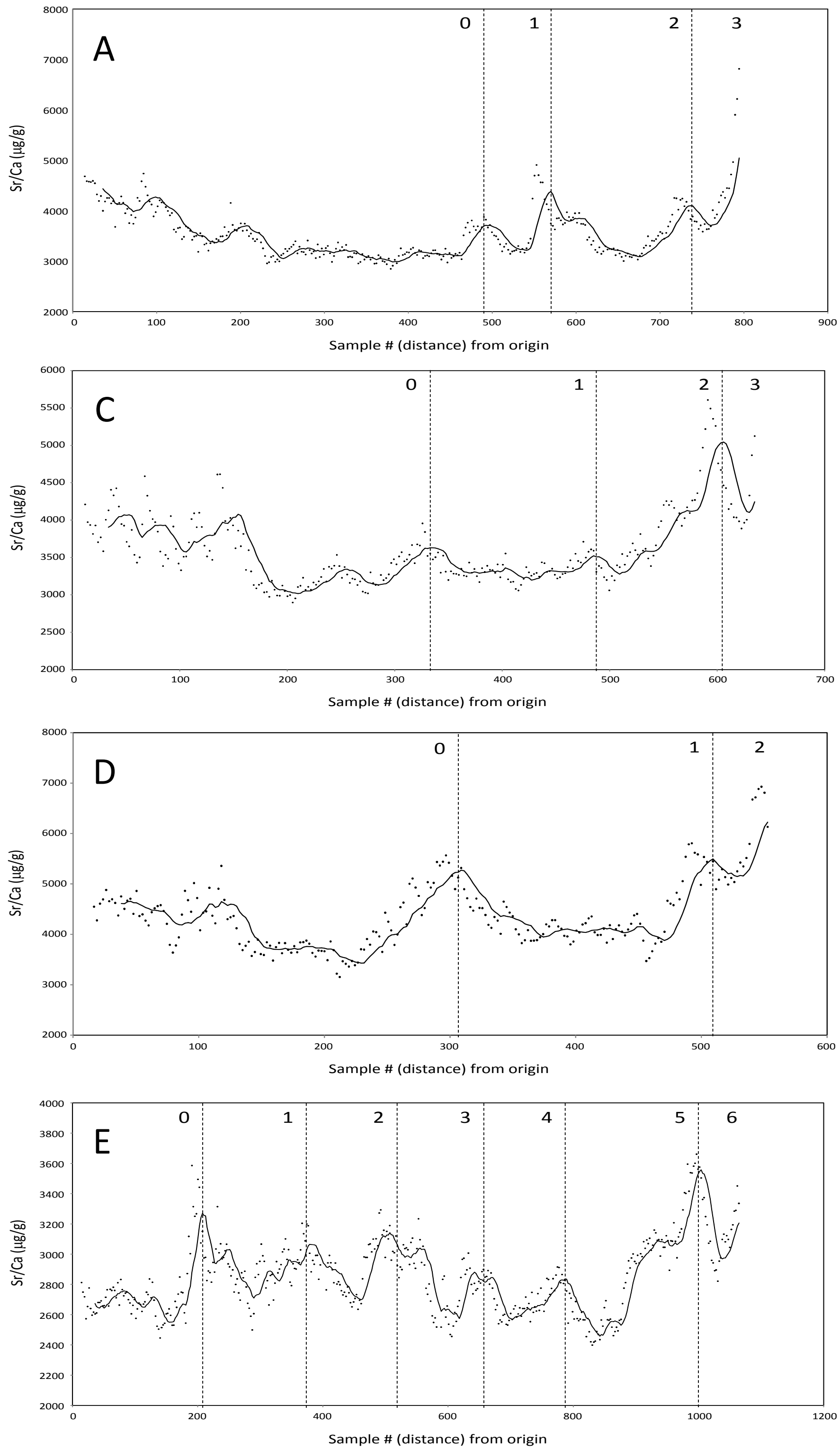

Figure 3. Vertebrae Sr/Ca trends for samples A,C,D and E. Left side of graphs represents origin (center) of vertebrae. Assumed annuli (warm/cold cycle or low/high Sr/Ca) are annotated.

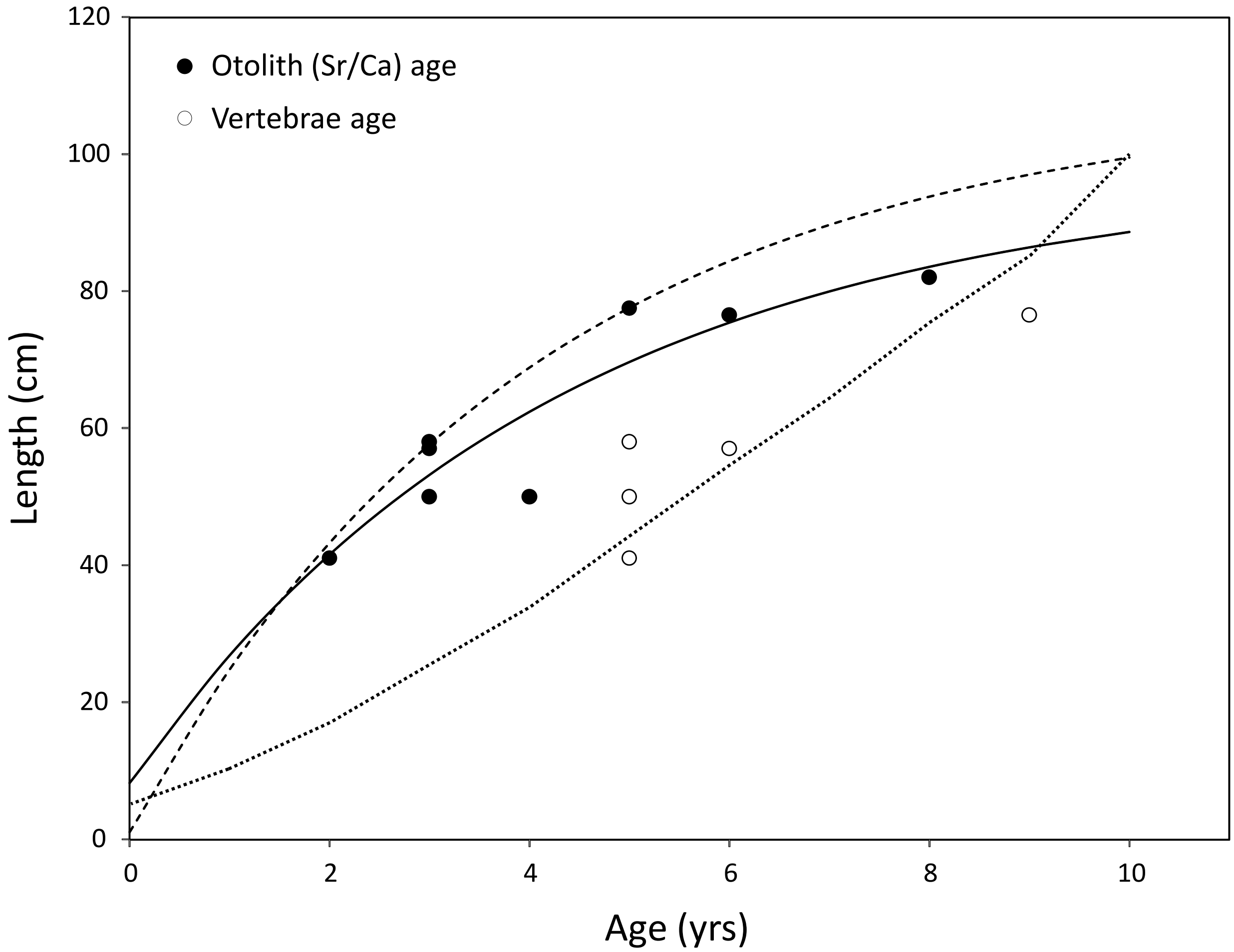

Figure 4. Growth curves for ages estimated by the Sr/Ca method (solid line), the vertebral method (no line) and comparisons to the growth curve used in the monkfish assessment (dotted line; Richards et al. 2008), and a growth curve generated from tagging data (dashed line; Sherwood et al. *in prep*).