Validation of Annual Growth Zone Formation in Gray Triggerfish (Balistes capriscus) Dorsal Spines, Fin Rays, and Vertebrae

Robert J. Allman, Carrie L. Fioramonti, William F. Patterson III and Ashley E. Pacicco

SEDAR43-WP-01

10 March 2015

Please cite this document as:

Allman, R.J., Carrie L. Fioramonti, William F. Patterson III and Ashley E. Pacicco. 2015. Validation of Annual Growth Zone Formation in Gray Triggerfish (Balistes capriscus) Dorsal Spines, Fin Rays, and Vertebrae. SEDAR43-WP-01. SEDAR, North Charleston, SC. 18 pp.

Validation of Annual Growth Zone Formation in Gray Triggerfish (*Balistes capriscus*) Dorsal Spines, Fin Rays, and Vertebrae

Robert J. Allman[1], Carrie L. Fioramonti, William F. Patterson III[2] and Ashley E. Pacicco[3]

[1]National Marine Fisheries Service, Southeast Fisheries Science Center, Panama City Laboratory, 3500 Delwood Beach Road, Panama City, FL 32408

[2]University of South Alabama, Dauphin Island Sea Lab, 101 Bienville Blvd., Dauphin Island, AL 36528

[3]Riverside Technology Inc., National Marine Fisheries, Panama City Laboratory, 3500 Delwood Beach Road, Panama City, FL 32408

ABSTRACT

The goal of this study was to validate annual growth zone formation in the Gray Triggerfish dorsal spines, fin rays and vertebrae. Adult Gray Triggerfish (n= 4) were chemically marked by injecting with 50 mg of oxytetracycline (OTC) per kg body mass and reared in a 2,300-L aquaculture tank. Fish were exposed to ambient light and water temperature mimicked bottom temperatures observed at approximately 30 m depth in the northern Gulf of Mexico. Fish were sacrificed after 262 days and their first dorsal spines, fin rays and vertebrae were extracted and sectioned. One translucent zone formed distal to the OTC mark in all hardpart types during winter. Additional fin rays and vertebrae with corresponding dorsal spines were sampled from fish (n =27 and 59, respectively) during fishery-independent surveys to compare translucent zone counts between hardparts. There was a significant difference between translucent zone counts between fin ray and dorsal spine sections ($t_{1,25} = -3.15$, $P = 0.004$), with fin ray counts being on average 1 zone higher than dorsal spines. Translucent zone counts in vertebrae were similar to those counted in dorsal spines with no significant difference between structures ($t_{1,57} = 1.90$, $P = 0.062$). The percentage of dorsal spines with translucent margins increased in winter months, peaking in February, and dropped to the lowest values in summer. The combined results of this study validate annual translucent zone formation in Gray Triggerfish hardparts, with dorsal spines being the most reliable ageing structure.

INTRODUCTION

The Gray Triggerfish, *Balistes capriscus*, is a conspicuous member of the northern Gulf of Mexico (GOM) reef fish community (Dance et al. 2011; Patterson et al. 2014). Historically, Gray Triggerfish were not heavily targeted or considered an important food resource in the reef fish fishery. However, they have become increasingly targeted both commercially and recreationally due to increased regulations on other reef fishes, such as snappers and groupers (Valle et al. 2001; Bernardes 2002). Landings for Gray Triggerfish in the GOM increased substantially from the mid-1980s to the late 1990s and declined thereafter due to stock depletion (SEDAR 2006, 2012).

Recent stock assessments for Gray Triggerfish have been performed using age-based statistical catch at age models (SEDAR 2006, 2012), with triggerfish ages estimated via counts of translucent zones in dorsal spines (Johnson and Saloman 1984; Ofori-Danson 1989; Ingram 2001; Burton et al. 2015). However, this is problematic due to the fact that annual growth zone formation has not been validated for Gray Triggerfish spines or any other hardpart (e.g., otoliths, fin rays, vertebrae). The first dorsal spine has been the preferred ageing structure for Gray Triggerfish because otoliths are difficult to locate, extract and process due to their small (< 5 mm) size and irregular shape (Bernardes 2002; Moore 2001). Consequently, age estimation has been accomplished by counting translucent zones in sectioned dorsal spines that are presumed to be formed annually but without direct validation . Validation of annual growth zone formation in fish hardparts is critical to ensure accurate estimation of growth rates and for estimating catch at age as data inputs for stock assessments (Beamish and McFarlane 1983; Campana 2001).

Validation can be accomplished by counting growth zones in hardparts of known-aged fish or by chemically marking hardparts with calcium-binding compounds, such as oxytetracycline (OTC), calcein, or alizarin (Campana 2001). Chemical marking requires examination of growth zone formation following some period of growth, either in sacrificed captive-reared fish or recaptured tagged fish (Beamish and McFarlane 1983). Hood and Johnson (1997) attempted to validate the periodicity of translucent zone

formation in the first dorsal spine of Gray Triggerfish by injecting fish with OTC and rearing them in an indoor aquaculture facility under constant light and temperature. Dorsal spine sections of those animals did not show translucent zone deposition following OTC marks, but deviation from natural light and temperature fluctuations may have altered normal physiological processes. Outdoor enclosures or tanks may be a better approach to replicating natural conditions, particularly light cycles (Natanson 1993; Campana 2001).

The primary goal of this study was to validate annual growth zone formation in the first dorsal spines of Gray Triggerfish, with secondary goals being validation of annual growth zone formation in fin rays and vertebrae. The specific objectives were to: 1) validate dorsal spines, fin rays and vertebrae as ageing structures for Gray Triggerfish by rearing OTC-marked fish; 2) examine marginal condition of dorsal spine sections of wild-caught Gray Triggerfish to verify annual translucent zone formation, and 3) compare translucent zone counts among dorsal spines, fin rays, and vertebrae to evaluate the utility of each as an ageing structure. Fin rays were of particular interest as potential non-lethal aging structures (Cass and Beamish 1983; Koch and Quist 2007; Murie et al. 2008).

METHODS

Age Validation Experiment

Gray Triggerfish (n = 4) were collected in October 2009 with fish traps off the coast of Panama City, Florida. Fish were transferred to and reared in a 2,300-L aquaculture tank with a recirculating biofiltration system. The tank was housed in a building constructed with a translucent vinyl covering that allowed natural light to penetrate. Each fish was tagged with a Floy FM-95 stainless steel internal anchor tag and chemically marked by injecting with 50 mg of OTC per kg body mass. Fish were exposed to ambient light and diurnal rhythms. Water temperature was maintained with heaters during the winter months to replicate mean bottom temperature observed in the northern GOM at approximately 30 M depth. Salinity was monitored and maintained at 32-34

psu. Gray triggerfish were fed cut squid, shrimp, or fish every other day throughout their captivity. Following their death, fish carcasses were placed in plastic bags and frozen whole until processing.

Hardparts were extracted from thawed fish in a darkened room to prevent degradation of OTC marks due to light exposure. First dorsal spines were extracted by inserting a knife just posterior to a spine and cutting medially approximately 2.5 cm into the fish. Another identical cut anterior to the spine effectively cut out a notch of flesh that included the entire condyle of the dorsal spine. Dorsal spines were prepared for sectioning by boiling in water for 1 min to remove soft tissue and scraping the posterior groove free of tissue. Each spine was glued to cardstock and 3 transverse sections (0.5-0.7 mm thickness) were cut simultaneously with four 10-cm diamond coated blades on an Isomet low-speed saw. Prepared sections were fixed to microscope slides with mounting medium.

Dorsal and pectoral fin rays were extracted by cutting below the pterygiophores of each fin with a scalpel. Fin rays were cleaned of tissue by submerging the basal portion of rays in boiling water for up to 20 seconds. Soft tissue was removed with forceps and a soft-bristled brush and then laid flat to dry. Once dry, fin rays were embedded in epoxy for sectioning. Embedded fin rays were sectioned using a single, 5-cm blade on an Isomet saw. Each fin ray was sectioned to between 0.5-0.7 mm thickness. Sections were mounted on microscopic slides with mounting medium.

The 3 anterior-most abdominal vertebrae were dissected from each fish for ageing as Künzli and Tachihara (2012) reported that translucent bands were more apparent in anterior vertebrae than those of more posterior vertebrae in Picasso Triggerfish, *Rhinecanthus aculeatus*. Vertebrae were boiled for 3-5 minutes to remove soft tissue. Each was sectioned with an isomet low-speed saw equipped with two 10-cm blades with a 0.5-mm spacer. Vertebrae were sectioned in the dorsal-ventral plane and then sections were mounted on microscope slides with mounting medium. A few sections were stained with a solution of crystal violet in an attempt to improve resolution between opaque and translucent zones, but this step did not improve readability and thus was abandoned.

Dorsal spine, fin ray and vertebral sections were aged by counting the number of translucent zones present. Dorsal spine and vertebral sections were viewed with a dissecting microscope under 10-40 x magnification with transmitted light, and fin ray sections were viewed with a compound microscope under 100 x magnification using transmitted light and a green filter. For all three structures, opaque zones representing faster growth are relatively wide, and zones corresponding to slow growth periods are narrow and appear translucent under transmitted light (Lessa and Duarte-Neto 2004; Brusher and Shull 2009; Künzli and Tachihara 2012). The margin of each section was recorded as translucent or opaque and dorsal spines were assigned a readability code of good, fair, poor, or unreadable. Broad opaque zones in vertebrae often contained faint translucent zones which were considered checks and were not counted for ageing (Künzli and Tachihara 2012). Oxytetracycline marks in dorsal spine, fin ray and vertebral sections were examined as described above but with transmitted UV light.

Hardpart Comparison

Dorsal spines, fin rays and vertebrae were extracted from Gray Triggerfish sampled during fishery-independent surveys to compare translucent zone counts among hardparts. All hardparts were processed for ageing as described above. Ageing of all three structures was conducted by two readers independently without knowledge of FL (mm) to prevent bias. Average percent error (APE; Beamish and Fournier, 1981) was used to estimate precision between reader ages. Any disagreement in ages was resolved by reader consensus. If a consensus could not be reached the hardpart was rejected. Differences in translucent zone counts between fin rays and vertebrae versus dorsal spines were tested with paired t-tests ($\alpha = 0.05$). Bias plots were constructed to detect any systematic differences in translucent zone counts between ageing structures (Campana et al. 1995).

Ageing Verification in Dorsal Spines

Dorsal spine sections were selected for marginal condition analysis from archived Gray Triggerfish samples collected from 2003-2010. Samples were restricted to sections

which were assigned readability codes of fair to good. The percentage of dorsal spines with a translucent margin was plotted versus month to examine the temporal progression of translucent zone formation.

RESULTS

The 4 OTC-marked Gray Triggerfish were reared from October 31, 2009 to July 20, 2010. The original intent was to rear fish for at least one year but an unexpected pump failure resulted in low dissolved oxygen levels and all fish died. Nevertheless, results of the OTC marking experiment indicate that one translucent zone formed in dorsal spines, fin rays and vertebral sections during winter months (Fig. 1). An opaque margin on each structure also indicated opaque zone formation had begun prior to experiment termination in July.

Gray Triggerfish selected for hardpart comparisons included the most common sizes seen in the fishery and ranged from 75 to 450 mm FL for fin ray samples (n = 27) and from 108 to 481 mm FL vertebrae samples (n = 59). Translucent and opaque zones were apparent in all structures and translucent zone counts were the same between hardparts for many fish (Fig. 2). APE between readers was 10.8% for dorsal spines, 12.3% for fin rays and 18.8% for vertebrae. A paired t-test indicated a significant difference existed between translucent zone counts in fin ray versus dorsal spine sections ($t_{df = 1,25} = -3.15$, $P = 0.004$). On average, one more translucent zone was counted in rays than in spine sections, which is apparent in the bias plot for those two structures (Fig. 3A). Translucent zone counts in abdominal vertebrae were similar to those counted in dorsal spines, and no significant difference in counts existed between those structures ($t_{df = 1,57} = 1.90$, $P = 0.062$) (Fig. 3B).

Marginal condition analysis of dorsal spine sections indicated translucent zones began forming in fall, with the highest percentage of translucent margins occurring in winter (February and March) (Fig. 4). Fish sampled in summer months (June and July) had the lowest percentage of translucent dorsal spine margins, hence most margins in summer were opaque.

DISCUSSION

This study represents the first attempt to directly validate annual translucent zone formation in Gray Triggerfish dorsal spines, as well as in fin rays and vertebrae. All hardparts of experimental fish that were injected with OTC in the fall 2009 demonstrated translucent zone formed during winter, followed by opaque zone formation in spring and summer. Rearing experimental fish for a second year likely would have provided even more robust results, as would have tagging OTC-injected fish in the wild for subsequent recapture. However, results from the OTC marking experiment, as it was conducted, clearly validate one translucent zone being formed in winter.

Verification of annual translucent zone formation in wild fish via marginal condition analysis also provided meaningful results with respect to the efficacy of ageing Gray Triggerfish with dorsal spines. The percentage of spines with translucent margins was greatest in the winter months and lowest in the summer, with an annual periodicity of translucent zone formation apparent in the data. Ingram (2001) reported a similar result for fish captured off Alabama in the late 1990s, while Moore (2001) and Burton et al. (2015) inferred from marginal condition analysis that translucent zone formation was completed by June for Gray Triggerfish from the South Atlantic U.S. While direct validation of annual growth zone formation is the gold standard for ageing studies, marginal condition trends reported here provide additional evidence for annual translucent zone formation in Gray Triggerfish dorsal spines.

Despite validation of annual translucent zone formation in fin rays, there was considerably more variance in translucent zone counts from fin rays versus dorsal spines. Given the validation of translucent zone counts as being formed annually, the difference in counts between fin ray and dorsal spine sections indicates that fish aged with fin rays would be estimated to be one year older, on average, than if aged with dorsal spines. This difference is likely due to difficulty identifying the first translucent zone in fin ray sections. The first counted zone looked distinctly different from others and may have been either part of the core or a settlement mark. Examination of fin ray sections from individuals of age-0 and age-1 may aid in the identification of the first annulus.

Preparation and processing of the fin rays also was more laborious than for either spines or vertebrae due to their small size. Furthermore, sample extraction to produce a readable transverse section of a fin ray required rays to be removed at the insertion into the pterygiophore, thus increasing the potential for infection for the fish. Therefore, the invasive nature of fin ray extraction may preclude their use as a non-lethal means of age determination.

Experimental rearing also validated abdominal vertebrae as forming one translucent zone in winter followed by an opaque zone after the OTC mark. No significant difference was found between translucent zone counts of vertebrae sections and spine sections, and Künzli and Tachihara (2012) previously reported high (96.9%) agreement in translucent zone counts between dorsal spines and abdominal vertebrae of the Picasso triggerfish (*Rhinecathus aculeatus*) for age classes 0-14. Despite the validation of annual translucent zone formation in Gray Triggerfish vertebrae and the high agreement with counts in dorsal spines, vertebrae should probably only be viewed as complimentary aging structures at this stage. Comparisons of translucent zone counts between vertebral and dorsal spine sections currently are lacking for fish >6 years old, dissection of vertebrae is more labor-intensive than spines, and vertebral sections take approximately 3 times longer to prepare than those of dorsal spines. Therefore, even if translucent zone comparisons for older ages indicate similar numbers of zones in vertebrae versus spines of older fish, vertebrae are unlikely to replace dorsal spines as the hardpart of choice for production ageing.

Further support for dorsal spines as the preferred hardpart for ageing triggerfish is the lower reader error (APE) for dorsal spines compared to both fin rays and vertebrae. APE for dorsal spines (10.8%) was also consistent with the overall APE reported by Burton et al. 2015 (11%).

In conclusion, the most significant contribution of this study is the validation of annual translucent zone formation in Gray Triggerfish dorsal spines. Dorsal spines have been employed to age triggerfish in previous studies (Johnson and Saloman 1984; Hood and Johnson 1997; Ingram 2001; Moore 2001; Burton et al. 2015), but no direct validation of annual translucent zone formation was previously conducted. Results of

marginal increment or condition analysis have been cited as verification of translucent zones forming annually, but directly validating age estimates via chemical marking of hardparts is much more definitive (Beamish and McFalane 1983; Campana 2001). Such validation is imperative for examining Gray Triggerfish population ecology, as well as for age-based stock assessment. This latter requirement is even more critical given the fact that Gray Triggerfish are currently estimated to be overfished in the northern Gulf of Mexico from the results of the most recent age-based stock assessment (SEDAR 2012). Results provided here strengthen the inference that Gray Triggerfish can be aged accurately based on translucent zones in dorsal spines, thus also providing validation for age-based assessment of this fishery resource.

ACKNOWLEDGMENTS

We thank the federal and state port agents for sampling dorsal spines from fishery-dependent landings and Doug DeVries, Chris Gardner and Patrick Raley for providing fishery-independent samples. We are also grateful to Bill Walling for sampling and processing ageing structures. Debra Murie provided much appreciated guidance on the processing and ageing of fin rays and Beverly Barnett supplied database support.

LITERATURE CITED

Beamish, R. J., and D. A. Fournier. 1981. A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences 38:982-983.

Beamish, R. J., and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society 112(6):735-743.

Bernardes, R. Á. 2002. Age, growth, longevity of the Gray Triggerfish, *Balistes capriscus* (Tetraodontiformes: Balistida), from the Southeastern Brazilian coast. Scientia Marina 66(2):167-173.

Brusher, J. B., and J. Schull. 2009. Non-lethal age determination for juvenile Goliath Grouper (*Epinephelus itajara*) from southwest Florida. Endangered Species Research 7(3):1-9.

Burton, M. L., J. C. Potts, D. R. Carr, M. Cooper, and J. Lewis. 2015. Age, growth, and mortality of Gray Triggerfish (*Balistes capriscus*) from the southeastern United States. Fishery Bulletin 113:27-39.

Campana, S.E., M.C. Annand and J.I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determination. Transactions of the American fisheries society 124:131-138.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of fish Biology 59(2):197-242.

Cass, A. J., and R. J. Beamish. 1983. First evidence of validity of the fin ray method of age determination for marine fishes. North American Journal of Fisheries Management 3:182-188.

Dance, M.A., W.F. Patterson III, D.T. Addis. 2011. Fish community and trophic structure at artificial reef sites in the Notheastern Gulf of Mexico. Bulletin of Marine Science 87(3): 301-324.

Hood, P. B., and A. K. Johnson. 1997. MARFIN Final report: A study of the age structure, growth, maturity schedules and fecundity of Gray Triggerfish (*Balistes capriscus*), Red Porgy (*Pagrus pagrus*), and Vermilion Snapper (*Rhombolplites aurorubens*) from the eastern Gulf of Mexico. Florida Marine Research Institute, St. Petersburg, Florida.

Ingram, Jr., G. W. 2001. Stock structure of Gray Triggerfish, *Balistes capriscus*, on multiple spatial scales in the Gulf of Mexico. Doctoral dissertation. The University of South Alabama, Mobile, Alabama.

Johnson, A. G., and C. H. Saloman. 1984. Age, growth, and mortality of Gray Triggerfish, *Balistes capriscus*, from the northeastern Gulf of Mexico. Fishery Bulletin 82(3):485-492.

Koch, J. D., and M. C. Quist. 2007. A technique for preparing fin rays and spines for age and growth analysis. North American Journal of Fisheries Management 27:782-784.

Künzli, F. and Tachihara K. 2012. Validation of age and growth of the Picasso Triggerfish (Balistidae: *Rhinecanthus aculeatus*) from Okinawa Island, Japan, using sectioned vertebrae and dorsal spines. J. Oceanogr. 68:817-829.

Lessa, R., and P. Duarte-Neto. 2004. Age and growth of Yellowfin Tuna (*Thunnus albacares*) in the western equatorial Atlantic, using dorsal fin spines. Fisheries Research 69:157-170.

Moore, J. L. 2001. Age, growth, and reproduction of the Gray Triggerfish, *Balistes capriscus* of the southeastern United States, 1992-1997. Masters thesis. University of Charleston, Charleston, South Carolina.

Murie, D. J., D. C. Parkyn, C. C. Koenig, F. C. Coleman, J. Schull, S. Frias-Torres. 2008. Evaluation of finrays as a non-lethal ageing method for protected Goliath Grouper *Epinephelus itajara*. Endangered Species Research 7:213-220.

Natanson, L. J. 1993. Effects of temperature of band deposition in the Little Skate, *Raja erinacea*. Copeia 1:199-206.

Ofori-Danson, P. K. 1989. Growth of Grey Triggerfish, *Balistes capriscus*, based upon growth checks of the dorsal spine. FishByte:11-12.

Patterson, W.F., III, J.H. Tarnecki, D.T. Addis, L.R. Barbieri. 2014. Reef fish community structure at natural versus artificial reefs in the Northern Gulf of Mexico. Proceedings of the 66th Gulf and Caribbean Fisheries Institute. 66:4-8.

SEDAR (Southeast Data, Assessment, and Review) 2006. Stock Assessment Report of SEDAR 9 Gulf of Mexico Gray Triggerfish. Charleston, South Carolina, 195 pp.

SEDAR (Southeast Data, Assessment, and Review) 2012. Stock Draft-Assessment of Gray Triggerfish in the Gulf of Mexico: SEDAR Update Assessment. Miami, Florida.

Valle, M., C. M. Legault, and M. Ortiz. 2001. A stock assessment for Gray Triggerfish, *Balistes capriscus*, in the Gulf of Mexico. Department of Commerce, Miami, Florida.

FIGURE LIST

Figure 1. Digital images of OTC-marked Gray Triggerfish hardparts viewed with transmitted visible (left) and UV light (right). Dorsal spine (A,B) and pectoral fin ray (C, D) sections are from a 270 mm FL female, while the vertebral section (E,F) is from a 243 mm FL male. The light source was covered with a green filter for fin sections. Translucent zones are marked with circles in each section.

Figure 2. Digital images of A) dorsal spine and B) fin ray sections from a 249 mm FL Gray Triggerfish with 4 translucent zones present, and C) dorsal spine and D) vertebral sections from a 481 mm Gray Triggerfish with 7 translucent zones present.

Figure 3. Bias plots for Gulf of Mexico Gray Triggerfish for A) mean fin ray and B) mean vertebral section translucent zone counts for dorsal spine count numbers. Error bars represent the 95% confidence interval. Line represents 1:1 relationship between counts.

Figure 4. Percent translucent margin in Gulf of Mexico Gray Triggerfish dorsal spines collected from 2003-2010 (n = 2,411). Numbers indicate monthly sample size.

Figure 1.

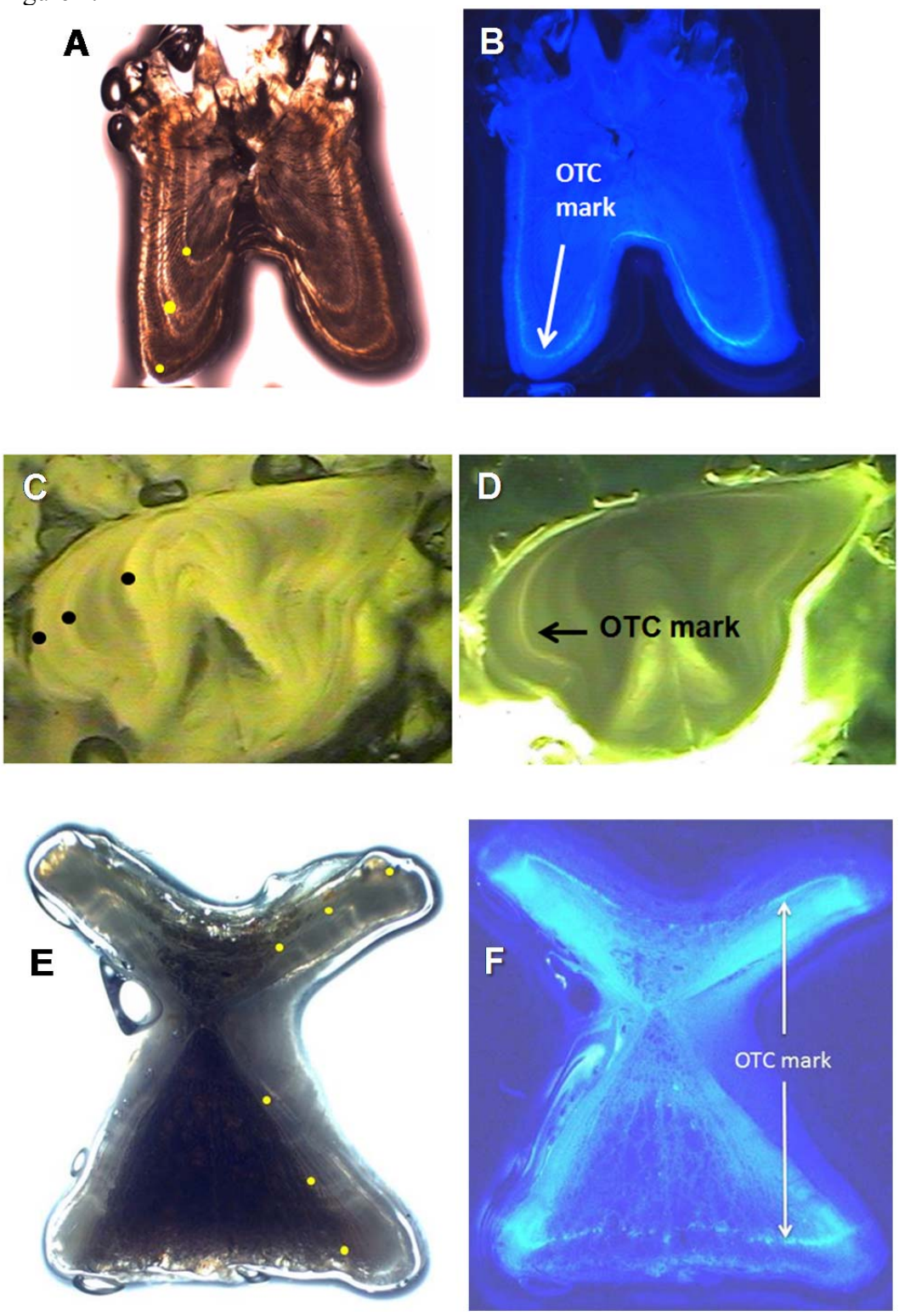


Figure 2.

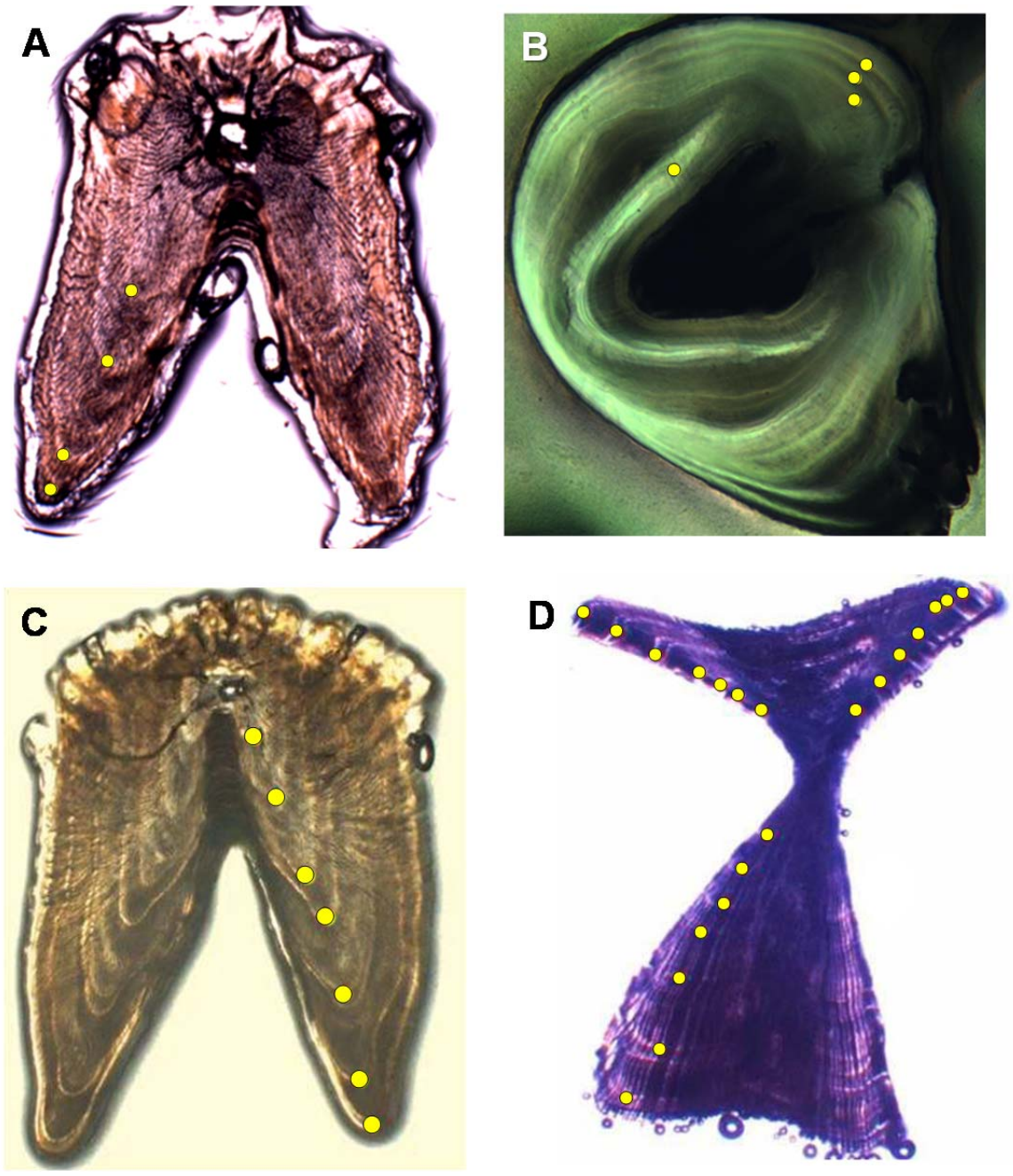
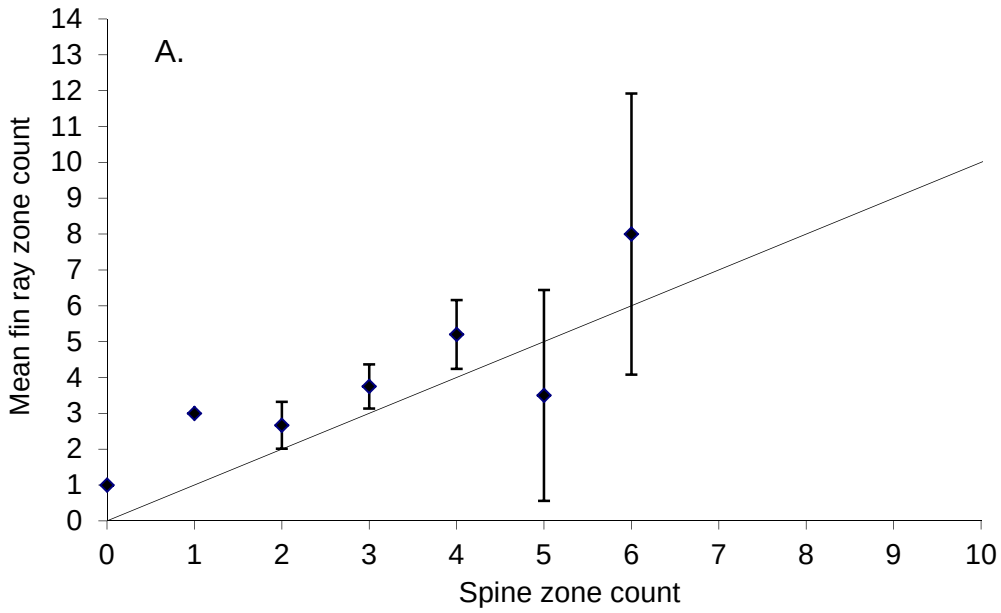


Figure 3.


A.
Mean fin ray zone count
Spine zone count
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

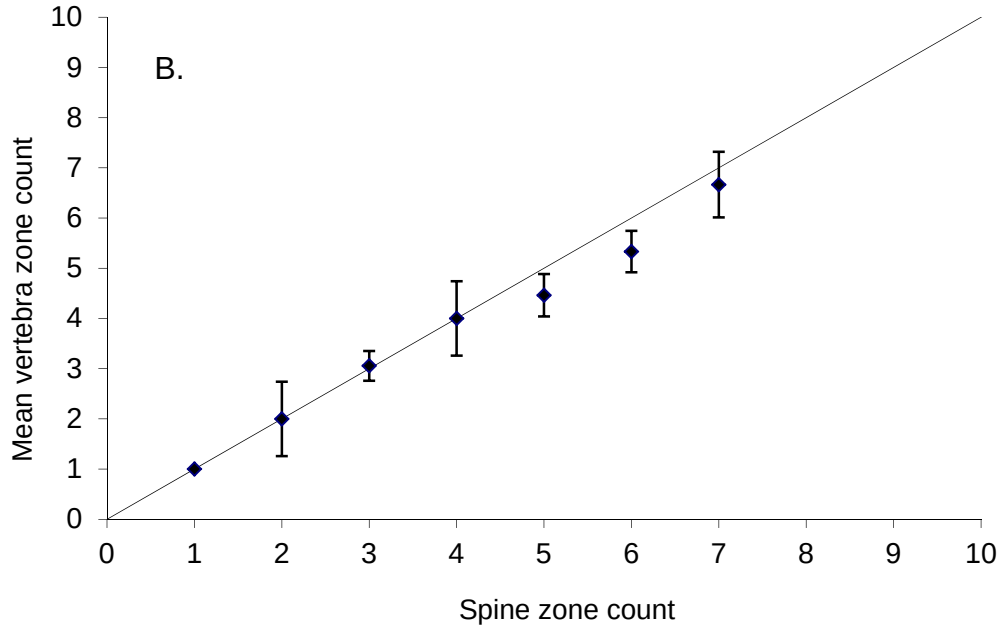
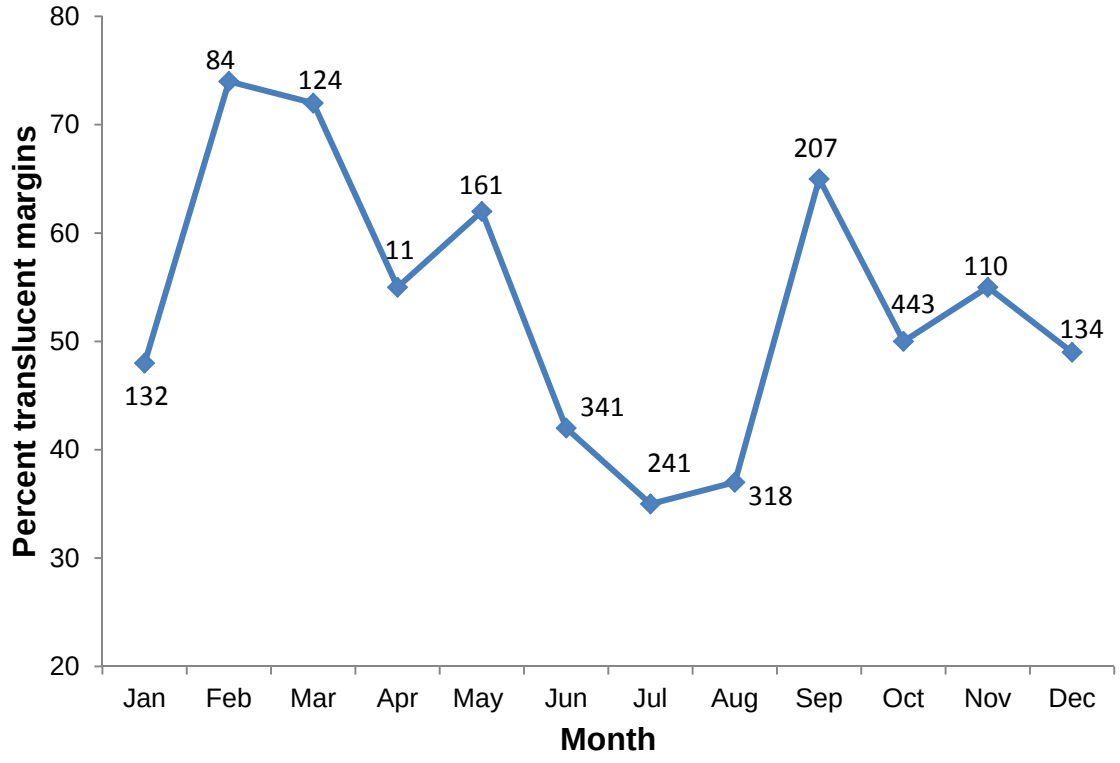

B.
Mean vertebra zone count
Spine zone count
0
1
2
3
4
5
6
7
8
9
10

Figure 4.


Percent translucent margins
80
70
60
50
40
30
20
84
124
11
161
207
110
443
134
132
341
241
318
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month