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A weight-of-evidence approach for validating age and growth in US monkfish (*Lophius americanus*) stocks

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EXECUTIVE SUMMARY

Reliable age determination is the cornerstone of effective age-based fisheries management. The most recent assessment 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 for two age validation methods that may shed light on interpreting annulus formation in monkfish growth structures: 1) chemical "marking" of bony structures (otoliths, illicia and vertebrae) via oxytetracycline (OTC) and fluorexon injection to examine recent annulus formation both in the lab and in situ; and 2) examination of strontium/calcium ratio (Sr/Ca) patterns in otoliths to examine annulus formation over the life of monkfish in situ. The following is a summary of our results:

1) 44 monkfish (38-63 cm) have been brought to the sea water lab and 21 have been injected with a chemical marker. Two specimens lived one year after injection with OTC: one specimen received a 25 mg/kg dose of OTC and lived 467 days after injection, the other specimen received a 75 mg/kg dose of OTC and is currently alive after 607 days. Two other specimens are currently alive and will soon pass the year mark: one received a 25 mg/kg dose of fluorexon and the other received a 50 mg/kg dose of OTC.

2) 194 monkfish have been injected in the field with a chemical marker: 149 received 75 mg/kg OTC, 20 received 50 mg/kg OTC, and 25 received 25 mg/kg fluorexon. To date (May 2012) 7 fish have been recaptured. The longest time at large was 248 days, a female with 7.5 cm growth.

3) Sr/Ca, a temperature proxy, follows a cyclical pattern in monkfish otoliths that corresponds to banding patterns (opaque = warm = low Sr/Ca; translucent = cold = high Sr/Ca).

4) Two-dimensional Mapping of Sr/Ca values on a single otolith revealed the position of the first annulus and aided in interpretation of Sr/Ca banding patterns.

5) Sr/Ca trends were used to aid in visual interpretation of annuli in otoliths for 33 monkfish ranging in size from 49 to 112 cm.

6) Resulting ages were used to construct length-at-age relationships for female and male monkfish. Von Bertalanffy Growth Function curves differed markedly between females and

males with females reaching much higher assymptotic length than males (L_{inf} = 110 cm versus 59 cm, respectively).

7) Our results call into question previous findings of linear growth in monkfish, point to major differences in growth between female and male monkfish, and provide an explanation for the lack of large male monkfish in the population (i.e., males rarely grow past ~ 60 cm).

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. Sherwood (unpublished) reviewed the available published scientific literature and found that studies considering monkfish biology are outnumbered by a factor of 33 by studies that consider cod biology (Table 1). Monkfish are about as well studied as the longhorn sculpin (*Myoxocephalus octodecemspinosus*), a sympatric species with virtually no economic value. 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 a preliminary age validation study of monkfish using a variety of complementary techniques. Specifically, the efficacy of chemical marking (OTC and fluorexon) was examined in the laboratory with the expectation that growth structures would record the time of marking so that annulus formation following marking could be recorded. We also extended this marking technique to the field by injecting monkfish with OTC and fluorexon (various concentrations) at the same time as surgically implanting data storage tags (to record depth and temperature) in monkfish that would be released as part of a parallel and ongoing movement study. Given highly visible outreach for this tagging project along with a high reward for whole, recaptured monkfish ($500), we expected a high return rate and an invaluable opportunity to examine annulus formation in monkfish for known time at large. At the same time as conducting chemical "tagging" studies, we also undertook a study of natural markers of annulus formation in monkfish otoliths. 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). However, both chemical injections (OTC and fluorexon) and Sr patterns can be used together in a complementary fashion particularly to provide more reliable estimates of recent annulus formation (i.e., for time at large since chemical injection). If both agree, then Sr ageing may be deemed reliable over the entire range of ages. The purpose of this study was to explore and perfect both of these techniques so that future studies may apply these in a more routine fashion. We also provide a preliminary analysis of age and growth in monkfish to compare to standard ages estimated by visual ring counts in vertebrae.

2. METHODS

2.1. Holding Study for OTC and Fluorexon Injections

Live monkfish were collected from research cruises and during commercial fishing trips on otter trawl vessels and gill net vessels. The fish were transported in a 100 gallon, 4 x 4 foot tank with oxygenated water and then placed in 400 gallon circular tanks at the SMAST sea water lab. The first specimens were kept in a flow-through sea water system with basic sand filters, and the later specimens were kept in a semi-closed, re-circulating system with multiple filtration systems.

Feeding techniques varied throughout the study. Live fish (mummichogs, silversides, golden shiners) were introduced into the tank one or two at a time to see if normal feeding behavior was exhibited and if the esca (fishing lure on the first dorsal spine) was used to attract the fish before striking. Different types of dead fish (mackerel scad, herring, anchovy) or squid were dangled in front of the specimen to elicit a striking response. If the first two methods failed, dead fish or squid were nudged into the corner of the mouth to trigger a feeding response. Feeding attempts occurred every few days, or more frequently if they were eating. Records were kept of each feeding attempt: technique and species used, weight of ingested food if successful, and water temperature.

Protocols for injections adapted to emerging concerns about fish health and sampling constraints. After a period of acclimation, each fish was weighed, measured, and given an intraperitoneal or intramuscular injection of either oxytetracycline or fluorexon. Both chemicals were tested at three different concentrations: 25 mg/kg, 50 mg/kg, and 75 mg/kg (McFarlane and Beamish 1987, Gelsleichter 1997). During one period in the study, protocols changed to determine if oxytetracycline was increasing mortality in the fish. Monkfish were injected in groups of three, and each fish received a different treatment. One was given a 75mg/kg intramuscular injection of either oxytetracycline or fluorexon, one an intraperitoneal injection of the same dose, and the third was a control. When it became difficult to get numerous healthy samples from the fishing industry, and it became apparent we would not have enough groups to statistically determine if oxytetracycline was increasing mortality, protocols changed again to treat each fish individually, test lower doses of both chemical markers, and only use intraperitoneal injections.

2.2. Field Injections of OTC and Fluorexon

Injections in the field were conducted during the 2008 and 2010 Monkfish Research Set Aside Data Storage Tag (DST) trips and followed protocols developed in the lab. Both chemical markers were used: oxytetracycline at 75 mg/kg and 50 mg/kg, and flourexon at 25 mg/kg.

2.3. Image Analysis for Chemical Markings

When a specimen died it was dissected immediately. Length, weight, gut content, and sex were recorded. Illicium, otolith and vertebrae were removed, embedded in epoxy (5:1 mixture; West Systems©) and allowed to harden in the dark in silicon molds. A transverse section of the otolith through the nucleus, and a transverse section of the illicium 0.5 cm above the basal bulb (Duarte 1997) were cut with a double-bladed isomet saw (Buehler©) and mounted on glass slides. A sagittal section (0.3mm), centered on the focus of the vertebral centra was cut and mounted using the same method.

An Olympus BX51 microscope with a UV-light attachment was used to detect the presence of the chemical mark and images of the sections were taken using the CoolSNAP Pro color digital camera. A second vertebrae was kept intact and viewed under a Nikon SMZ 1500 microscope fitted with a UV-light attachment and Nikon digital sight ds-fi1c camera. Images of

the intact vertebrae were taken with imaging software, NIS-elements, under reflected light to show the banding pattern and under the UV light to show the chemical mark. Images of the age structures from control fish (no injections) were compared to images from treated fish to determine if the chemical had been incorporated into each structure and if the mark was visible.

2.4. Otolith Preparation for Strontium Ageing

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, Appendix) 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 fine (180 grit), extra fine (400 grit) and ultra fine (600 grit) 3M Pro Grade ® sandpaper. Grinding was done on both the proximal and distal surface to the primordium resulting in a ~ 1 mm flat "disc".

2.5. Strontium Analysis (Laser Ablation ICPMS)

Prepared monkfish otoliths were analyzed for strontium/calcium (Sr/Ca) ratios by laser ablation inductively couple plasma mass spectrometry (LA-ICPMS) at the Bruneau Centre for Research and Innovation, Memorial University, St. John's, Canada. Strontium concentrations are typically standardized to calcium concentrations (Gauldie et al. 2005). Prior to analysis, otoliths were mounted on 2.5 × 4.5 cm glass slides (4 per slide) using Cytoseal™ 60 low viscosity mounting medium. Marks were made on each otolith using a fine tipped pencil to guide ablation track (otherwise it was difficult to find start and end points under high magnification microscope coupled to LA-ICPMS system). Ablation tracks were run from primordium (start) to edge (end) in a diagonal, dorsal to ventral direction and varied from 3.3 to

5.8 mm in length. Laser fire rate was one per second and speed was 0.001 cm · sec^{-1} or 10 μm · sec^{-1}. Thus, sampling points for Sr/Ca were 10 microns apart. In addition to measuring strontium (Sr-88) and calcium (Ca-43) concentrations, we also measured lithium (Li-7), magnesium (Mg-24, Mg-25), manganese (MN-55), copper (Cu-63), zinc (Zn-66), cadmium (Cd-111), barium (Ba-137, Ba-138) and lead (Pb-208). Spatial variability in all of these elements will be examined but are not part of this study.

2.6. Image Analysis of Ablated Otoliths

Digital images of prepared, ablated monkfish otoliths were generated using an image analysis system: Nikon dissecting scope (using 10x magnification) coupled with Nikon Digital Sight DS-5M camera. Lighting was reflected. Captured images were imported into Image-Pro Express 6.0 where they were digitally enhanced by a series of filters including local equalization which enhances pixel contrast based on the histogram of the local neighborhood (i.e., evens out intensity) and despeckling which removes impulse noise. It was found that these imaging and filtering methods resulted in the best contrast for interpreting both annuli and the laser ablation track which was crucial for superimposing Sr/Ca trends (see Appendix for resulting figures); Sr/Ca was plotted as a function of distance from start (sample number) and the cyclical trend in Sr/Ca was visualized by fitting a lowess curve (3 % of points at a time) in SPSS version 16.0.

For one otolith, multiple ablation tracks were run (N= 22) in a radial pattern from the suspected primordium to the edge. In reality, the primordium was missed slightly but not by much. These tracks were then used to create a surface map of Sr/Ca values (temperature) to aid in identification of annuli and most importantly, the first annulus. Resulting data was linearly interpolated from the 22 ablation lines (6,207 data points) in Matlab. A contour plot was then created based on interpolated data, each contour representing a change in Sr/Ca of 1.8 units.

2.7. Verifying Strontium – Temperature Relationship and Otolith Sr Ageing Principles

Strontium/calcium ratio is assumed here as a proxy for temperature based on previous published findings (Gauldie et al. 2005, Townsend et al. 2005); i.e., Sr/Ca is negatively correlated with temperature (high values represent cold and low values warm temperatures). An opportunity to test this assumption came from a DST returned from a monkfish that was at large for 248 days or two thirds of a year. The otolith from this fish was prepared in the manner

described above and analyzed for Sr/Ca. Two thirds of an assumed annuli (i.e, trough/peak cycle in Sr/Ca) was superimposed on the DST temperature record for comparison. In this case temperature values were digitally matched to individual overlying Sr/Ca points and the relation between the two was examined by linear regression analysis.

Based on the assumption (also tested here) that Sr/Ca tracks seasonal fluctuations in temperature with only one warm/cold cycle per year (Figure 7), the following methods and principles were used to age monkfish otoliths using both visual cues (i.e., traditional hyaline and opaque zones) and Sr/Ca. First, monkfish are likely born in the fall in the Gulf of Maine (e.g., October) and the summer in the northern Mid-Atlantic Bight (e.g., July). Percentages of ripe and post-spawning females in each of these areas by month support this supposition (Richards et al. 2008). All fish in the north Atlantic are assigned a January 1st birth date. Thus, monkfish would have a shortened 0 year (i.e., only 5-3 months) before they turn one year old on January 1st. Water temperature on January 1st in the northeast is rapidly cooling and is about half way to yearly low values which occur in about March for waters at 20-50 m depth (Figure 9). There is a lag in this cycle for deeper waters such that annual temperature maxima occur around Jan 1st at 100 m (Figure 9). Gulf of Maine water temperatures are available online from the Northeastern Regional Association of Coastal and Ocean Observing Systems (NERACOOS) historical data portal (www.neracoos.org). Since most monkfish live at depths greater than 50 m (although DST data from our ongoing DST study supports extensive inshore movements to shallower water), we used the 100 m temperature trend and assumed that troughs (i.e., low values) in Sr/Ca correspond to January 1 or the conventional birth date. Given the previous, it is likely that the 0 year in the Sr/Ca record would be narrow and the year 1 band comparatively wider. We also assumed that successive bands are likely narrower than the first full year's growth.

Resulting ages from the above protocol were used to construct length-at-age relationships for female and male monkfish (N = 26 for females and N = 7 for males). Strontium ageing was conducted by one reader blindly (i.e., without any knowledge of fish length or sex). Due to the low sample sizes, growth was modeled using a modified Von Bertalanffy growth function (VBGF) equation where the curve was forced through the origin (i.e., $t_0 = 0$):

$$L_t = L_{inf} \cdot L_{inf} \cdot \exp(-kt)$$

where L_t is length at age t, L_{inf} is assymptotic length, and k is the Brody growth coefficient. To test for differences among curves generated for females and males we conducted an analysis of the residual sum of squares (ARSS) which is based on an F test (Chen et al. 1992).

3. RESULTS

3.1. Holding Study for OTC and Fluorexon Injections

Forty-four monkfish have been brought to the sea water lab, and twenty-one have been injected with a chemical marker. The size range was from 38 – 63 cm with the average size being 52 cm. Two specimens lived one year after injection with oxytetracycline: one specimen received a 25 mg/kg dose of oxytetracycline and lived 467 days after injection, the other specimen received a 75 mg/kg dose of oxytetracycline and is currently alive after 607 days. Two other specimens are currently alive and will pass the year mark on June 6, 2012: one received a 25 mg/kg dose of fluorexon and the other received a 50 mg/kg dose of oxytetracycline and is at the New England Aquarium.

Short-soak gill net sets during commercial fishing operations were the most successful for obtaining healthy specimens with little to no damage from fishing gear. If there was any damage from fishing gear on the fish or any visible injuries, they did not survive in the lab. Minimal handling between the vessel and lab was also important in their long term survival. All transfers were done either with a plastic bag "sling" or by moving fish directly from one container to the next without a net or picking them up. Although monkfish inhabit a wide range of temperatures (Richards, et al 2008; Bigelow and Schroeder, 1953), the water temperature in the laboratory's flow-through sea water system was too variable and extreme, and no fish survived. A semi-closed, re-circulating system was built to control the temperature and throughout the study continual improvements have been made to the system.

In the recirculating system, a single monkfish was kept in a 400 gallon circular tank with sterilized sand, approximately three inches deep covering the bottom so they can burrow and lie flat. Survival rates improved when fish were housed individually. The sea water supply in the re-circulating system and the incoming replacement water passes through two sand filters, two bag filters (50 um and 25 um) and an ultra violet sterilizer. Within the re-circulating system

there is a protein skimmer, bio-filtration system, and an additional ultra violet sterilizer on each tank. Temperature is controlled by a heating system in the winter and a chiller in the summer. The temperature simulates seasonal changes ranging from 7˚ C to 14˚ C.

A temperature probe and a high / low water mark are connected to a sensa-phone system that automatically called up to four people if there was a problem in the system. All three feeding techniques were successful, however the specimens went through phases when one method was preferred over the other. For example, if monkfish were striking at live fish they did not strike at dead fish. If they were eating dead fish nudged in the corner of their mouth, they did not typically strike at live fish. The most common food and feeding technique was mackerel scad nudged in the corner of the mouth.

Laboratory specimens that received an intramuscular injection of 75 mg/kg of oxytetracycline developed a localized reaction causing swelling and tissue necrosis around the injection site. This same reaction was observed in recaptured fish (Figure 2). Intramuscular fluorexon injections of the same dose did not produce a swelling reaction but did cause extensive tissue discoloration. There were no immediate deaths following the oxytetracycline or fluorexon injections in the lab but long term survival rates for both injected fish and control fish were low. Results from the treatment groups to determine if oxytetracycline was increasing mortality were inconclusive due to small sample size (Table 2). We eliminated intramuscular injections and continued testing both chemical markers with intraperitoneal injections.

3.2. Field Injections of OTC and Fluorexon

On commercial fishing trips, 194 monkfish have been injected in the field with a chemical marker: 149 received 75 mg/kg oxytetracycline, 20 received 50 mg/kg oxytetracycline, and 25 received 25 mg/kg fluorexon. To date (May 2012) seven fish have been recaptured (Table 3). The longest time at large was 248 days, a female with 7.5 cm growth.

3.3. Image Analysis for Chemical Markings

Oxytetracycline was incorporated into the age structures at 25 mg/kg, 50 mg/kg, and 75 mg/kg leaving a visible mark. There does not seem to be a difference in the intensity of the mark between the lower and higher dose, however the mark appears to be strongest in the illicium and weak or sometimes absent in the otolith (Figure 3). The lab specimen that lived 467 days after a

25 mg/kg injection of oxytetracycline and grew 6 cm showed a visible mark on the vertebra centrum (Figure 4), and the transverse section of the otolith, and the illicium (Figure 5).

Fluorexon was incorporated into the age structures at 25 mg/kg and 75 mg/kg. The intensity of the fluorexon mark appears greater than the intensity of the oxytetracycline mark even at the lower dose. The mark is also sometimes visible without ultra violet light (Figure 6).

3.3. Verifying the Strontium – Temperature Relationship

An inverse relationship between Sr/Ca and temperature is shown in Figure 7. In this case, a monkfish (# 17, Table 4) had been tagged with a data storage tag (DST 4078) and released on November 10, 2009 (Table 3). The same monkfish was recaptured on July 16, 2010 after being at large for 248 days or two thirds of a year. The DST showed a cyclical pattern in recorded temperature with a single maximum temperature (around December 1st) and single low temperature (around April 1st). The Sr/Ca record was superimposed on the temperature record and a strong negative correlation was found (Figure 8). The temperature record for this fish shows good agreement with the temperature cycle for water at 100 m depth (Figure 9); that is, in mid-July (the date of recapture for this fish) the water temperature should be warming and Sr/Ca should be decreasing as was the case.

Figure 9 shows an overlay of Sr/Ca values for another monkfish (# 31, Table 4) and ambient water temperature in the Gulf of Maine at various depths. In this case, Sr/Ca data for the entire ablation track is shown (see Figure 10 for location of track, Line A). The purpose of this figure is to orient the age reader to how temperature varies seasonally in the Gulf of Maine so that Sr/Ca variations can be interpreted on a seasonal basis; unfortunately no temperature data from depth in the SFMA was available on the NERACOOS website. A number of peaks and troughs in Sr/Ca were evident that appear to correspond well with temperature variations at 100 m in the Gulf of Maine. Since this fish was likely born during a warm period in the fall (appropriate for the Gulf of Maine), we expect the primordium to show “warm” Sr/Ca values which they indeed do. Given that temperature maxima at 100 m depth (a common depth for monkfish) corresponds to Jan 1st, Sr/Ca minima (inverse to temperature, Figure 8) can be considered birthdays. We counted 4 Sr/Ca minima (with Sr/Ca decreasing/warming at the edge) and therefore interpreted

this fish to be 4 years old. Note the width of the age 1 Sr/Ca minimum suggesting very high rate of fish growth, otolith accretion, or both during this stage.

3.4. 2-D Strontium (Temperature) Mapping

To validate our age estimate for monkfish #31 (Figure 9), we conducted extra LA-ICPMS sampling. Specifically, we sampled along 22 separate lines originating from the primordium and terminating at the edge. Sr/Ca over the entire surface of the otolith was interpolated from the resulting measured Sr/Ca values (total N = 6,207). A surface map of Sr/Ca (proxy for temperature) was thus generated and showed clear banding patterns that aided in identification of the first annulus (year 0) and first full year (year 1), as well as subsequent annuli (Figure 10). From Figure 9, we argued that the first annulus (year 0) should be small, given actual birth in the fall, and we interpreted a very wide second annulus (year 1). Our 2-D rendering of this otolith lends support to our interpretation of very rapid growth/accretion in year 1; the wide warm band is consistent throughout the otolith and not just along Line A (Figure 10). Two more warm bands were noted with a narrow warm edge, consistent with our interpretation in Figure 9, although the warm edge was not always evident. The inconsistency of the warm edge in the 2-D rendering may be an artifact of the linear interpolation method. Given the capture date (August 31st) we should assume a warm edge based on seasonal ambient temperature variations shown in Figure 9 which should be warming. Additionally, the warm edge was present along Line A. In contrast to the Sr/Ca map, the banding patterns (hyaline versus opaque zones) in the otolith were prominent (Figure 10), but it was less clear which bands were real annuli versus pseudo-annuli. Finally, this 4 year old fish, for which we have the best available visualization of annulus formation, was a 50 cm male. Age 4 fish from vertebrae ageing (Richards et al. 2008) are 22-41 cm (mean 34 cm). A 50 cm monkfish (both sexes) is typically aged as 5 or 6 years old by the vertebrae method.

3.5. Growth

The Strontium method was applied to ageing 33 monkfish representing both management areas and sexes. See Appendix for Sr/Ca trends superimposed on otolith sections and inferred annuli. Age curves for male and female monkfish are shown in Figure 11 (see figure caption for VBGF parameters). The VBGF curves differed significantly among sexes (ARSS, $F_{3,28} = 29.3$, *p*

< 0.0001). Assymptotic size was 50 cm larger in females than males. Compared to NMFS vertebrae-derived age estimates (direct comparisons for N = 14), Sr age estimates were generally lower for younger ages but agreed well at older ages (Figure 12).

4. DISCUSSION

4.1. Holding Study for OTC and Fluorexon Injections

Our results suggest that an intraperitoneal injection of 25 mg/kg of fluorexon is the preferred method to mark the age structures of *L. americanus*. There are advantages to using fluorexon in the field: 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. The pH of fluorexon is around 6.5 whereas oxytetracycline is more acidic, with a pH of 1.6. The acidity of oxytetracycline could explain why the intramuscular injections caused tissue necrosis. The majority of the literature regarding intramuscular oxytetracycline injections do not mention any adverse reactions or tissue damage around the injection site, however one study did report high volumes (100 mg/ml) of oxytetracycline solution into muscle tissue produced tissue necrosis (Bush et al. 1996). Another advantage to 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 can. There have been two previous studies (Gelsleichter et al. 1997, Monaghan 1993) comparing calcein (fluorexon) to oxytetracycline that drew the same conclusion; calcein was the better marker due to the intensity of the mark. Monaghan (1993) also noted that oxytetracycline appeared detrimental to the health of the fish since the fish treated with it became lethargic and stopped eating whereas the calcein treated fish did not change their behavior. During our study, injections from both chemicals caused stress and alterations in feeding behavior, leading us to believe that it was the stress from the procedure that compromised the health of the fish, at least temporarily. The one disadvantage of fluorexon is the cost. Five grams of fluorexon is $115 whereas 100 grams of oxytetracycline is $310.

Oxytetracycline can successfully mark the age structures at doses ranging from 25 to 75 mg/kg, and we recommend only using intraperitoneal injections. Although we could not determine if oxytetracycline was increasing mortality, there are well documented cases

confirming that high doses (> 100 mg/kg) increase mortality (McFarlane and Beamish 1987). There were no immediate deaths following the oxytetracycline injections in the lab, and we have recaptured fish with 75 mg/kg intramuscular injections and have one fish alive in the holding study that has lived over a year after a 75 mg/kg intraperitoneal injection.

Monkfish are difficult to keep alive in captivity. The two major challenges are preventing stress and infection, and feeding. Throughout this study we developed methods from the initial capture and handling techniques to the laboratory system that increases the longevity of monkfish in captivity. The multi-leveled filtration system and temperature control substantially improved the health of the fish. However the critical step is obtaining healthy, undamaged fish from the commercial gear. Working closely with a few fishermen who have taken the time to save fresh undamaged fish and transport them carefully made the difference in the survival rates.

4.2. Image Analysis

Armstrong's (1992) age estimation method was used to determine the age from the vertebrae pictured in figures 3 and 5. According to Armstrong's method, a year of growth is a broad, multi-ringed, opaque band combined with a narrow translucent band that has a distinct step. The key to the annuli is the step, he mentions false annuli appeared as dark (translucent) bands but were not associated with a step. It was difficult to find the steps and translucent bands on the vertebra pictured in Figure 3. This vertebra is from the laboratory male fish that grew 6 cm and lived through a seasonal cycle with temperatures ranging from 7˚ C to 14˚C. We expected to see more growth on the vertebra after the mark and a full annulus formation, because this fish was injected in September and lived through the following September until the end of February. According to Armstrong (1992) the annuli is formed in May and the period of growth is during July-December. There was not a distinct step associated with the year of growth after the mark. There could be a few explanations for not seeing a similar annulus formation: laboratory fish are subjected to unnatural stress which alters behavior and natural feeding patterns and vertebrae are subject to resorption, possibly that process is accelerated in stressful laboratory environments. However, researchers studying other *Lophius* species hypothesized different factors are driving annulus formation. Griffiths and Hecht (1986) concluded that the numerous opaque and narrow translucent rings that make up an annulus indicate the annual growth of the species is a multiple, sporadic phenomenon rather than the more normal condition of alternating periods of slow and

fast growth. Griffiths and Hecht (1986) also inferred that annulus formation could be related to feeding habits and reproductive seasonality. Maartens et al (1999) supported the hypothesis that feeding behavior is a principle factor in the multi-ring formation. In our holding study there were periods when the fish would start eating and then suddenly stop again. These periods of increased appetite and fasting did not seem to follow a seasonal or temperature cycle.

The vertebra in figure 5 had visible translucent bands, and the steps were easier to find. This fish (sex unknown) was injected and released in July, recaptured in November and grew three centimeters. Surprisingly, the growth after the mark is similar in both fish even though the laboratory fish lived four times as long and the growth was doubled. From such a small sample size it is hard to draw any definitive conclusions, but the preliminary results do question the current ageing method for *L. americanus*.

Now that we are able to mark the age structures, and the holding studies have been successful, the next step is to recapture a marked fish that has been at large for over a year. The continuation of this work will lead to a better understanding of annulus formation and the ultimate goal of age validation. This work will be continued with support from the 2011 and 2012 Monkfish Research Set Aside projects.

4.3. Verifying the Strontium – Temperature Relationship

Here we propose the use of otolith strontium/calcium ratios to verify annulus formation in monkfish otoliths. In essence, Sr/Ca banding patterns can be used to evaluate the likelihood of whether alternating opaque zones (OZ) and translucent zones (TZ) represent true annuli or checks (pseudoannuli). Practically speaking, if visual bands (e.g., one OZ + one TZ) align with one temperature cycle (warm/cold or high/low Sr/Ca), we expect that those zones represent an annulus. On the other hand, if multiple visual bands (e.g., two OZ and two TZ) occur within just one temperature cycle, then there is a high likelihood that at least one of those bands represents a psuedoannulus. In this way, environmental data is overlaid with traditional interpretations of banding patterns to provide a more robust evaluation of true annulus formation. However, in order for the Sr/Ca method to function, we need to first be confident that variations in Sr/Ca represent variations in temperature, and second to be sure that monkfish experience only one major temperature cycle in a year. Sr/Ca values in otoliths have been shown to be related to temperature in other species and applied to ageing and/or ecological study. For example,

Townsend et al. (1995) verified the Sr/Ca – temperature relationship in cod otoliths and used these values to hindcast likely origin of larvae from different water masses in the Georges Bank region with different ambient temperatures. Similarly, Gauldie et al. (1995) review the Sr/Ca – temperature relationship in fish otoliths and other aragonitic structures and apply this to evaluating annulus formation in orange roughy (*Hoplostethus atlanticus*). They concluded that "*otolith strontium content provides a useful tool for evaluation of fish ages estimated from structural and chemical variation in otoliths*". The present study provides further evidence for a relationship between Sr/Ca ratios and temperature in monkfish otoliths. Figures 7 and 8 show a tight correspondence (r^2 = 0.85) between these two variables from a monkfish with known temperature history and Sr/Ca concentrations. In fact, our study may be the first to demonstrate this relationship *in situ* thanks to coordination of this study with our ongoing DST study. Further DST recaptures should only reinforce this result.

Interestingly, Townsend et al. (1995), in a laboratory setting, demonstrated an exponential relationship between Sr/Ca and temperature with Sr/Ca values tending to flatten at temperatures above 10 degrees celcius. In other words, the negative relationship was more pronounced for colder temperatures than for warmer temperatures, and eventually at high enough temperatures Sr/Ca would not depend heavily on temperature. Although we presented a linear relationship between Sr/Ca and temperature in Figure 8, an exponential curve can also be fit with the same coefficient of determination ($Sr/Ca = 60.6 \times \exp(-Temp/20.1)$, $r^2 = 0.85$). Overall, there is good evidence from previous published studies (Gauldie et al. 1995, Townsend et al. 1995) and this study to the support the use of otolith Sr/Ca as an independent temperature proxy.

Finally, Sr/Ca values should only record one major temperature cycle (alternating warm/cold) per year to be useful for ageing. It was expected that monkfish would experience only one major temperature cycle per year based on NERACOOS buoy temperature records at various depths (Figure 9). Indeed, Figure 7 is consistent with this expectation; about two thirds of a warm/cold cycle (from Sr/Ca values) was observed in the otolith of monkfish DST 4078 (Table 3) which was at large for a period of 248 days (i.e., ~ two thirds of a year). Future DST recaptures are expected to further confirm this relationship.

4.4. 2-D Strontium (Temperature) Mapping

Based on our findings confirming the Sr/Ca temperature relationship and cyclicity in monkfish otoliths, we applied Sr/Ca to evaluating annulus formation. For one otolith we generated extremely high resolution imaging (over 6,000 data points in the space of ~ 0.3 cm^2) of Sr/Ca values over the surface of the entire otolith preparation (Figure 10). This rendering of Sr/Ca variations was extremely useful for examining annulus formation that may not have been evident from single ablation tracks. Particularly, we learned from this exercise that the first full annulus in monkfish otoliths is relatively large. The center large red (warm) area or "donut" seen in Figure 10 is indicative of high growth in the first full year (year 1). Year 0 (encompassing the primordium) is characterized by a much smaller warm zone consistent with the birthdate of these fish likely in the fall. In other words, very little growth would occur between the true birth date (fall, perhaps even late fall) and the conventional birth date (January 1st). Following the year 1 wide band, two more warm zones with a warm edge were noted (verified by single ablation track shown in Figure 9) such that age of this fish was estimated to be 4 years old. Although less evident, this age estimate seems to be in general agreement with the pattern of opaque and translucent zones seen structurally in the otolith (Figure 10 bottom). Specifically, despite the appearance of multiple checks, there appears to be 4 general TZ's and four general OZ's. The value in the Sr/Ca method was in more confident assignment of the first annuli (quite wide) and in being able to interpret the edge which was somewhat ambiguous from the point of view of structural patterns. Overall, we are confident in the age assigned to this fish (4) and applied what was learned from this fine-scale examination to interpreting 30 more single ablation tracks for Sr/Ca values, superimposed on prepared otolith sections (Appendix).

4.5. Growth

Growth patterns for 31 monkfish aged using the strontium method are shown in Figure 11. Our results point to a much different growth trajectory for *L. americanus* than that suggested by age results obtained from reading vertebrae (Richards et al. 2008). In particular, we demonstrate an assymptotic growth pattern as opposed to previous results which show a linear growth pattern. Indeed, the linear growth pattern for *L. americanus* has been questioned (Northeast Data Poor Stock Working Group 2007) in light of expectations for fish in general (i.e., physiological determination of growth usually drives assymptotic patterns) and findings in other *Lophius*

species which generally show some kind of assymptotic growth pattern (Duarte et al. 1997, Maartens et al. 1999, Landa et al. 2001, Fariña et al. 2008). Our results, although preliminary, point to some potential problems with the current method for ageing *L. americanus*.

Fourteen of the otoliths analyzed here were also aged by the Northeast Fisheries Science Center using conventional methods (i.e., vertebrae sectioning and reading). Figure 12 show where divergence in age estimates is most likely to be problematic. In particular, monkfish 8 years old and younger (from the Sr/Ca method) were typically aged 2-3 years older by the vertebrae method. Also, the fish for which we feel that we have our most confident age estimate (monk #31) was aged as 4 years old and was a 50 cm male. Assuming the linear growth curve (Richards et al. 2008), a 50 cm fish (either male or female) should be 6 years old, again a 2 year difference. These differences suggest that the vertebrae method overinterprets the inner part of the age structure. Our finding for a wider than expected first annulus in monk #31 (Figure 10) is consistent with this discrepancy. In other words, there is a good chance that a number of pseudoannuli are being counted within this first wide growth band. To our knowledge, the Sr/Ca method has not been applied before to vertebrae. However, a future study of Sr/Ca variations in monkfish vertebrae in relation to assumed annuli may be warranted.

In addition to our results pointing to a more biologically and physiologically realistic growth curve, our findings also shed some light on what should be considered peculiar growth in male versus female monkfish and corresponding maximum size. Male monkfish in the U.S. northeast rarely exceed 70 cm and more often are found in the 50-60 cm range (Richards et al. 2008). On the other hand, female monkfish routinely exceed 100 cm (Johnson et al. 2008). Given that both sexes follow the same linear growth curve as determined from the vertebrae ageing method (Richards et al. 2008), it must follow that male monkfish do not reach old age (> 6-7 years old), whereas females are commonly found up to 12 years old. This begs the question, what happens to male monkfish such that no old individuals are present in the population? Do they die prematurely, either of natural causes or disproportionate fishing mortality? Or do they emigrate to an as yet determined and sampled location? A similar problem presented itself for Scotian shelf haddock (*Melanogrammus aeglefinus*) in the 1990's (Campana 1995). Although not related to sex differences, the resolution of the problem may have implications for male monkfish. Prior to strict scrutiny of age results, Scotian shelf haddock (most notably NAFO division 4VW) were rarely aged higher than 5 years old and rarely exceeded 55 cm in total

length, whereas maximum reported length and age for this species are 112 cm and 20 years, respectively (Froese and Pauly 2012). Prior to Campana's (1995) study it was believed that these haddock were either experiencing unusually high mortality rates or emigrating to an unknown location. When otoliths were examined by a collection of experts from a number of international labs, it was found that age was being underestimated in some cases by as much as 7 years. Therefore, neither high mortality nor emigration were to blame. To the contrary, the growth curve was misrepresented. Instead of following a more linear growth curve, haddock were experiencing a rather sharp asymptote or stunting. Our results for the difference in growth between female and male *L. americanus* is analogous to the findings of Campana (1995). It should be reiterated that Sr/Ca interpretations were conduced blindly with no knowledge of sex. Instead of disappearing from the population at 6-7 years old, male monkfish in the U.S. northeast appear to just follow a different growth curve than females (i.e., growth asymptotes at around 60 cm for males as opposed to ~ 110 cm for females). Our sample size, especially for male monkfish, is admittedly low. Future studies should follow up on this apparent size dimorphism which has been demonstrated in other *Lophius* species (Landa et al. 2001).

Differences in growth between females and males may also shed light on observed sex ratios. Richards et al. (2008) present sex ratios for the different management areas. Particularly for the southern fishery management area (SFMA), sex ratio starts out about even for monkfish less than 40 cm in length, and then becomes strongly biased towards males from 40-60 cm, after which sex ratio builds sharply towards 100 % females for fish greater than 70 cm. The pattern is somewhat similar for the NFMA except that the sex ratio stays relatively even up until about 60 cm (with a slight dip towards more mores in the 40-60 cm range) before it becomes 100 % females after 70 cm. One interpretation for this result is that there exists sex-specific differences in movement patterns (Richards et al. 2008). The more obvious explanation, that there exist sex differences in growth rate such that males accumulate in the last size grouping (< 70 cm), was dismissed by Richards et al. (2008) based on available age data (from vertebrae). However, based on our new age results, this explanation should now be reconsidered. Indeed, sex-based differences in growth rate are responsible for skewed sex ratios in other *Lophius* species (Duarte et al. 2001, Ofstad and Laurenson 2007). Overall, our growth results provide a biologically reasonable explanation for skewed sex ratios in *L. americanus*, but more importantly, skewed sex ratios provide an indirect corroboration of our age results; i.e., the sharp change in sex ratio from

dominant male to dominant female occurs at about 60 cm and the assymptotic size determined from our Sr/Ca ageing method is 58.7 cm, a fairly good agreement.

5. CONCLUSIONS

This study demonstrates that monkfish age structures can reliably be marked with oxytetracycline and fluorexon to examine annulus formation following chemical injections. Both chemicals were taken up by all age structures (vertebrae, illicia, otoliths). However, Fluorexon may cause less tissue damage which is an important consideration when injecting fish to be released back into the wild where the least amount of adverse effects should lead to higher survival and better recapture rates. Fluorexon marks may also be more visible and stable over time. Laboratory holding techniques were perfected during the course of this study so that longer-term growth patterns may be examined following injections. Strontium/calcium ratios were measured in otoliths and the relationship between Sr/Ca and temperature was verified in an individual for which detailed temperature data from a data storage tag (DST) was available. This step was important for implementing the Sr/Ca ageing technique which relies on a strong temperature dependency, as well as predictable seasonal temperature variations which were also verified in the same individual. Thirty one monkfish were aged using the Sr/Ca method and growth was compared to conventional age estimates (from vertebrae). The Sr/Ca method points to potential problems with the vertebrae method, particularly that age is currently overestimated for younger monkfish and that the vertebrae method is missing important sex-based differences in growth that are consistent with skewed sex ratios in the population. Our preliminary results show that monkfish growth follows an assymptotic and not linear pattern and that assymptotic length is about 50 cm smaller in males than females (~ 60 versus ~ 110 cm). Future studies should aim to confirm these results by directly comparing estimates of annulus formation in monkfish for which both Sr/Ca data and chemical markings (wither fluorexon or OTC) are available for individuals that have been at large for more than one year (preferably more than two). Unfortunately, no fish from this study met this criteria. However, given the number of monkfish still at large with DST's and chemical markings (from our parallel RSA tagging studies), we are optimistic about our ability to make these comparisons soon.

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Table 1. Survey of published literature on 3 contrasting fish species common to the U.S. northeast: Atlantic cod, monkfish, and longhorn sculpin (Sherwood, unpublished).

	Google Scholar	Canadian Journal of Fisheries and Aquatic Sciences	ICES Journal of Marine Science	Marine Ecology Progress Series	Journal of Fish Biology	Fisheries Research	Transactions of the American Fisheries Society	Total in these Journals
"*Gadus morhua*"	17,300	378	494	119	787	333	375	2486
"*Lophius americanus*"	**539**	**9**	**7**	**0**	**13**	**31**	**16**	**76**
"*Myoxocephalus octodecemspinosus*"	174	5	27	0	3	8	3	46

Table 2. Summary of monkfish treatment groups in the laboratory.

Group Identification Chemical Marker	INJECTION LOCATION	AMOUNT injected	LENGTH (cm)	Acclimation Time (days)	DAYS ALIVE AFTER TREATMENT	DAYS ALIVE IN LAB	DEAD LENGTH	Growth	SEX
OTC GROUP 1	control	none	55	6	256	262	57.5	2.5	female immature
OTC GROUP 1	IM	75 mg/kg	54.5	6	91	97	55.5	1	male
OTC GROUP 1	IP	75 mg/kg	55	6	69	75	53	-2	Male
OTC Group 2	control	none	52	34	44	78	51.5	-0.5	female immature
OTC Group 2	IM	75 mg/kg	50	34	70	104	50.5	0.5	female immature
OTC Group 2	IP	75 mg/kg	56	34	77	111	55.5	-0.5	male
OTC Group 2	IM - saline (control)	none	53.5	34	63	97	53.5	0	female immature
Fluorexon Group 1	control	none	53	41	168	209	54	1	male immature.
Fluorexon Group 1	IM	75 mg/kg	57	18	28	46	56	-1	male
Fluorexon Group 1	IP	75 mg/kg	47.5	18	93	111	48	0.5	male

Table 3. Summary of recaptured monkfish that were injected with a chemical marker.

		RELEASE		RECAPTURE				
DST #	Injection chemical and amount	date	total length	date	total length	Days at Large	Growth Increment	Gender
4114	OTC 75 mg/kg	11/10/2009	58	6/11/2010	60.5	213	2.5	M
4078	OTC 75 mg/kg	11/10/2009	68	7/16/2010	75.5	248	7.5	F
L12773	OTC 50 mg/kg	4/16/2011	59	10/18/2011	63	185	4	F
L12757	FL 25 mg/kg	7/11/2011	77	7/20/2011	78	9	1	F
L12775	FL 25 mg/kg	7/11/2011	72	9/21/2011	71	72	-1	F
L12766	FL 25 mg/kg	7/11/2011	54	11/7/2011	57	119	3	N/A
L12756	FL 25 mg/kg	7/11/2011	60	1/5/2012	N/A	178	N/A	N/A

Table 4. Summary of samples analyzed for Sr/Ca. Project refers to sample source. Management area and project specific ID are also shown. Sr/Ca age is age estimated using Sr/Ca variations and vert age is age estimated by the National Marine Fisheries Service using vertebrae. Days at large pertains to DST tagged fish only.

Sample	Project	Area	Fish ID	Sex	Length (mm)	Sr/Ca Age (yrs)	Vert Age (yrs)	Days at Large
1	Coop survey	SFMA	41	F	1120	11	13	
2	Coop survey	SFMA	40	F	940	10	10	
3	Monkfish Diet	NFMA	416	F	660	4		
4	Coop survey	NFMA	11	F	900	7	10	
5	Coop survey	SFMA	40	F	860	7	9	
6	Coop survey	SFMA	1	F	800	6	9	
7	Coop survey	NFMA	1	F	880	8	9	
8	Coop survey	NFMA	20	F	850	10	9	
9	Coop survey	SFMA	3	F	800	8	8	
10	Coop survey	SFMA	52	F	870	8	10	
11	Coop survey	SFMA	9	F	840	8	9	
12	Coop survey	SFMA	1	F	820	6	8	
13	Coop survey	SFMA	3	F	800	6	8	
14	Coop survey	NFMA	1	F	850	7	9	
15	Coop survey	SFMA	1	F	810	7	9	
16	DST Tagging study	SFMA	DST 12757	F	780	6		9
17	DST Tagging study	SFMA	DST 4078	F	760	5		248
18	Tank study	NA	B	M	530	8		
19	Tank study	NA	A	F	580	3		
20	DST Tagging study	NFMA	DST 4127	F	745	5		3
21	DST Tagging study	NFMA	DST 4128	F	680	6		3
22	DST Tagging study	SFMA	DST 4114	M	605	11		213
23	DST Tagging study	SFMA	DST 5429	F	640	4		33
24	DST Tagging study	SFMA	DST 5448	M	600	6		40
25	Monkfish Diet	NFMA	406	F	720	7		
26	Monkfish Diet	NFMA	409	F	797	6		
27	Monkfish Diet	NFMA	418	F	761	6		
28	Monkfish Diet	SFMA	169	F	1118	10		
29	Monkfish Diet	NFMA	369	M	492	5		
30	Monkfish Diet	NFMA	372	F	928	8		
31	Monkfish Diet	NFMA	373	M	500	4		
32	Monkfish Diet	SFMA	48	M	550	5		
33	Monkfish Diet	SFMA	72	M	536	6		

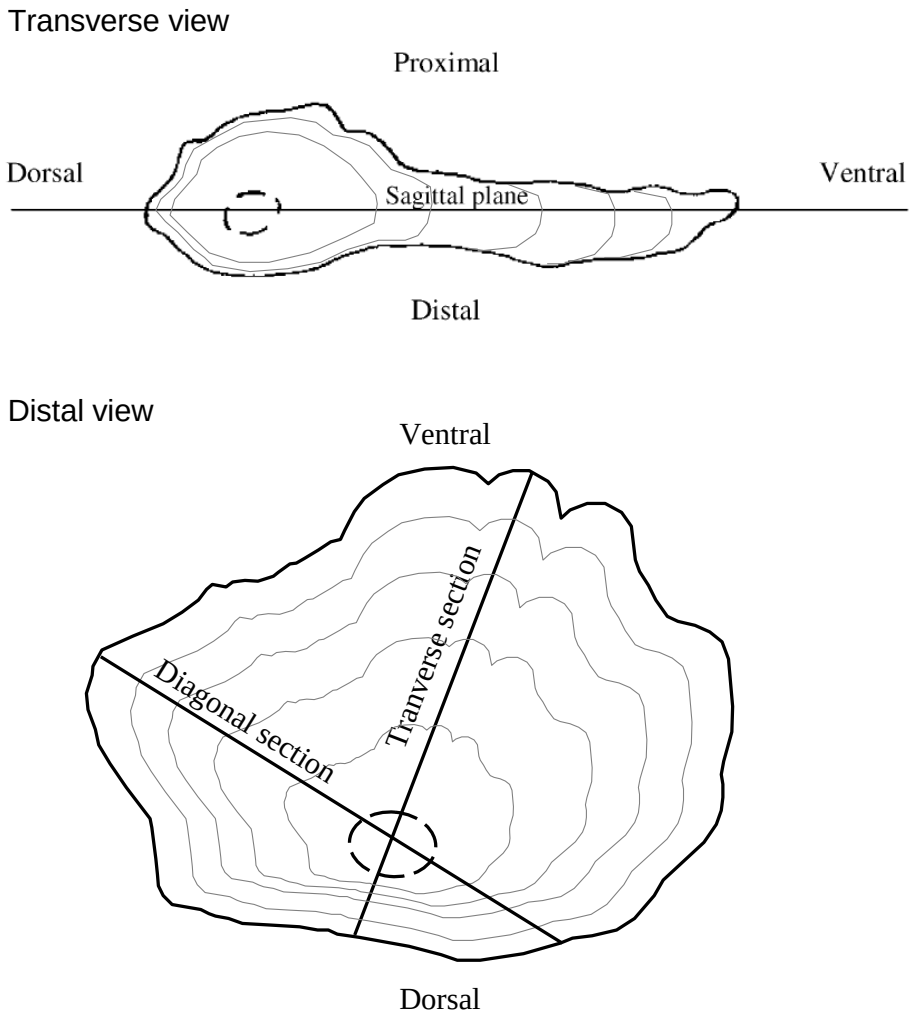


Figure 1. Illustration of different otolith sectioning techniques. Grinding down from the proximal and distal sides (parallel to the sagittal plane) creates a flat disk-like section which brings into view all rings. Sectioning the otolith perpendicular to the sagittal plane (either transverse or diagonal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

Figure 2. Tissue damage and discoloration caused by 75 mg/kg intramuscular oxytetracycline injection in a female fish at large for 248 days.

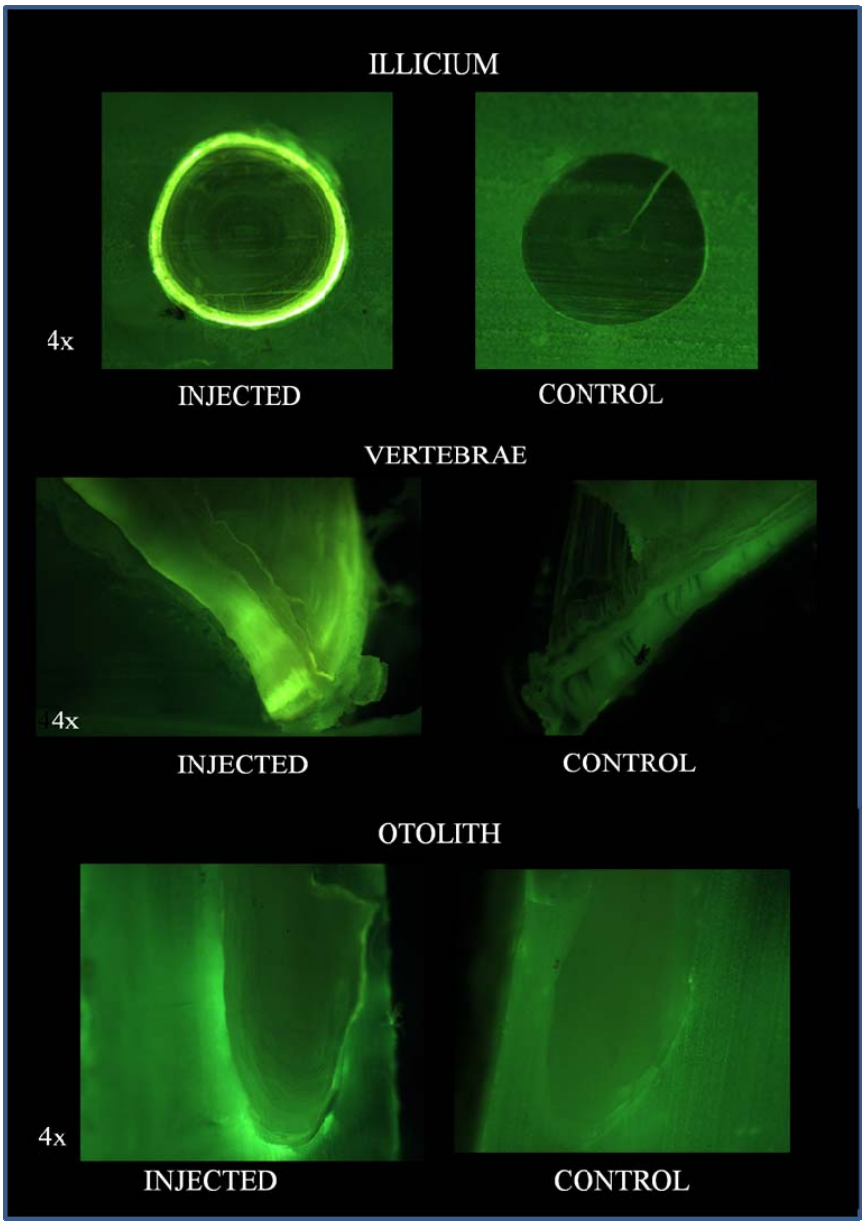
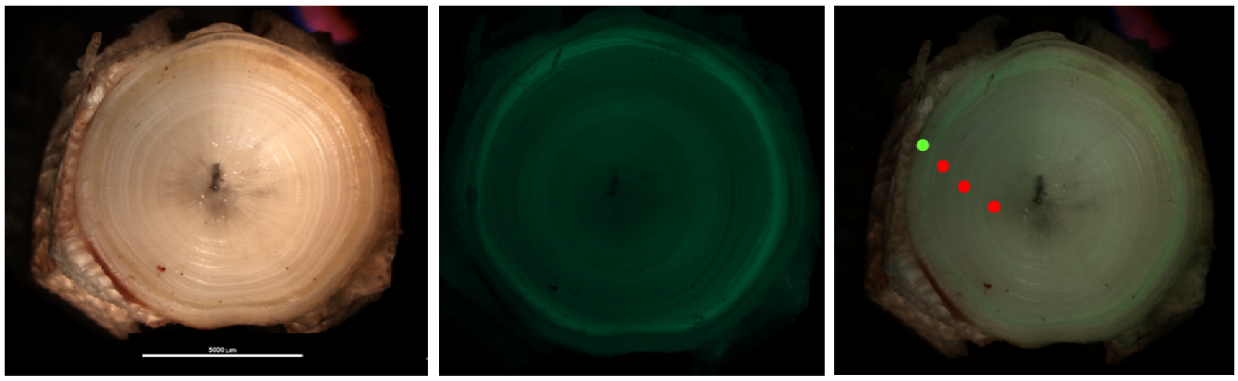


Figure 3. Age structures on the left from a male monkfish at large for 213 days after a 75 mg/kg injection of oxytetracycline and grew 2.5 cm; age structures on the right from a control fish (no injection).



a) Vertebra under reflected light b) Vertebra under UV light c) Vertebra images merged. Red dots indicate presumed annuli associated with a step; green dot indicates annulus formed after injection.

Figure 4. Vertebra images from a male fish that lived 467 days after a 25mg/kg oxytetracycline injection in the laboratory and grew 6 cm.

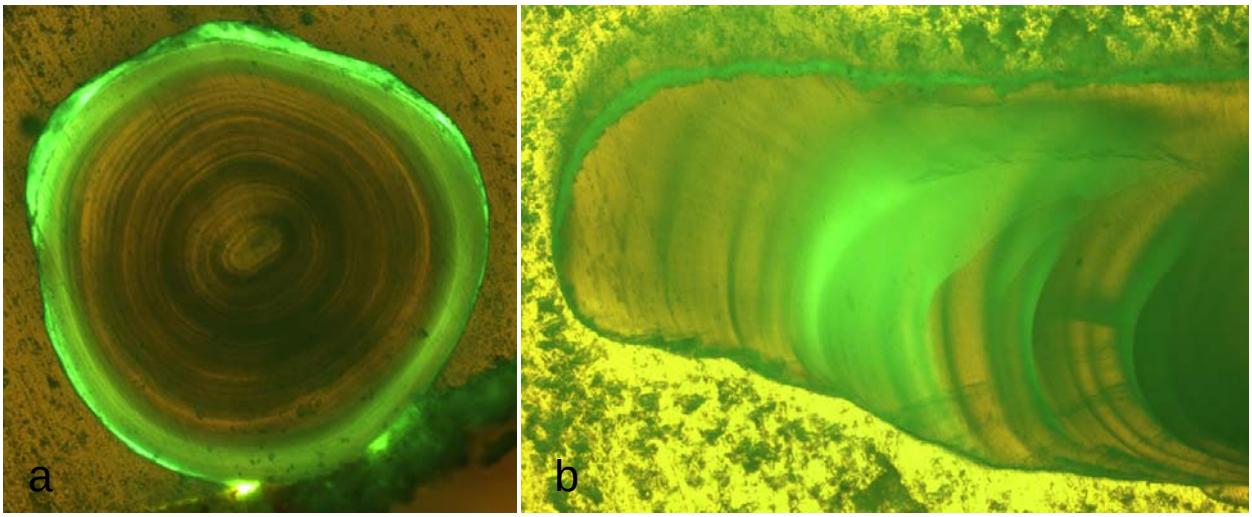
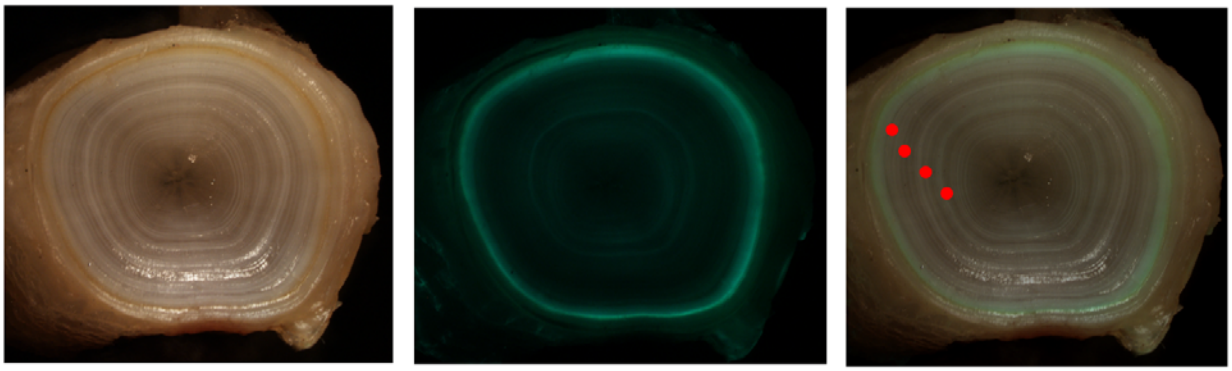


Figure 5. Illicium (a) and otolith (b) viewed under a mix of transmitted light and UV light. Structures are from a male fish that lived 467 days after a 25mg/kg oxytetracycline injection in the laboratory and grew 6 cm.



a) Vertebra under reflected light b) Vertebra under UV light c) Vertebra images merged. Red dots indicate presumed annuli associated with a step.

Figure 6. Vertebra images from a recaptured DST fish at large for 119 days after injection with 25 mg/kg of fluorescein. Gender is unknown, 3 cm growth.

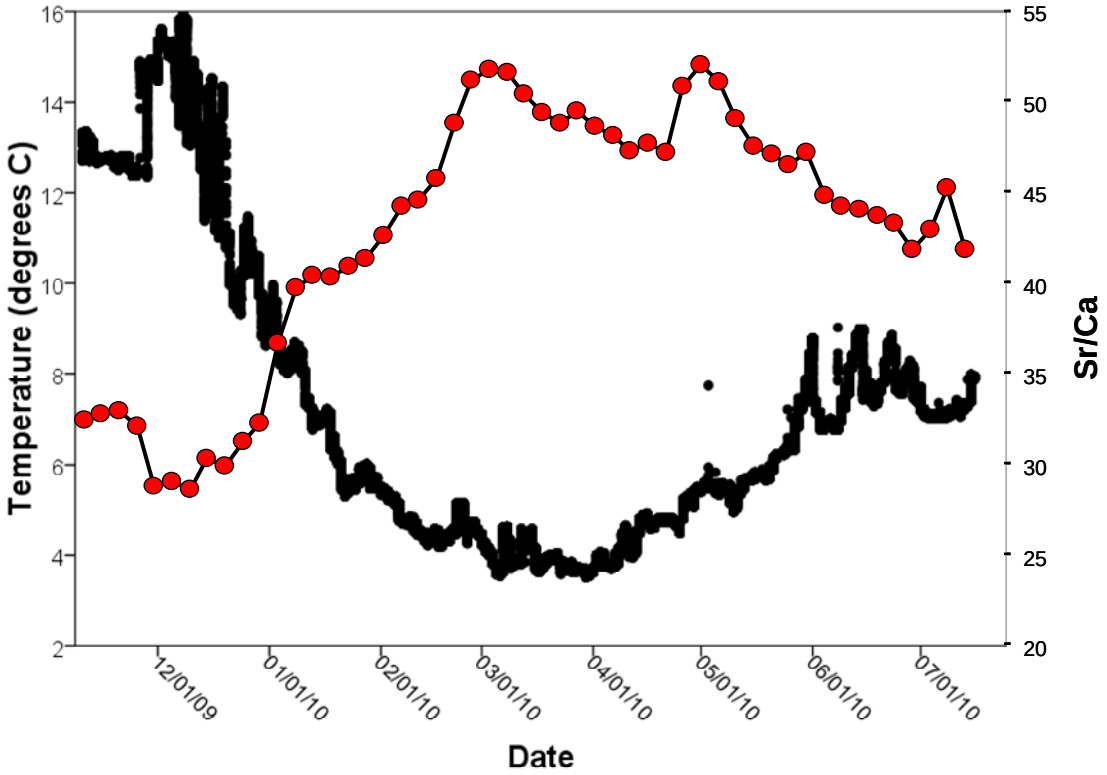


Figure 7. Temperature record for DST 4078. See Table 3 for release and recapture data. Sr/Ca, taken from most recent 2/3 of last annulus of Monk 17 (Appendix), is inversely related to temperature (see Figure 8).

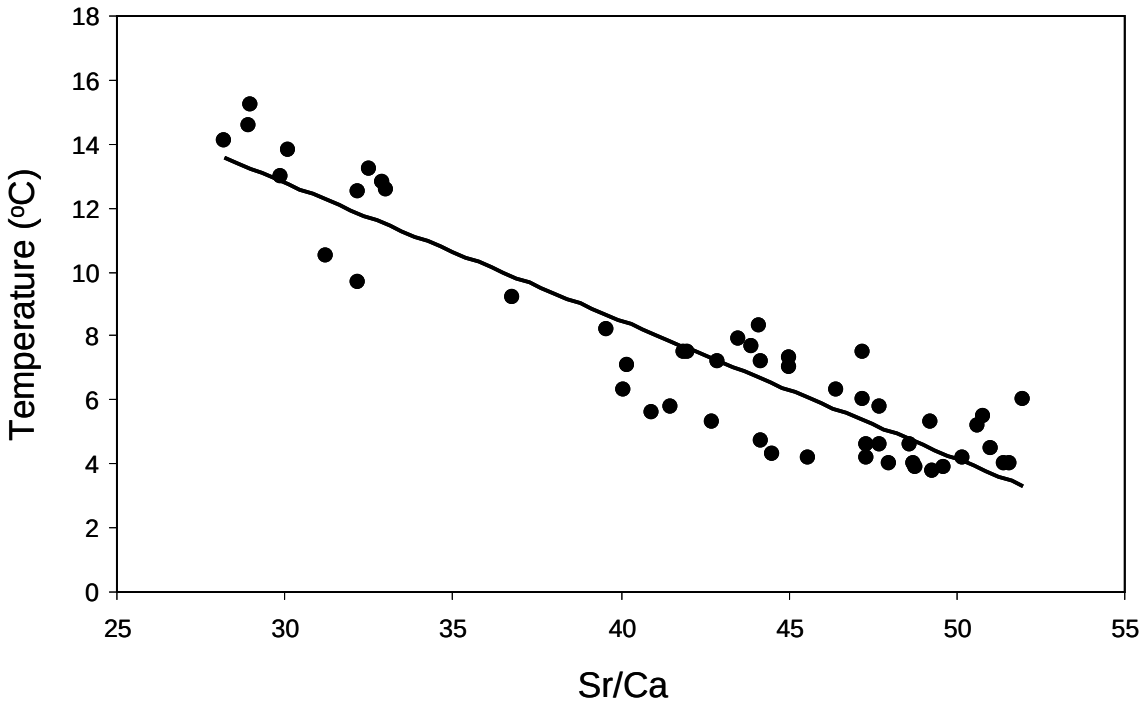


Figure 8. Interpolated temperature (T) versus Sr/Ca for relationship shown in Figure 7 (linear regression: T = 25.7 – 0.43 × Sr/Ca; $r^2 = 0.85$, $p < 0.0001$, N = 51).

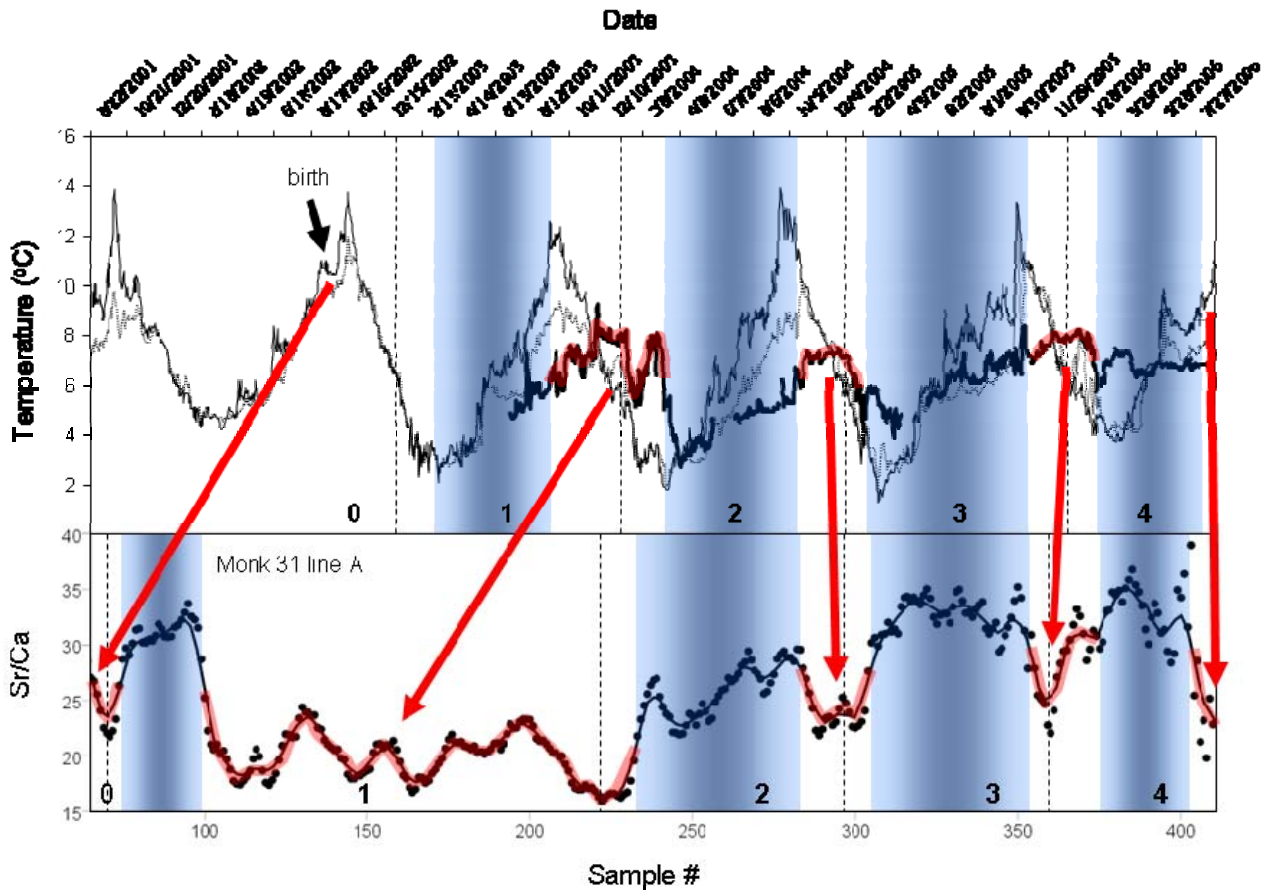


Figure 9. Overlay of water temperature and otolith strontium/calcium ratio (Monk 31 line A, see Figure 10). Capture date for this individual was 8/30/2006 in the western Gulf of Maine in 90 m of water. Water temperatures are from the NERACOOS website (historical data portal) for western Maine shelf (20 m, thin solid line), western Maine shelf (50 m, dotted line) and Jordan basin (100 m, thick solid line). Edge Sr/Ca agrees with warm temperature at capture. Four more warm periods were identified in Sr/Ca record and age is therefore estimated as 4 years old; birthdays (Jan 1st) are identified as vertical dashed lines; Jan 1st is assumed to correspond with temperature peak at 100 m depth. Therefore birthdays can be identified as Sr/Ca minima.

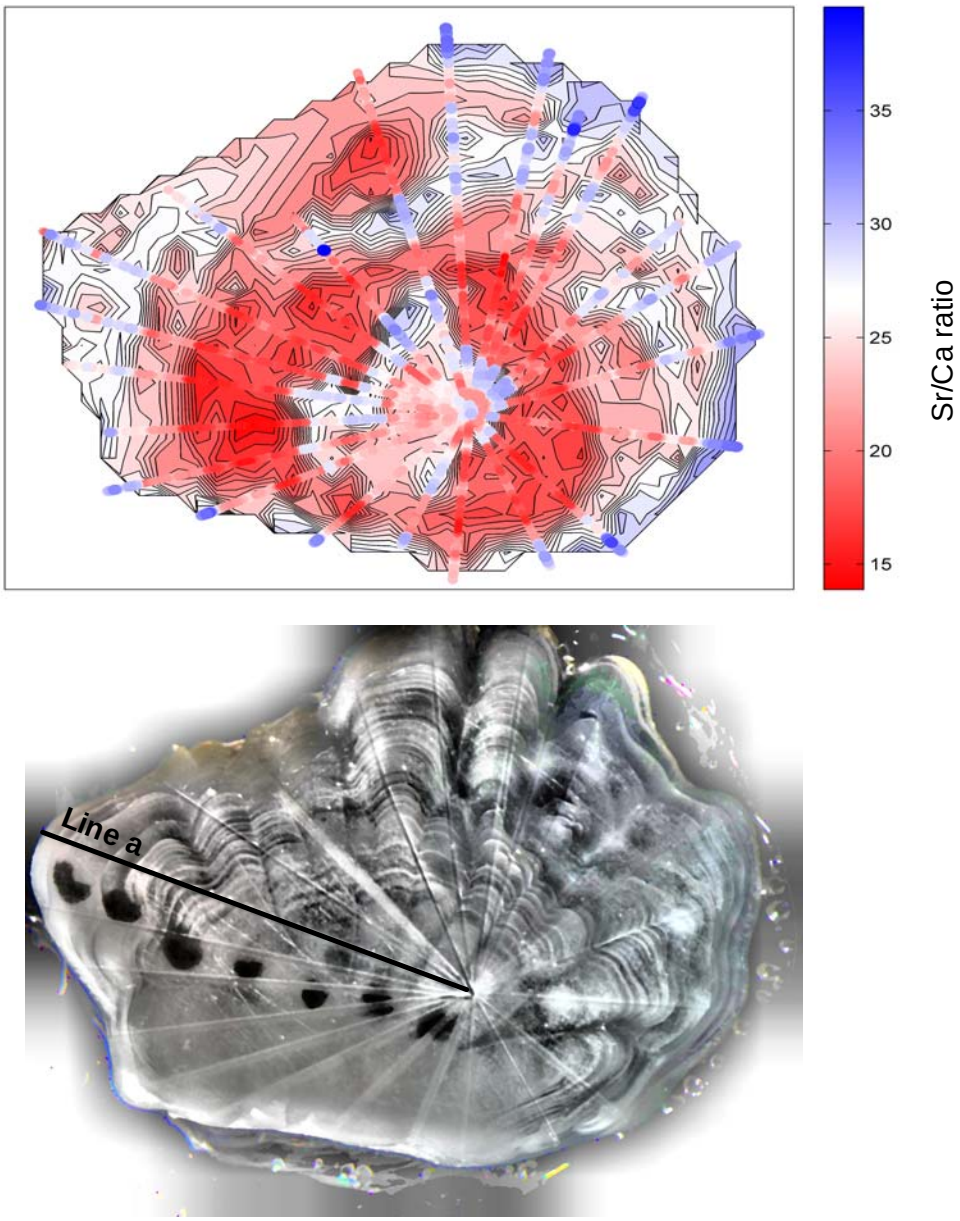


Figure 10. Top: Reconstruction of monkfish otolith from Sr/Ca values radiating from origin (see color bar for corresponding Sr/Ca values; red signifies warm conditions and blue cold). Prominent inner wide, red "donut" is interpreted as first full year of growth (age 1). Most inner red area (lighter) is age 0 (see Figure 9). Three more red areas (summers) are noticeable along line A and therefore this fish is estimated to be 4 years old. Bottom: Image of actual otolith and position of line A used in Figure 9.

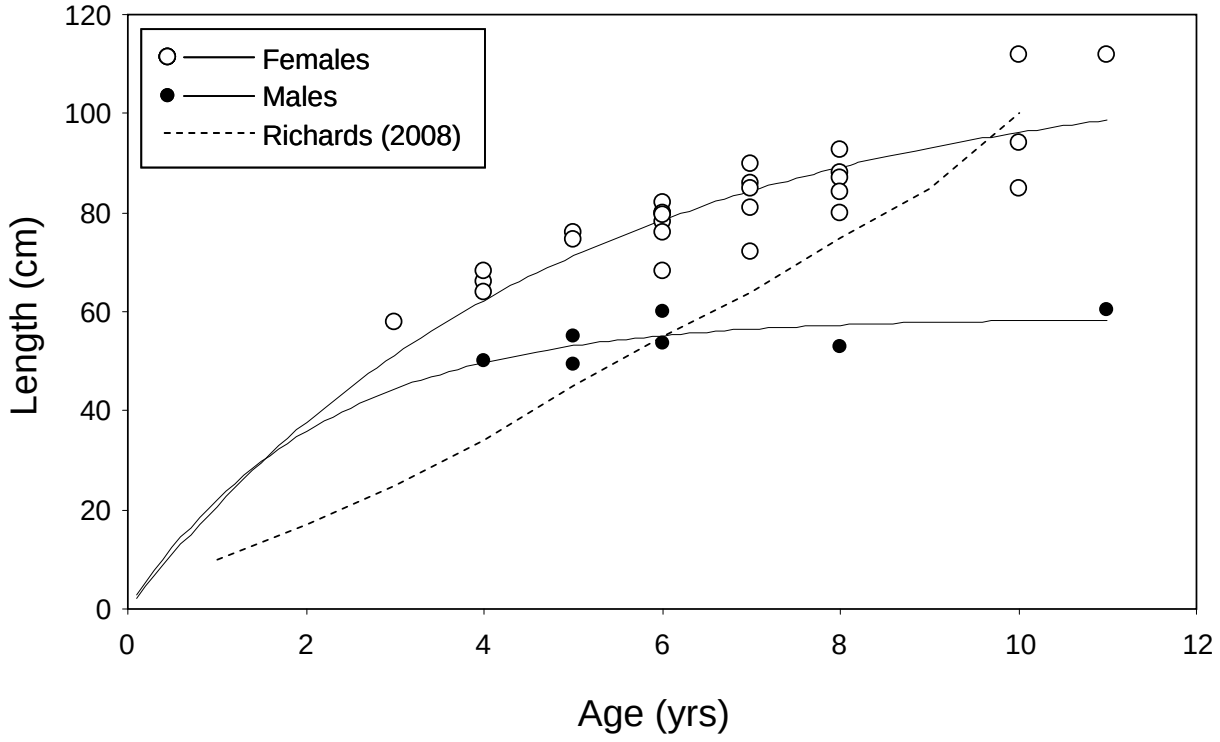


Figure 11. Age estimates from Sr/Ca method for female and male monkfish from both management areas. Curves are from Von Bertalanffy growth function and were forced through the origin (i.e., $t_0 = 0$); for females: $L_{inf} = 109.6 \pm 7.6$ cm, $k = 0.21 \pm 0.03$, $r^2 = 0.71$; for males: $L_{inf} = 58.7 \pm 3.2$, $k = 0.47 \pm 0.12$, $r^2 = 0.43$. Dashed line is growth curve from Richards et al (2008). There was no difference in growth among management areas. Growth curves are significantly different among sexes (ARSS, $F_{3,28} = 29.3$, $p < 0.0001$).

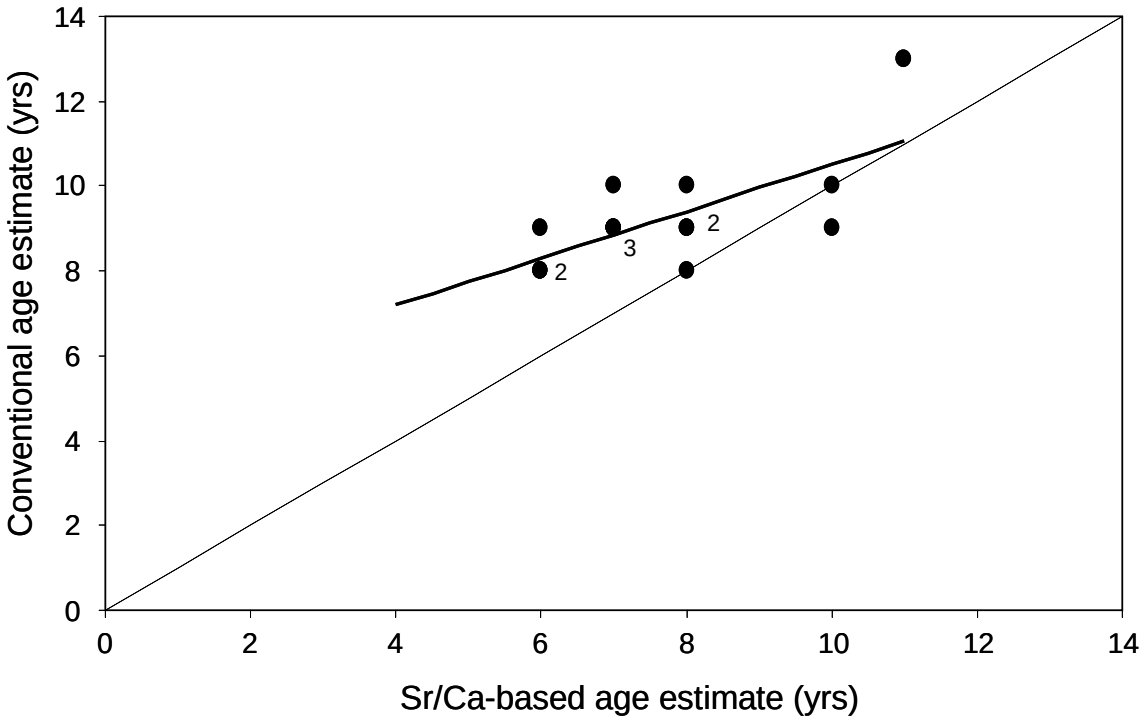
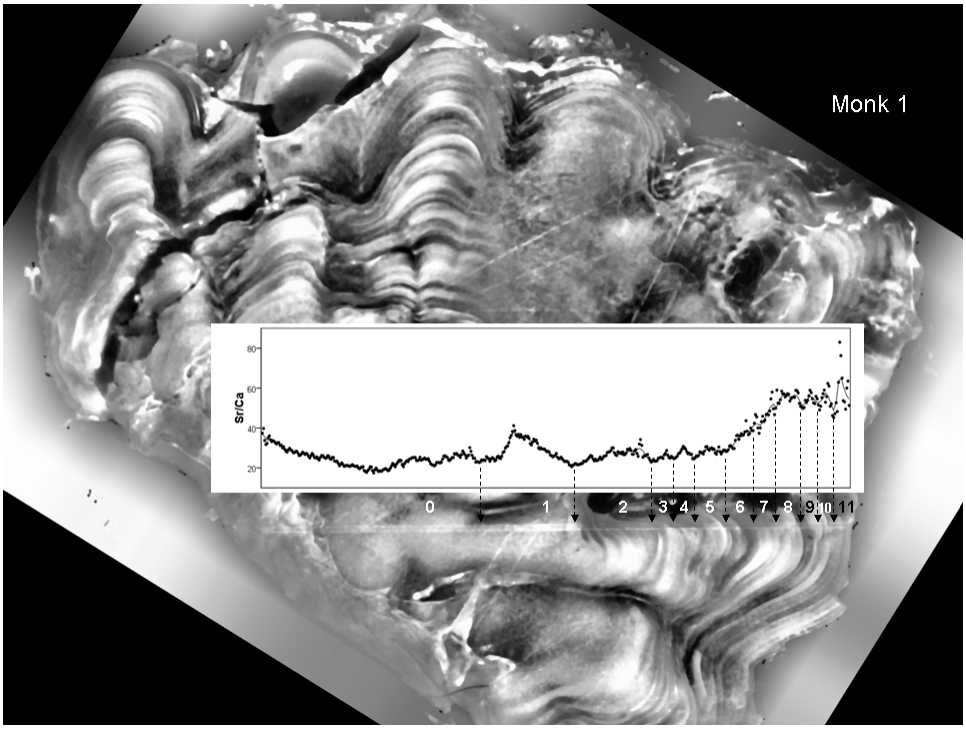
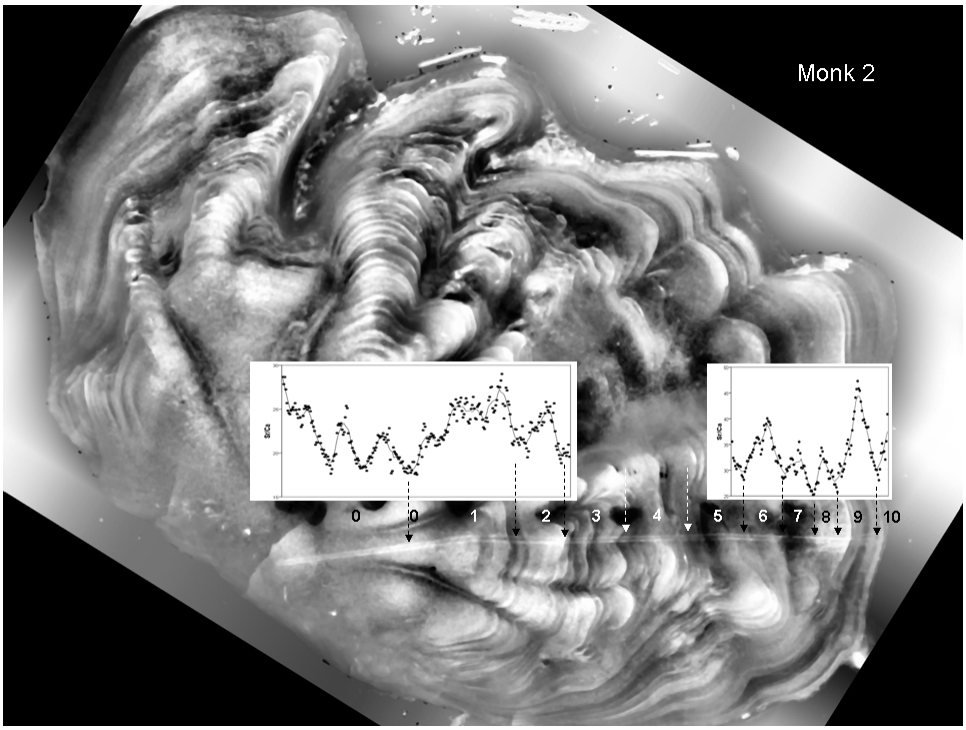


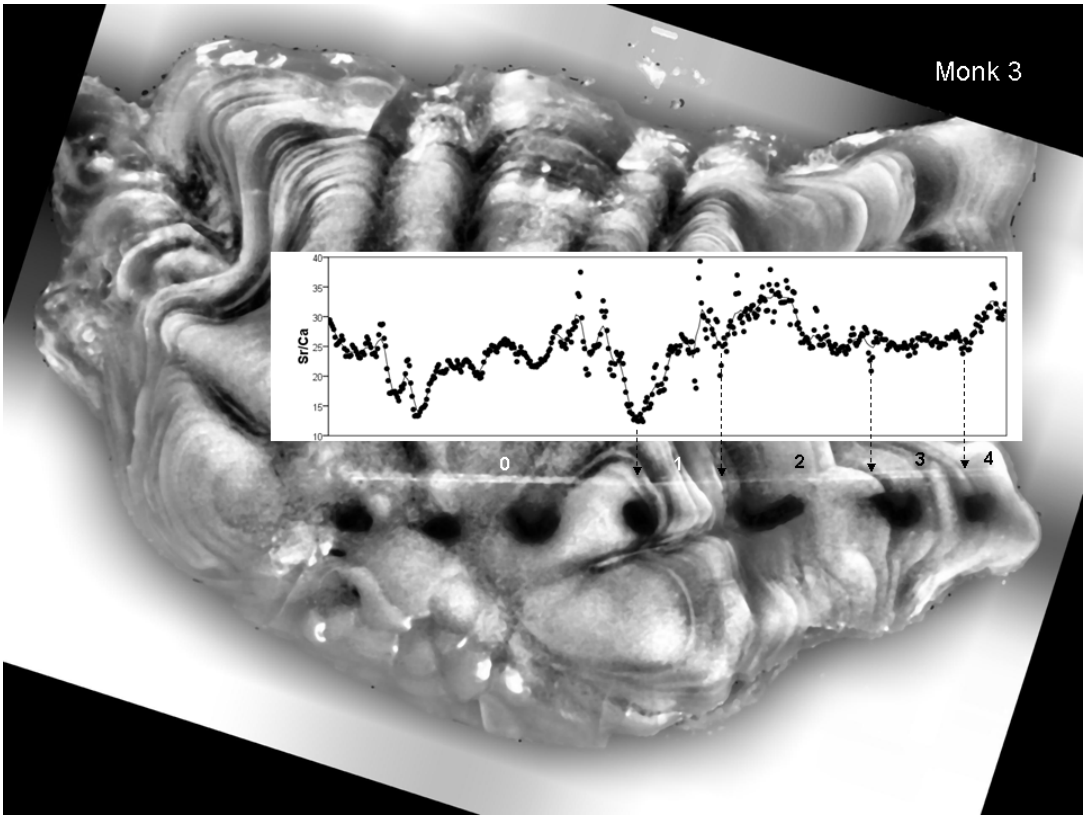
Figure 12. Strontium-based versus conventional (vertebrae-derived) age estimates for 14 samples which overlapped in this study. Dashed line is 1:1 line. Numbers beside points denote number of points occurring for that x-y value; no number means only a single point. Discrepancy occurs mostly at younger ages.

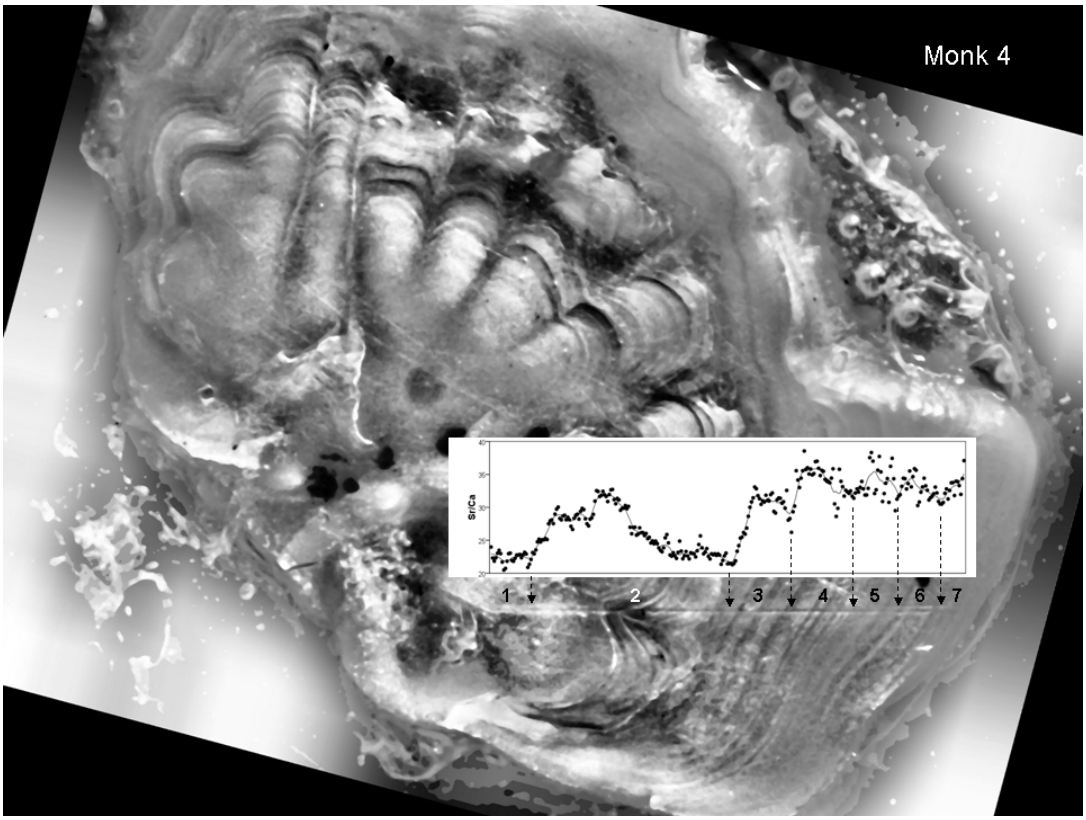
APPENDIX - Strontium to calcium ratio values (Sr/Ca) are shown superimposed on images of monkfish otoliths (x axis lines up with ablation track). See methods for age interpretation protocol.



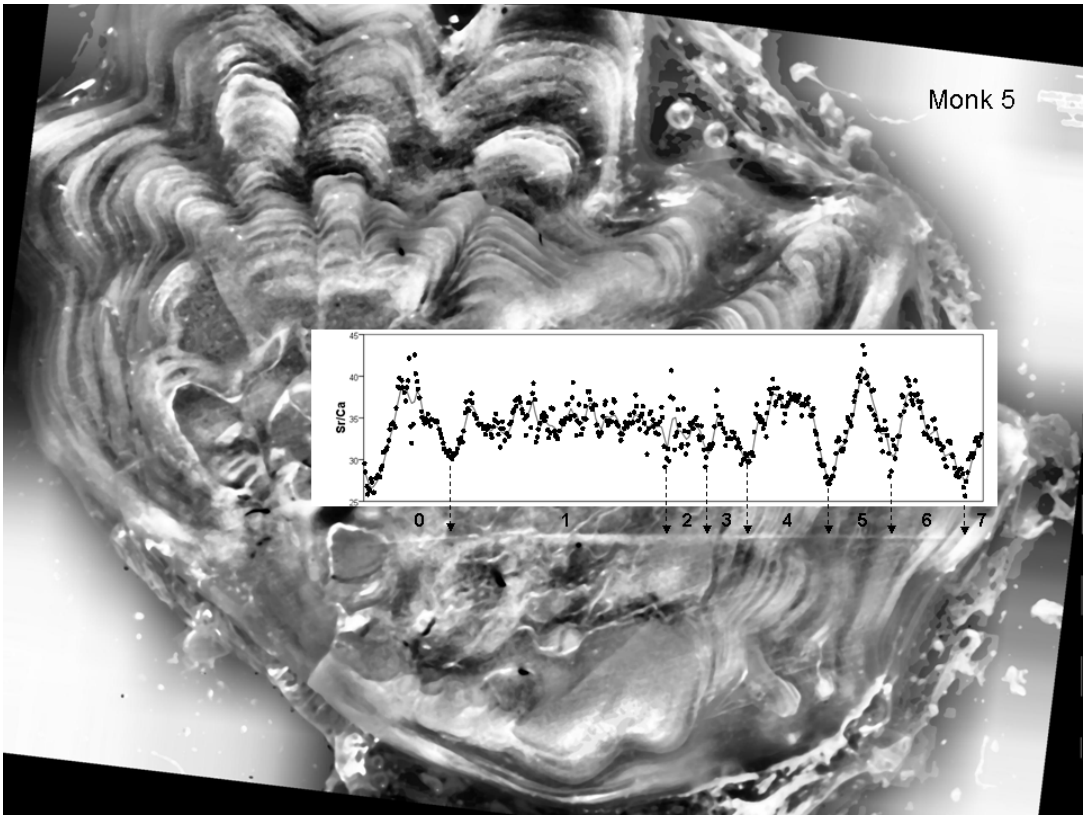


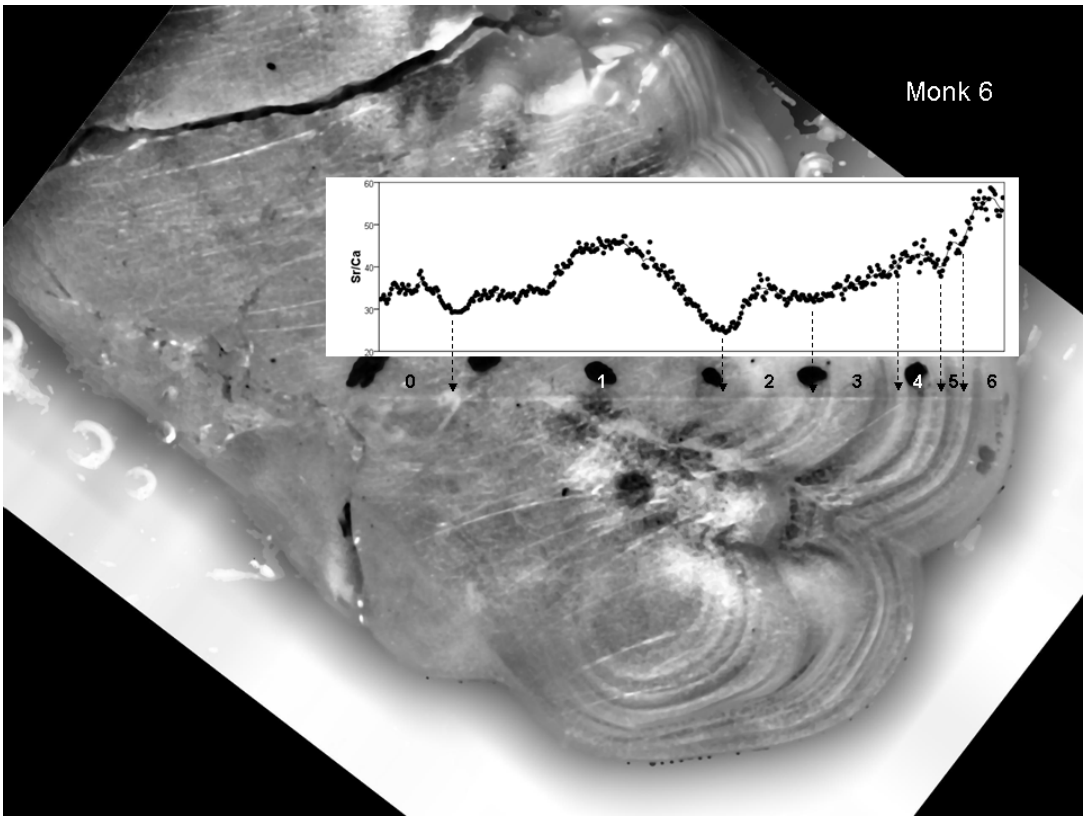
Note: Sr/Ca data for middle section not available. Annuli are interpreted without Sr/Ca here.



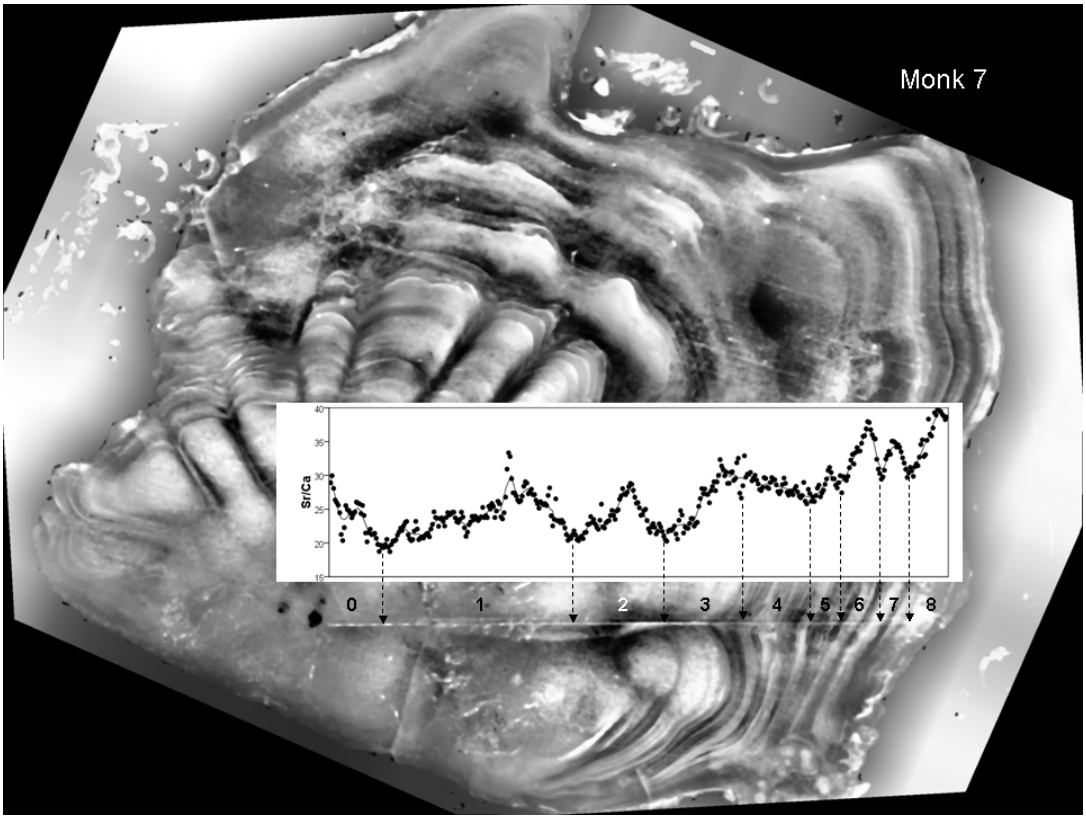


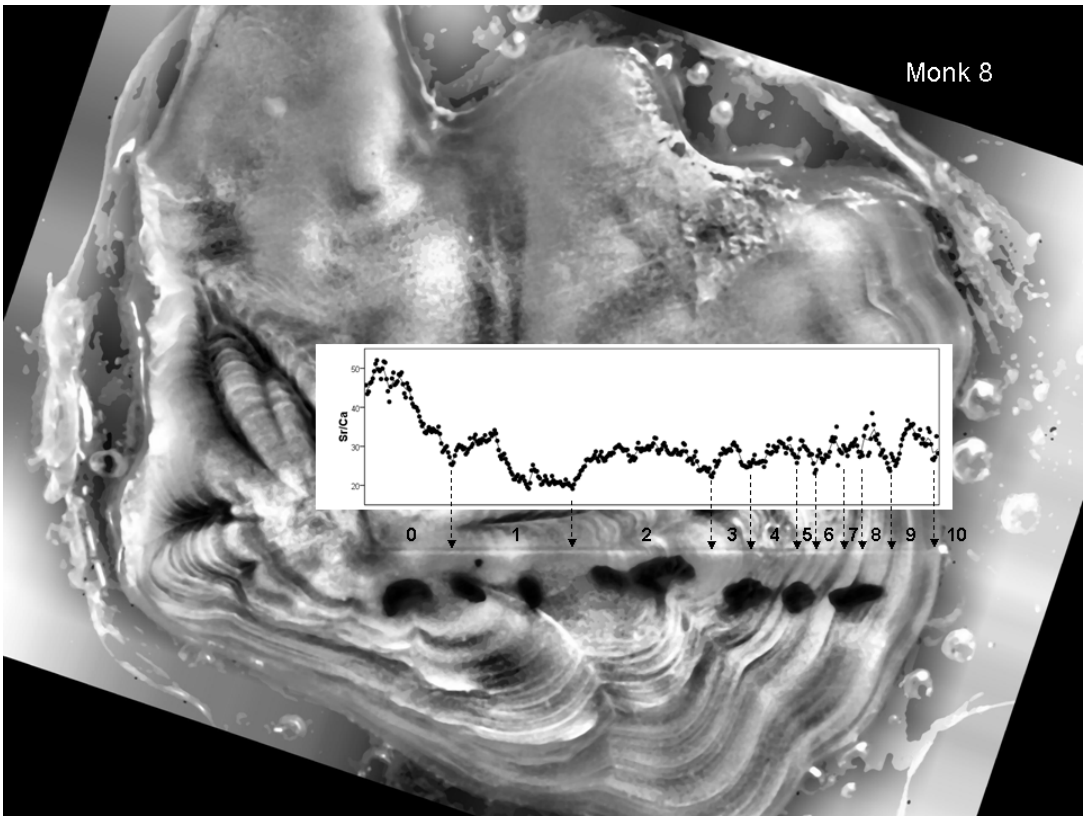
Note that black marks were made to guide laser track and do not signify age.





Note that black marks were made to guide laser track and do not signify age.





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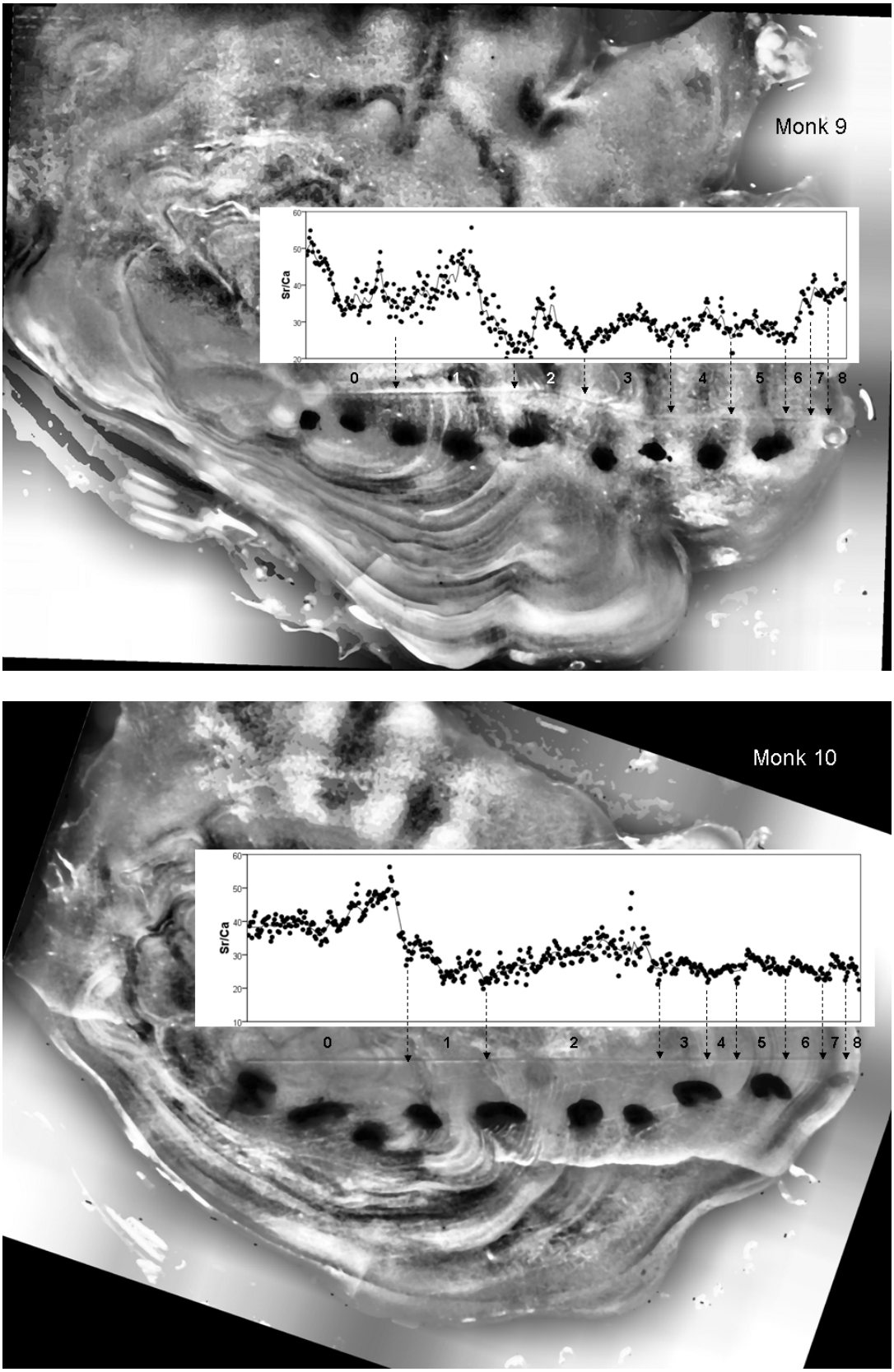

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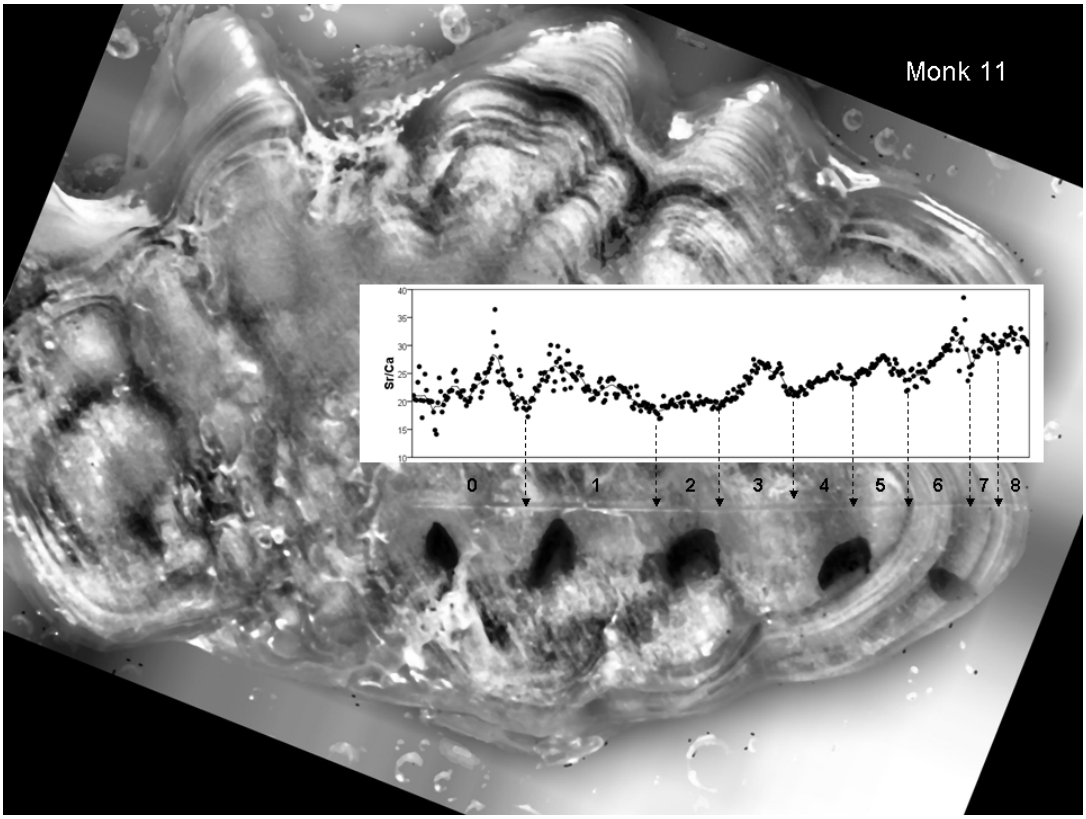

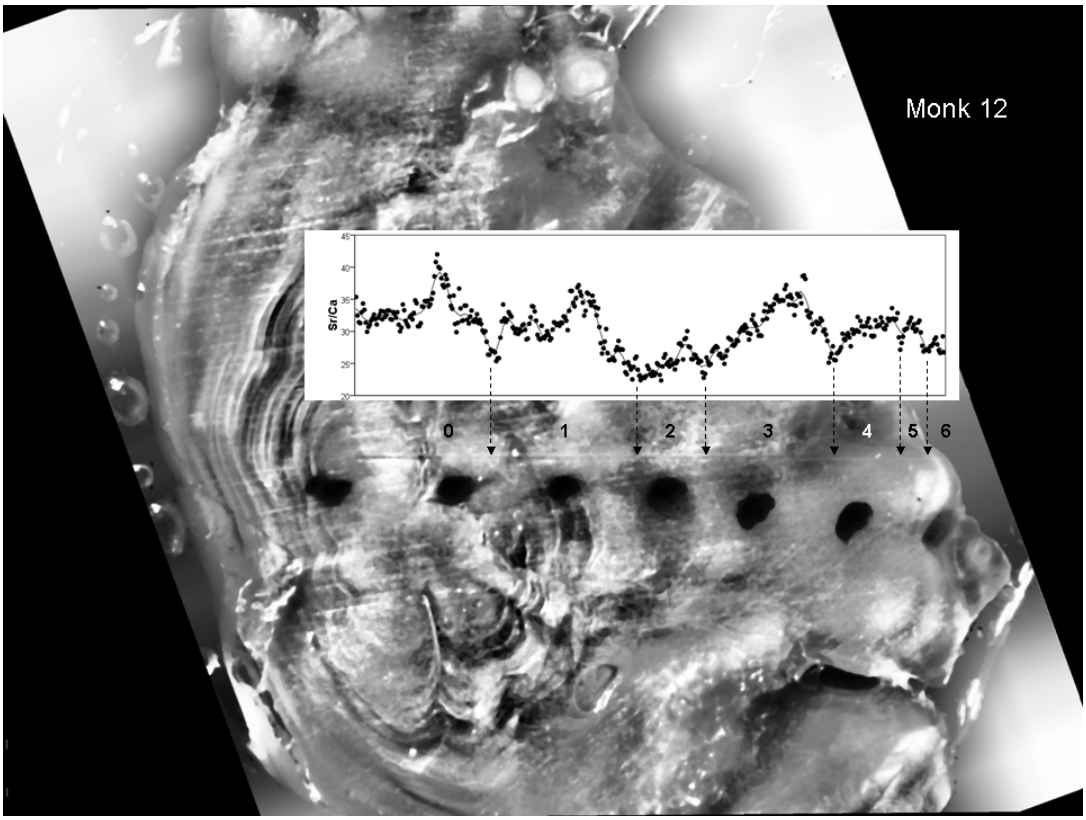

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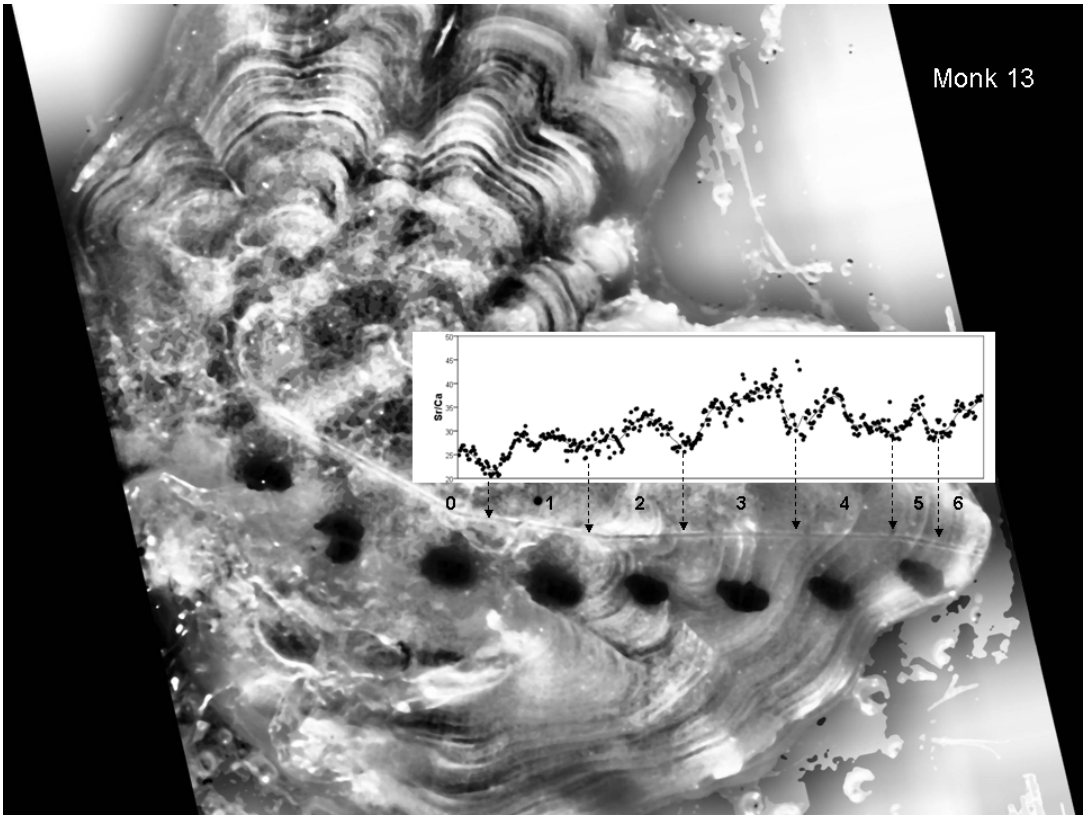

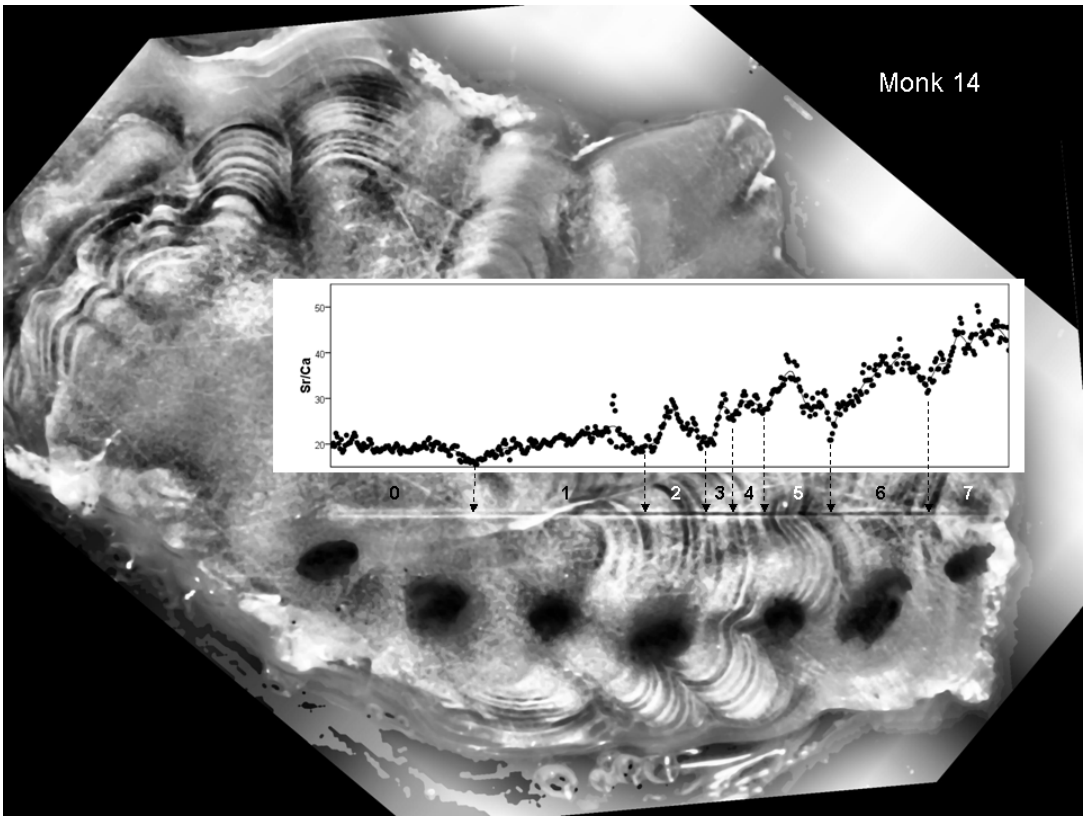

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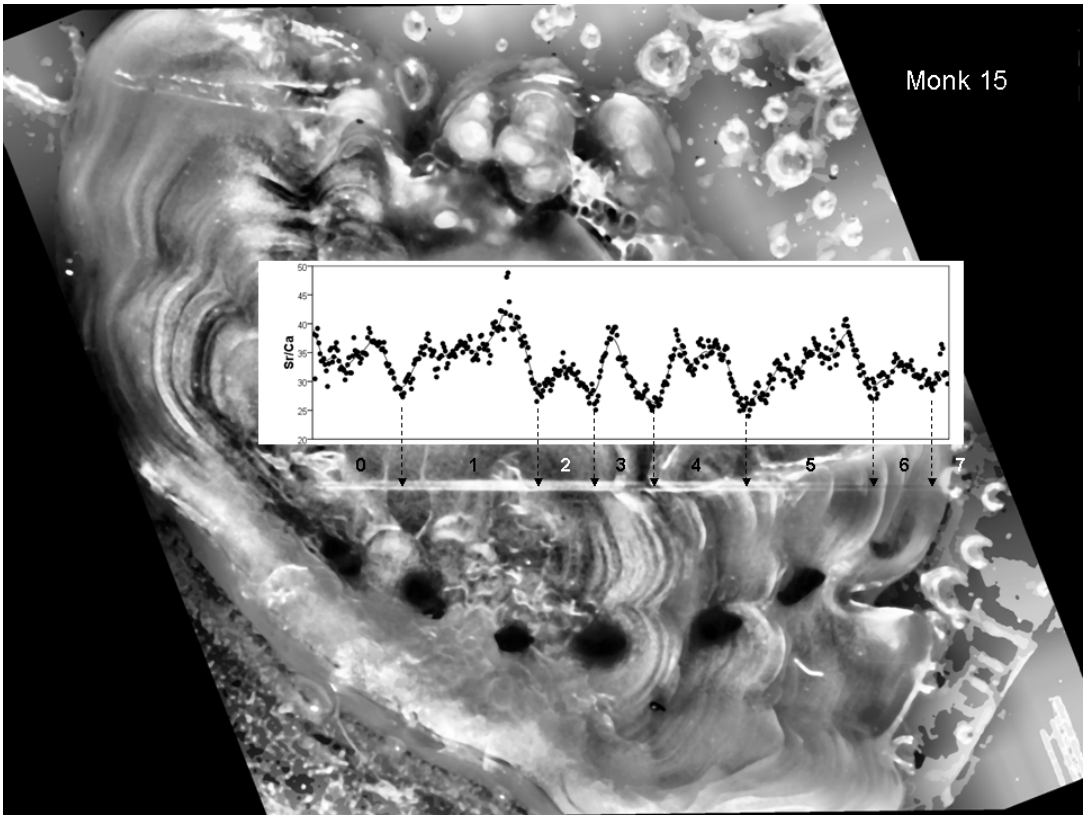

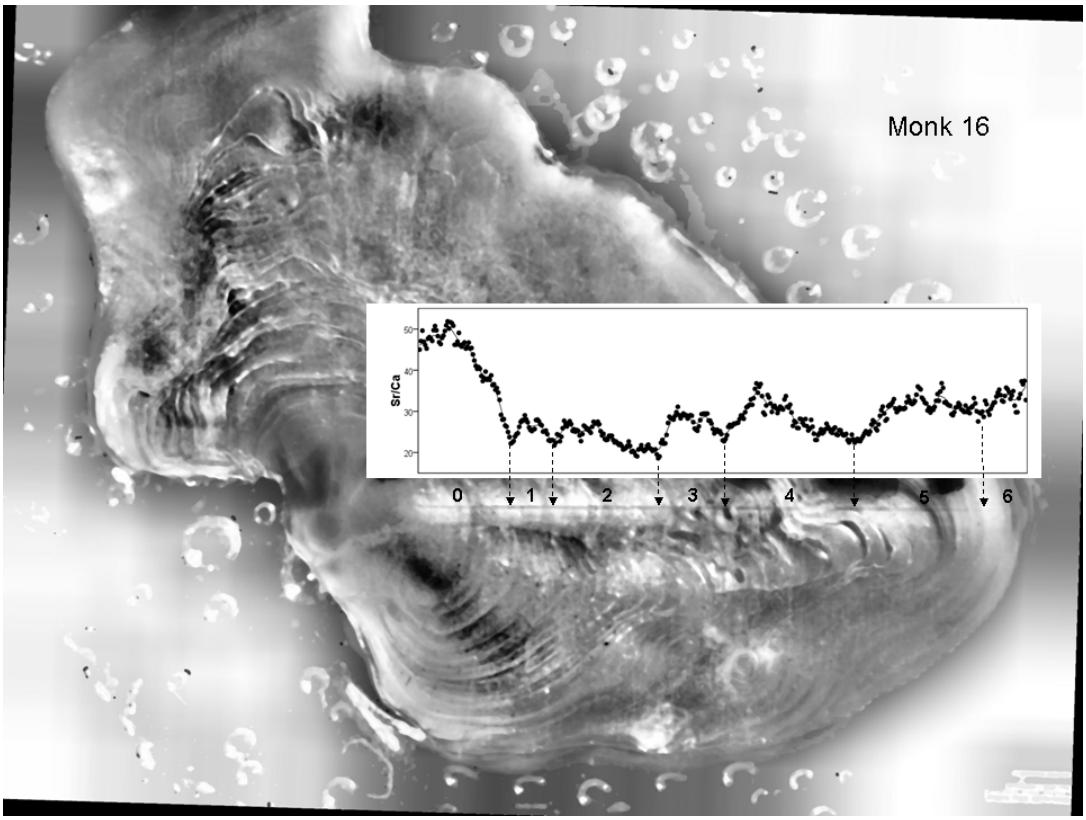

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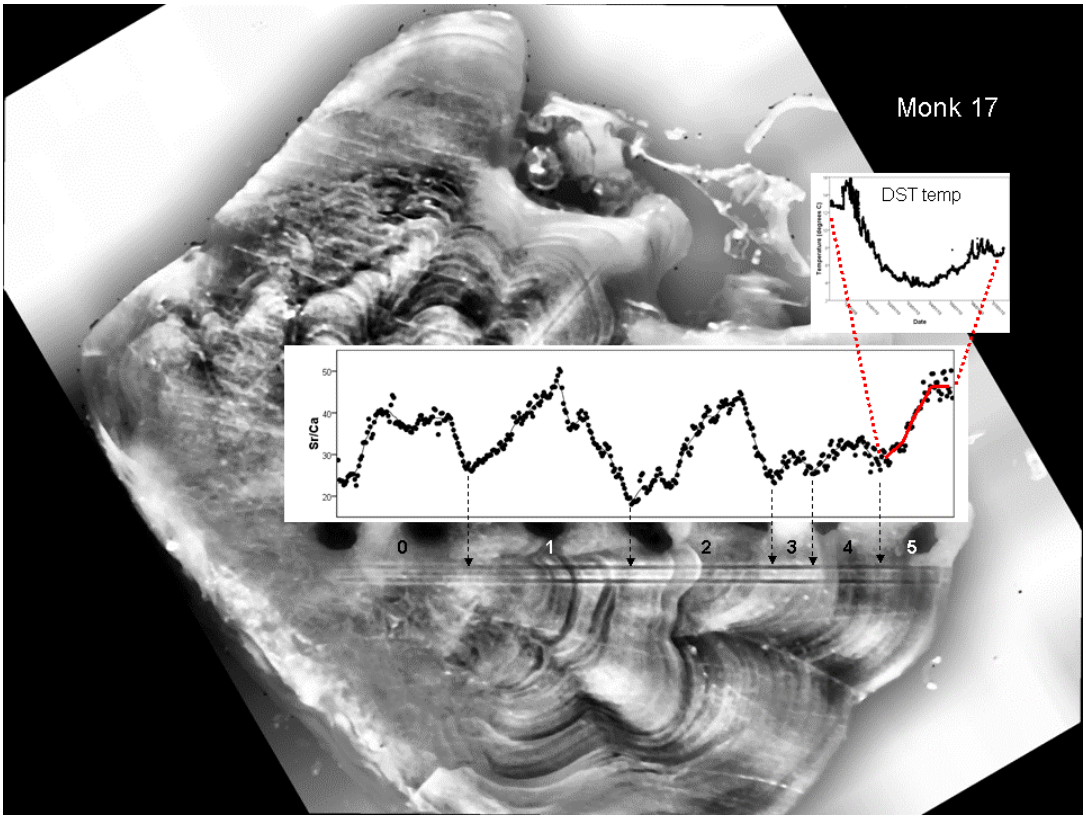
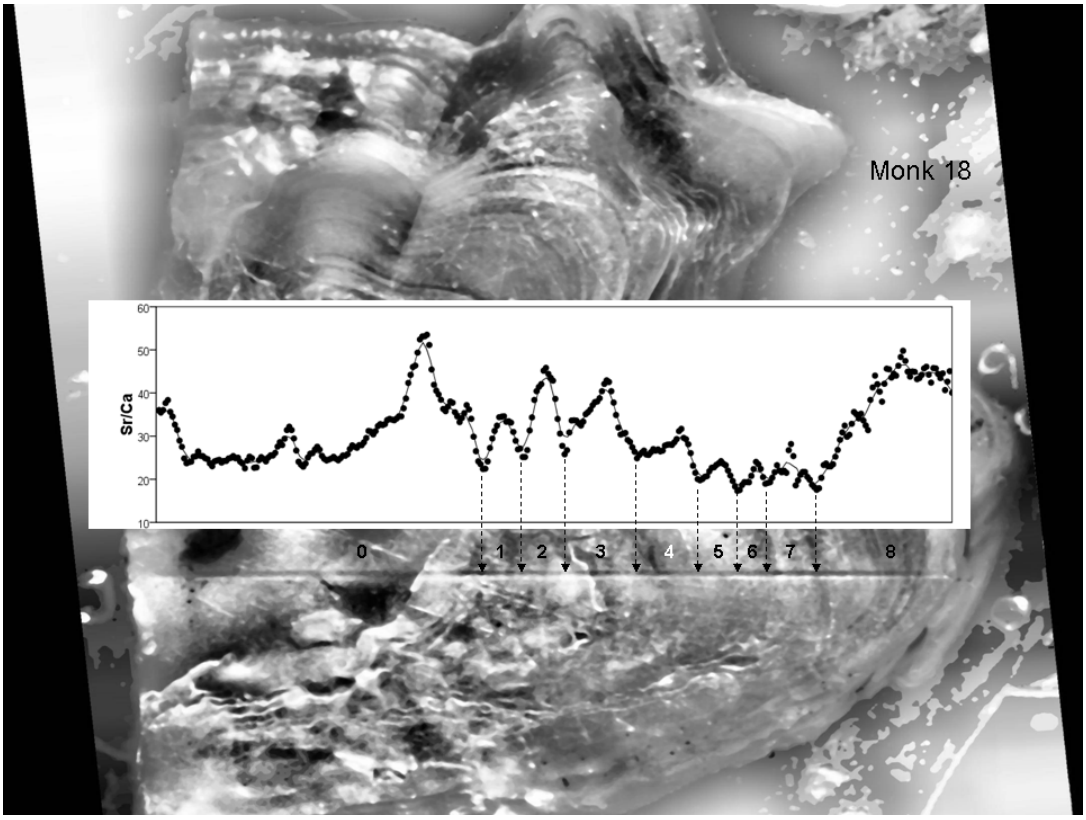


Temperature record from DST 4078 is superimposed. Note inverse relationship (see also Figure 7).



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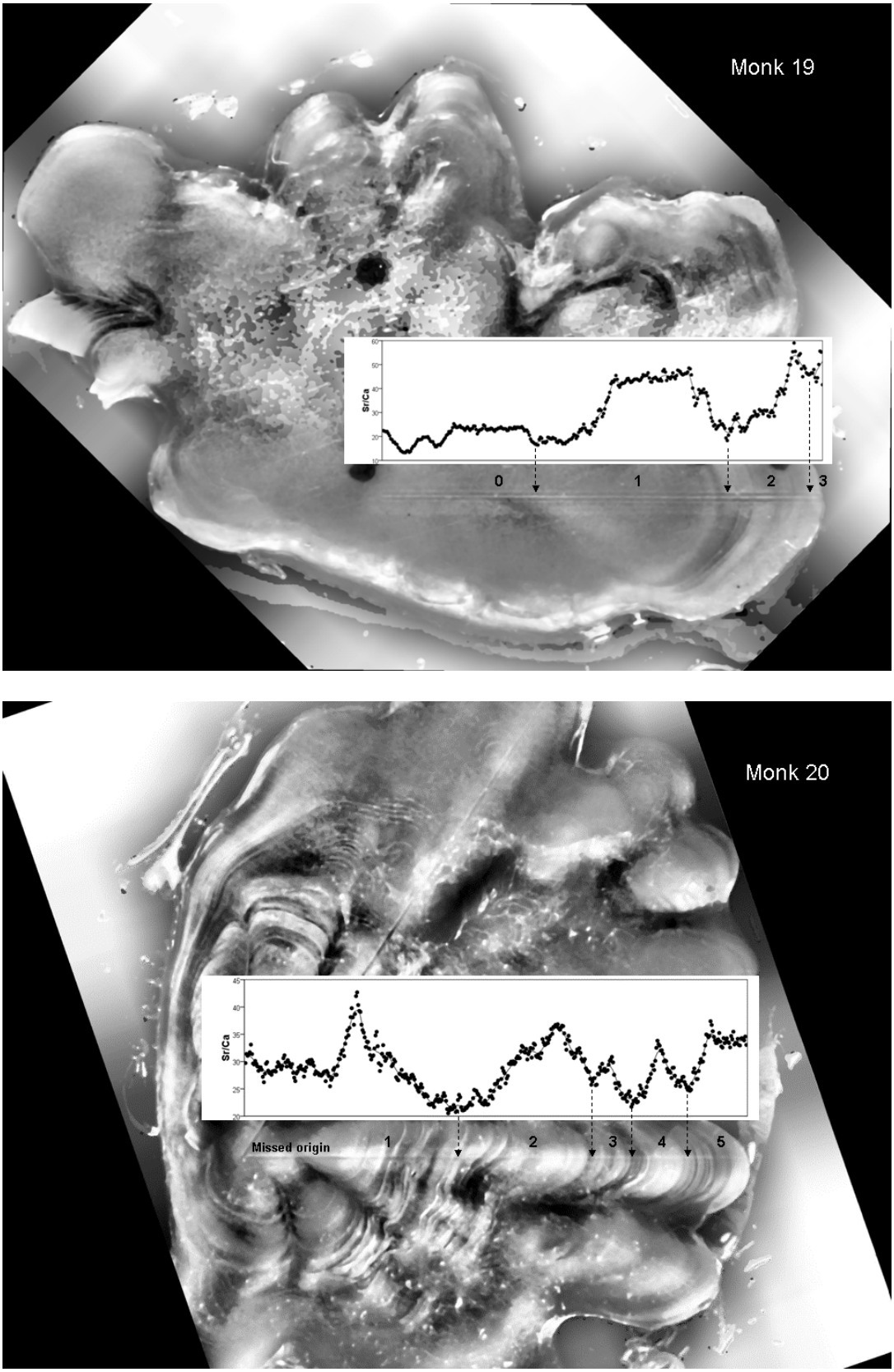

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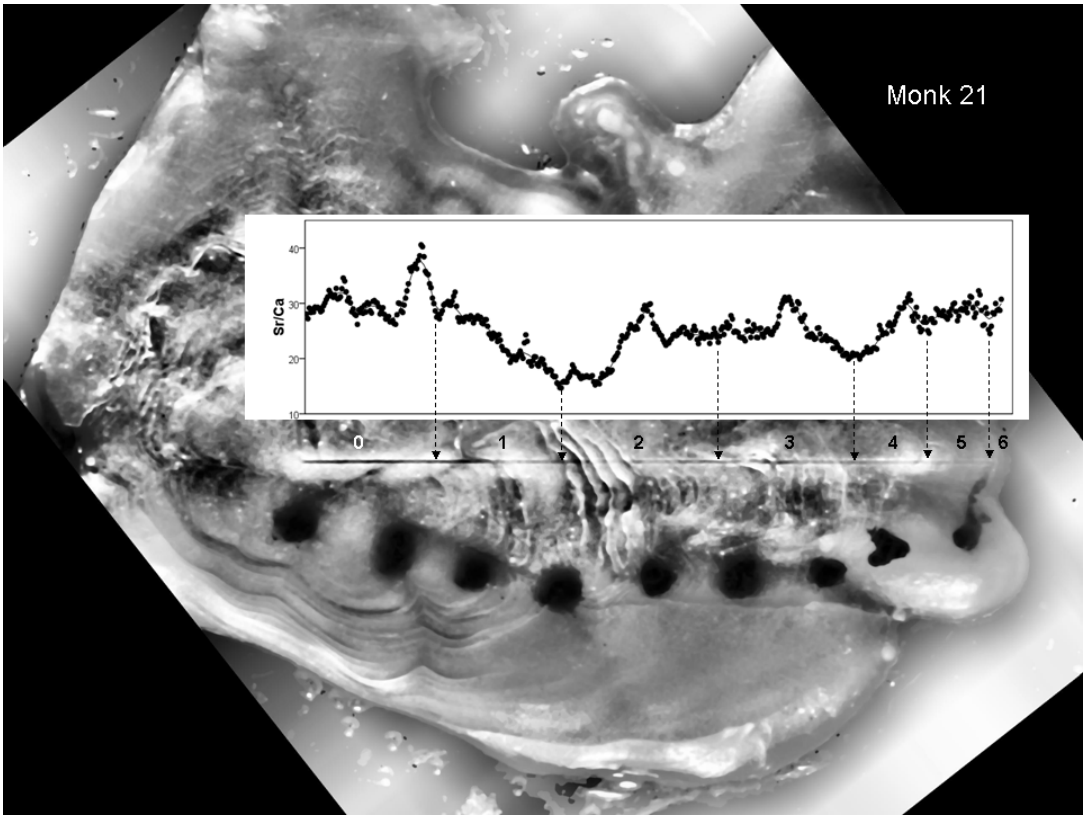

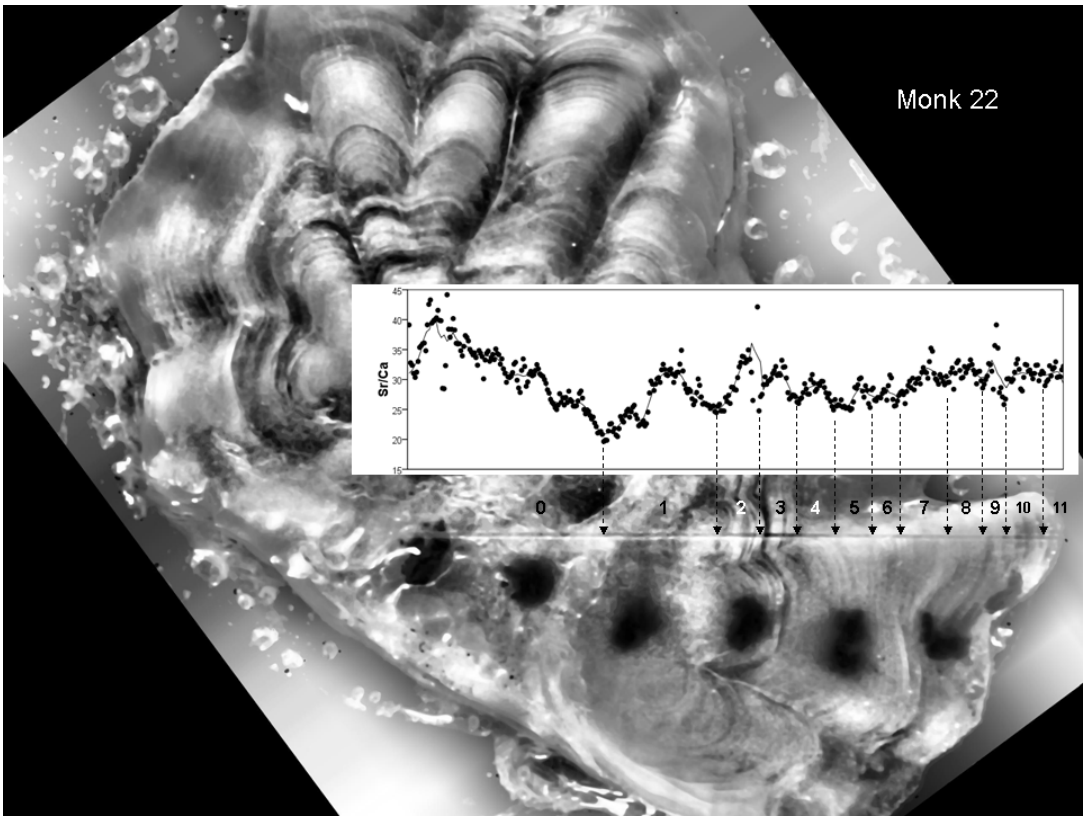

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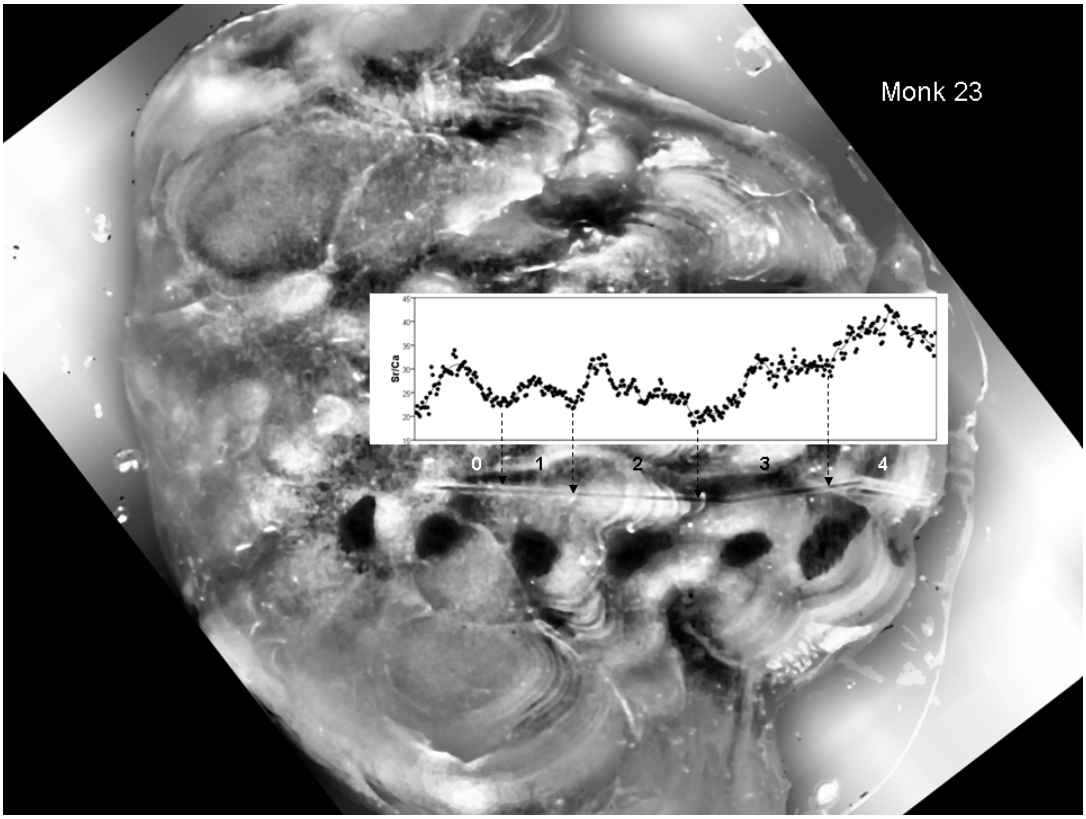

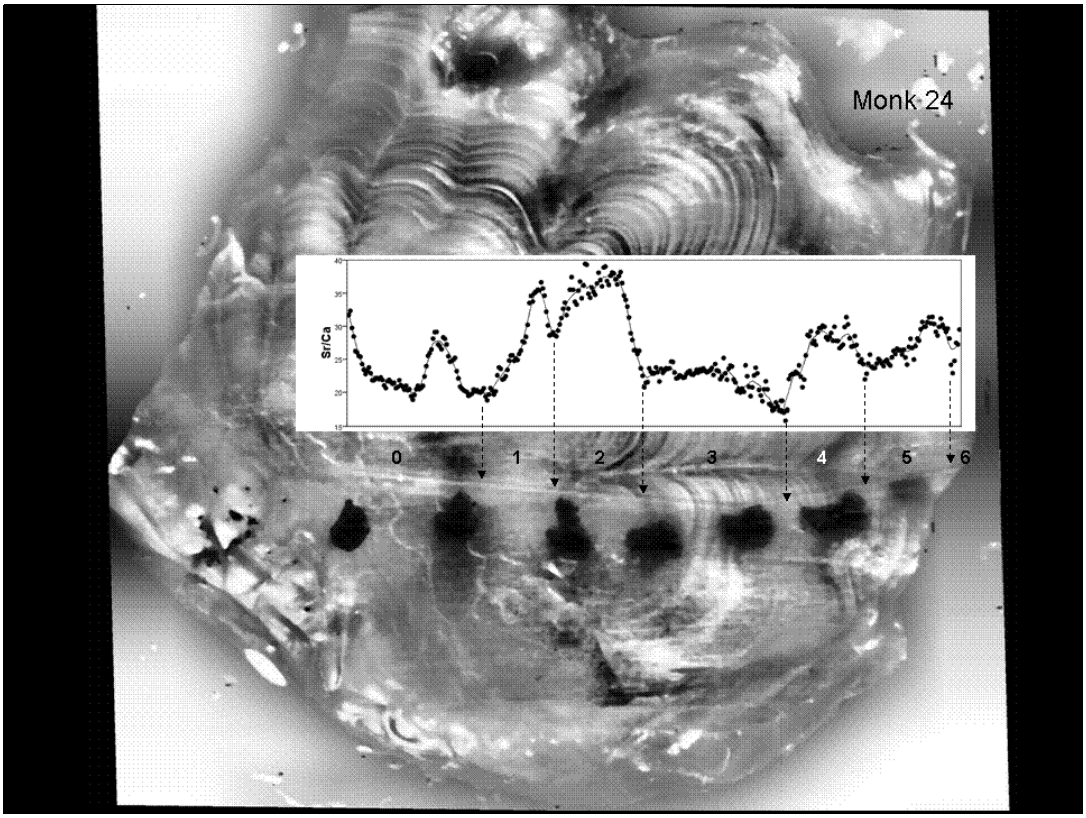

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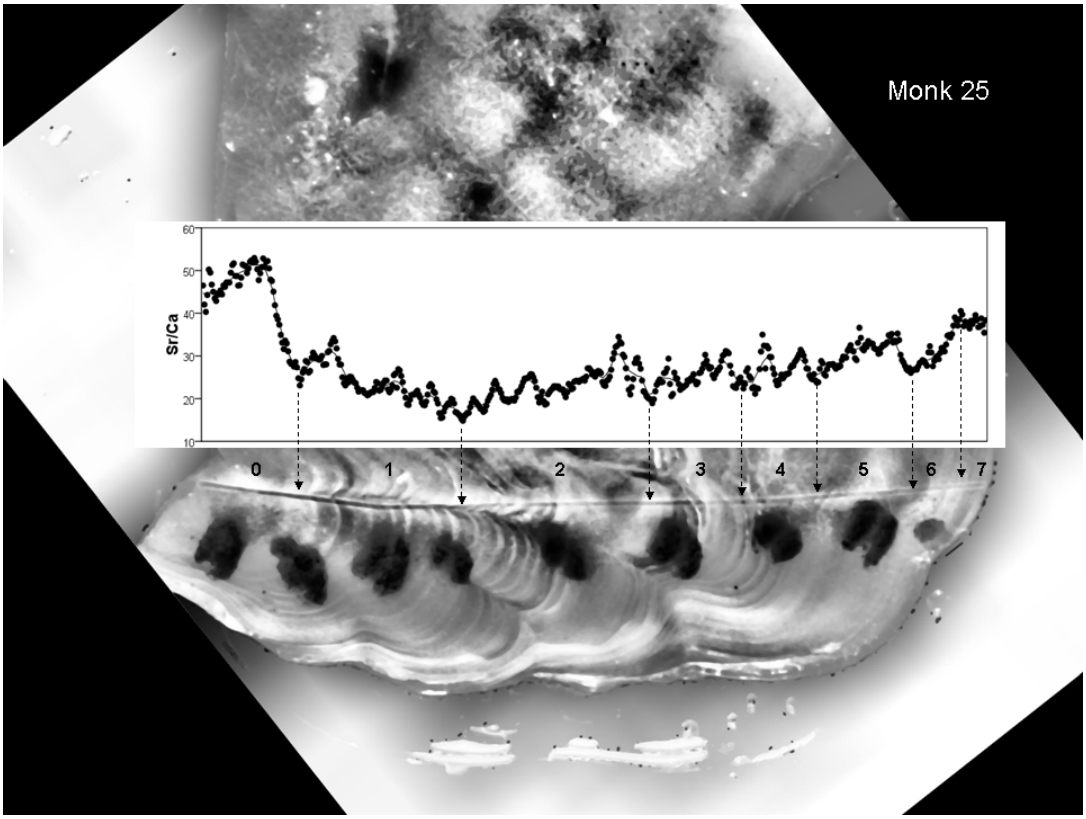

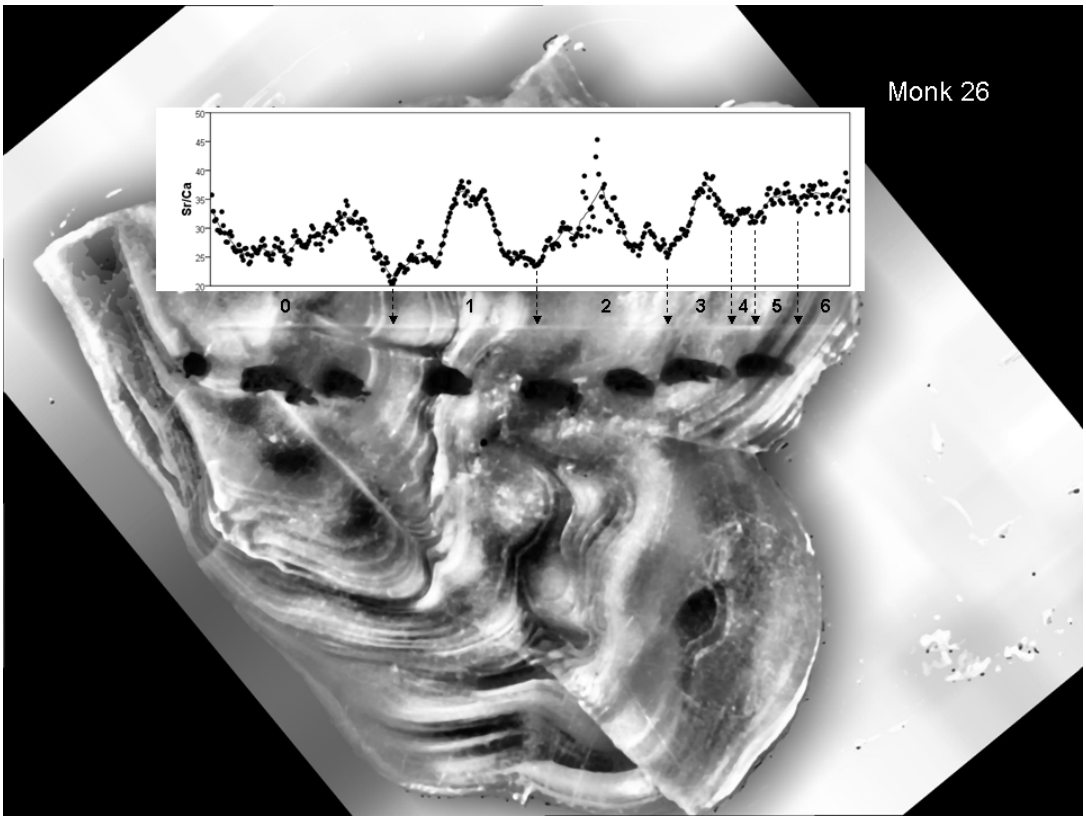

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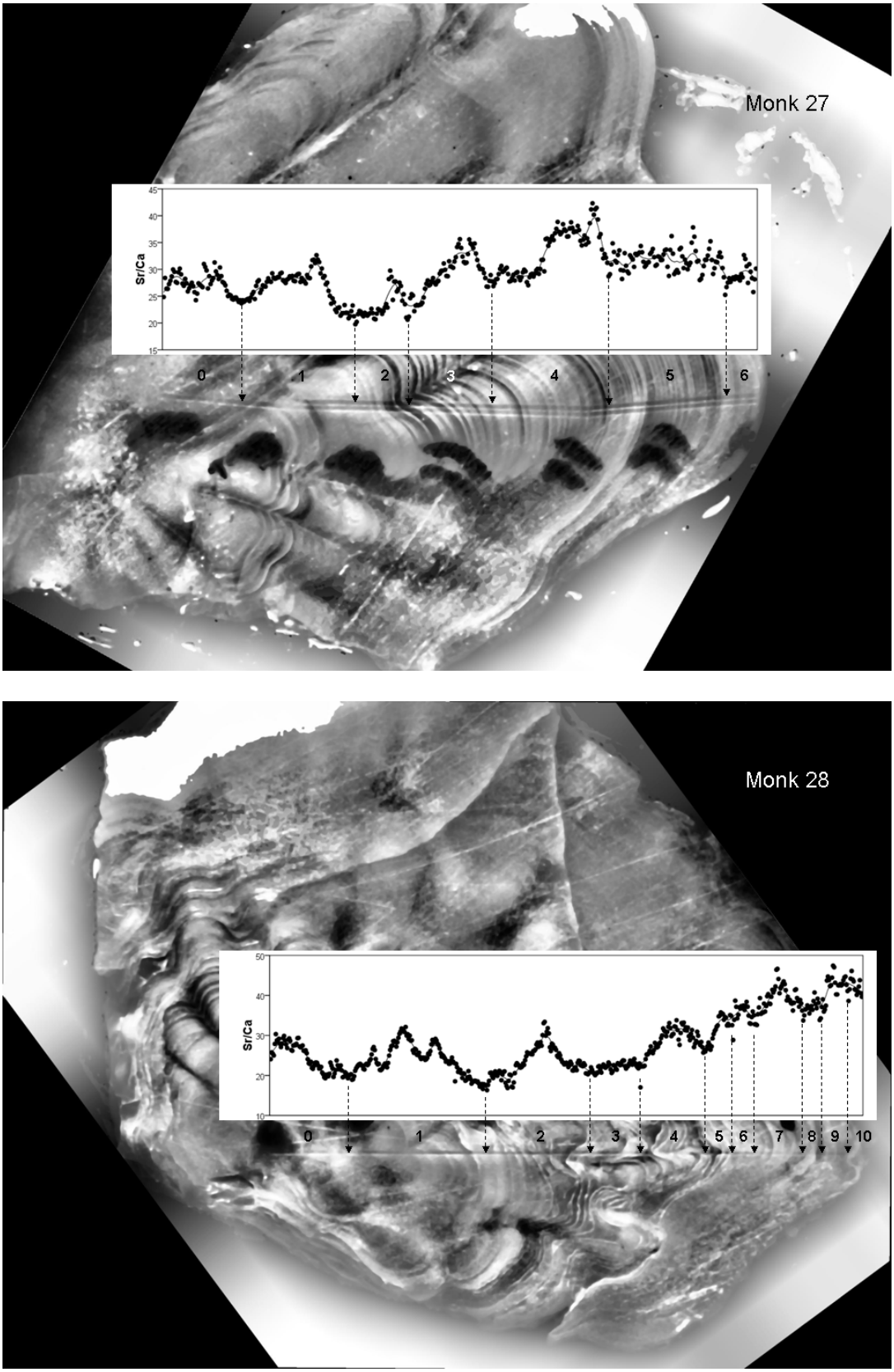

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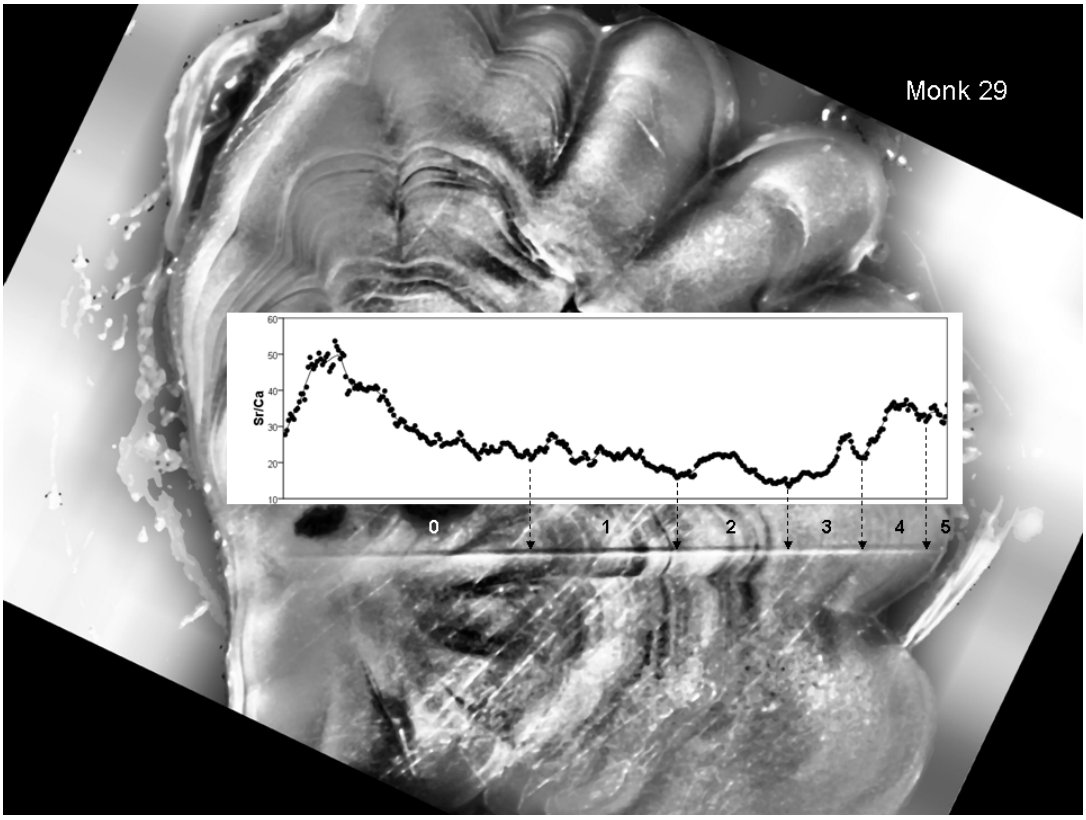
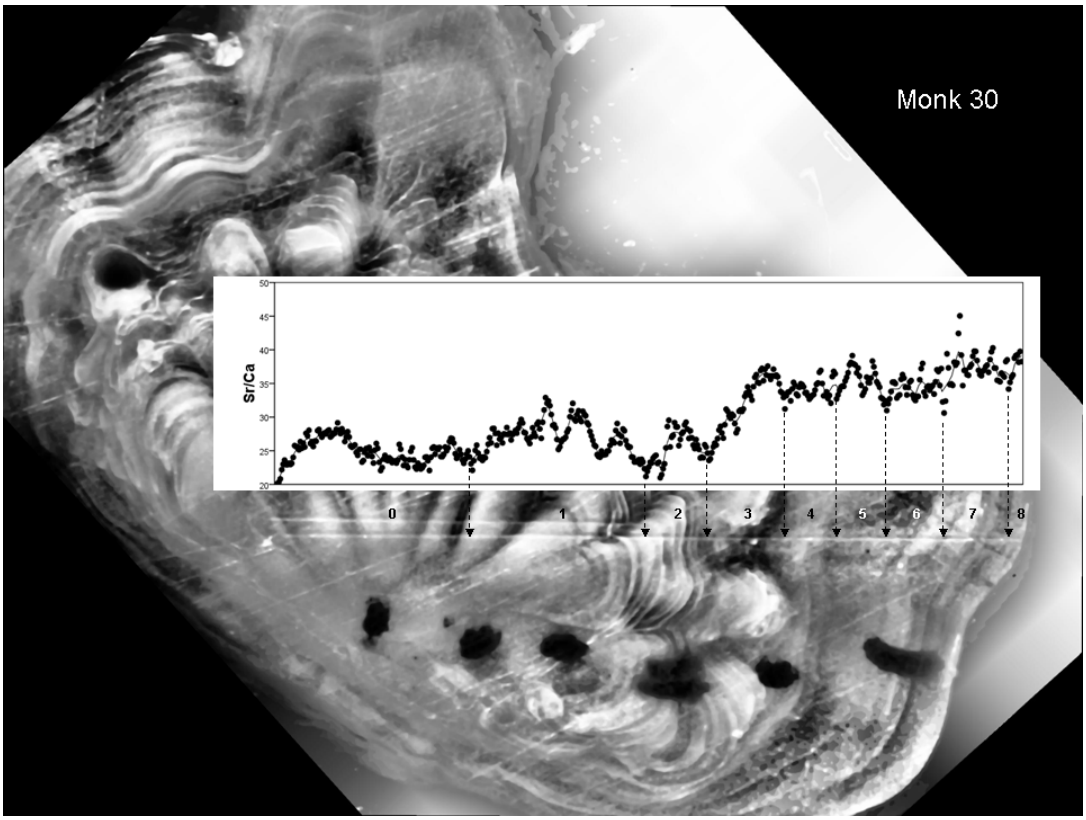
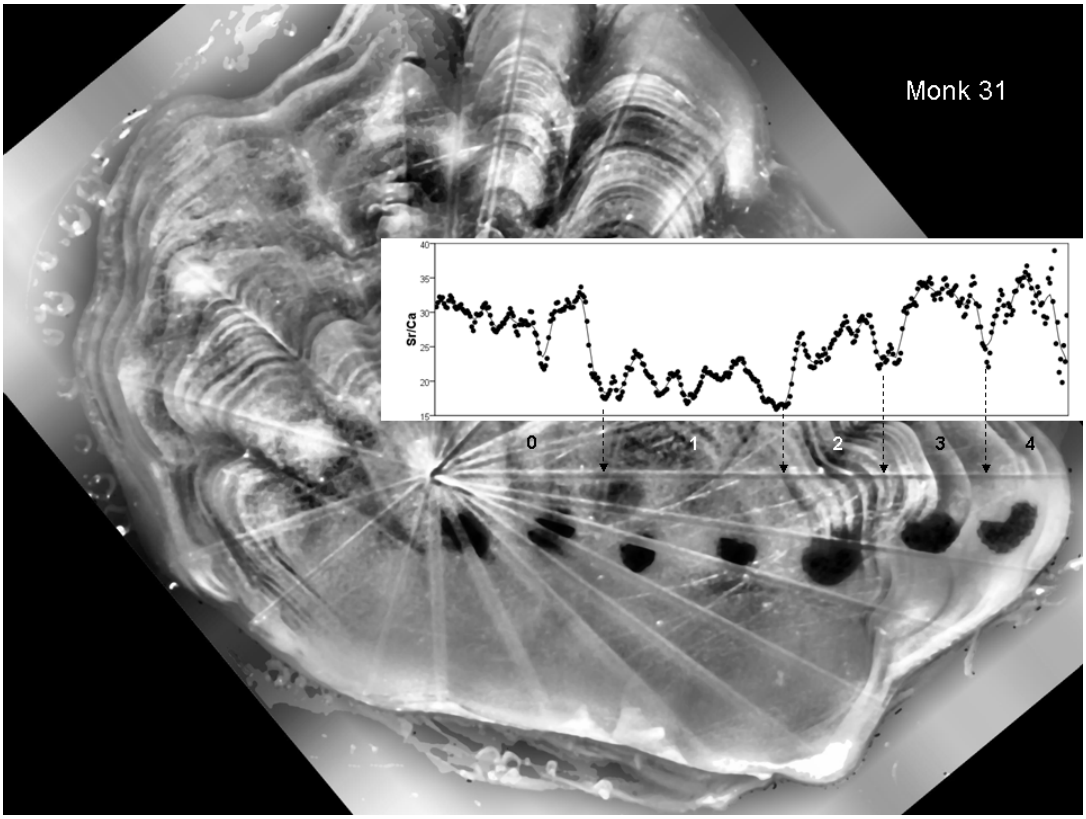
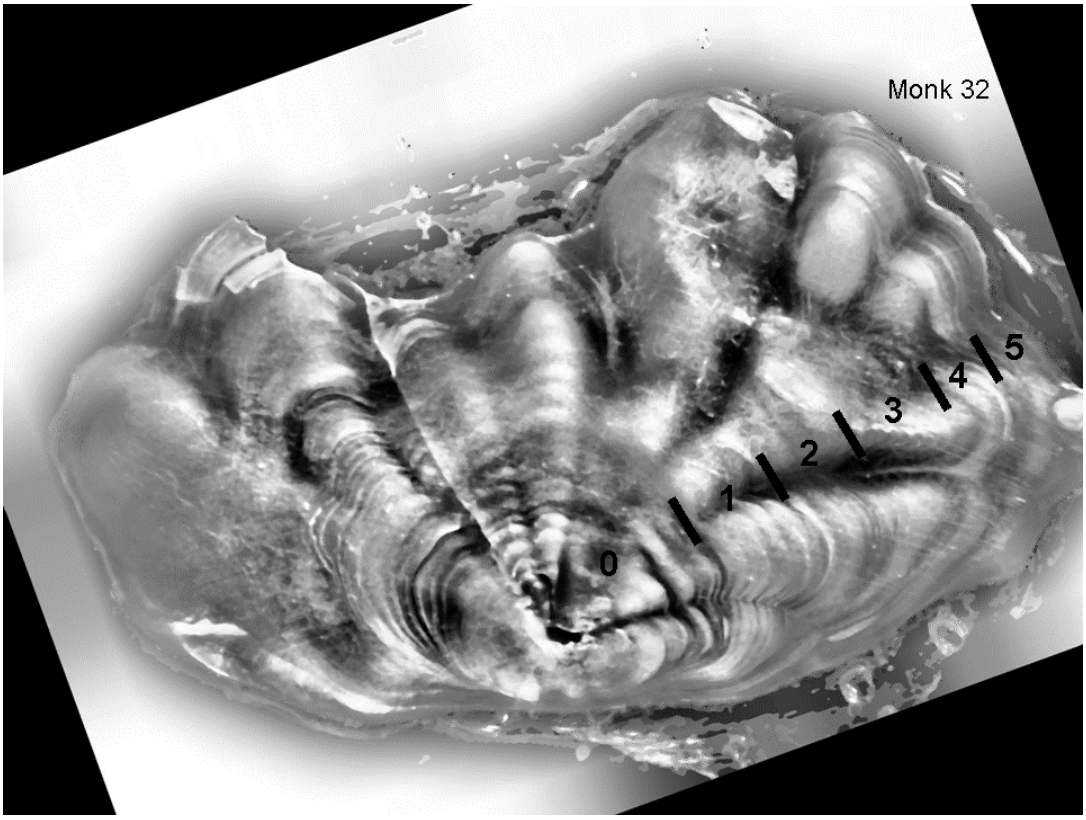




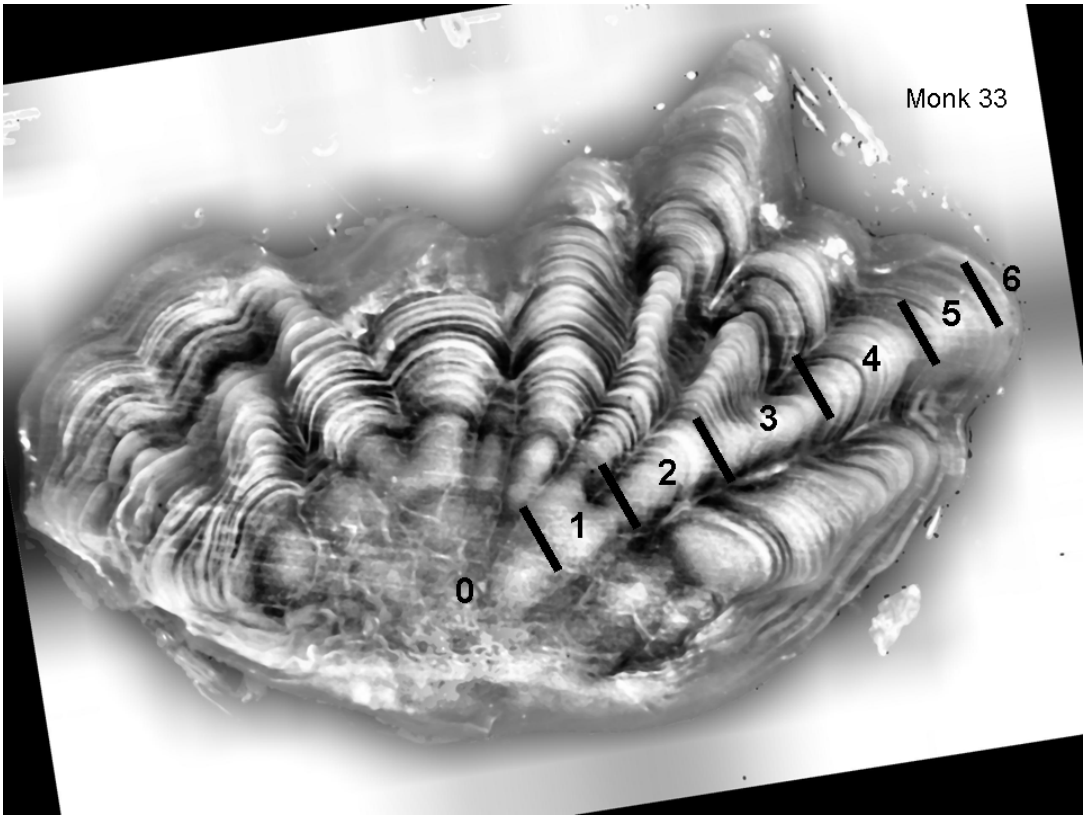
Note that black marks were made to guide laser track and do not signify age.



See also Figures 9 and 10. Black marks do not signify age.



Sr/Ca data not available for Monk 32. Age is based on visual ring counts.



Sr/Ca data not available for Monk 33. Age is based on visual ring counts.