Validation of annual growth zone formation in gray triggerfish *Balistes capriscus* dorsal spines, vertebrae, and otoliths

Validación de la formación de la zona de crecimiento anual en espinas dorsales, vértebras y otolitos del pez ballesta gris *Balistes capriscus*

Validation de la formation de la zone de croissance annuelle chez les balistes gris *Balistes capriscus* épines dorsales, vertèbres et otolithes

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EXTENDED ABSTRACT

Gray triggerfish *Balistes capriscus* is the most exploited balistid species along the coasts of the Southeastern U.S. Because gray triggerfish are exploited by recreational and commercial fisheries, stock assessments are essential in managing the resource. The most reliable stock assessments utilize fish age data to estimate age structure of the population, growth rates, and mortality. Reviews of fish ageing studies suggest that sagittal otoliths are the preferred ageing structures versus other bony structures (Campana 2001). Gray triggerfish otoliths are small, fragile, and difficult to extract and age. For these reasons, the first dorsal spine is used as the primary ageing structure for gray triggerfish stock assessments. Various ageing studies have noted difficulty in identifying the growth zones on gray triggerfish dorsal spines (Bernardes, 2002; Burton et al. 2015). The current study sought to validate the annual deposition of growth zones on dorsal spines, vertebrae, and otoliths of gray triggerfish through chemical marking (Potts et al, in prep).

Gray triggerfish were collected off of North Carolina from December 2014 through June 2016 and transported to, and reared in, the NOAA Beaufort Laboratory aquaculture facilities. Adult gray triggerfish were anesthetized and chemically marked with a 50 mg/kg body weight dose of calcein. Upon completion of the study, fish were sacrificed and dorsal spines, vertebrae, and otoliths were removed and sectioned for ageing. The three structures were aged by two independent readers without reference to fish size, date of death, or date of marking. Narrow, slow-growth zones (annuli) appeared translucent in spine and vertebra sections, and opaque in otolith sections. Consensus annuli counts were determined after both readers simultaneously re-examined samples for which annuli counts differed, and bias plots were created to visualize differences in annuli counts between the three structures. Statistical tests for symmetry and heteroscedasticity were performed to compare annuli counts between structures. The margin type of each section was recorded as translucent or opaque, and the percent-age of sections with an annulus on the margin was plotted against month of sacrifice to create a timeline of annulus formation.

A total of 74 gray triggerfish were marked with calcein and held in tanks for 5 days to 29 months after marking. Annuli appeared to form on all ageing structures during the captive rearing period. Annuli counts ranged from 0-11 for spines and vertebrae, and 1-12 for otoliths. The average percent error (APE) between readers was 8.5% for spines, 13.0% for vertebrae, and 13.7 % for otoliths.

Age bias plots showed strong agreement between spine and vertebra annuli counts for all observed ages, and counts of spines and vertebrae appeared to under-age beginning at age 4 when compared to otolith annuli counts. Annuli counts agreed perfectly for 57.3% (93.3% ± 1) of spine-vertebra pairs, 52.2% (82.6% ± 1) of spine-otolith pairs, and 53.2% (85.1% ± 1) of vertebra-otolith pairs. Tests for symmetry indicated that the paired age data were not biased ($p > 0.05$). The test of heteroscedasticity was highly significant for paired spine and otolith data ($p << 0.05$), but was not significant for paired spine and vertebrae data ($p > 0.05$).

Marginal increment analysis of ageing structures indicated that annuli form in spring and summer months. The highest percent of translucent margins occurred in May for vertebrae (80%) and in June for spines (58%). In otoliths, opaque margins occurred in July (14.3%). These findings are similar to results from previous studies showing annulus formation in late spring to early summer in South Atlantic (Burton et al. 2015) and Gulf of Mexico (Johnson and Saloman, 1984) gray triggerfish. According to the duration of fish survival after calcein marking, and month of sacrifice, an expected number of post-mark annuli was calculated and compared to the observed number of post-mark annuli. The expected number of annuli distal to the chemical mark was present on >90% of spines and vertebrae, and on 100% of the otoliths.

Some spine sections exhibited unusually wide translucent zones when viewed at lower magnification (15x - 20x). Examination of these spine sections under higher magnification (up to 40x) revealed that wide translucent areas contained compacted growth zones. As a result of this phenomenon, a new age reading methodology was developed and used to re-read a subset of spine sections used for age data in the most recent U.S. Atlantic stock assessment (SEDAR 41). Age readings generated from the older methodology appeared to underage when compared to those from the new methodology.

The results of this study reinforce the utility of the first dorsal spine as the practical ageing structure for gray triggerfish, but increased care needs to be taken when ageing sections from older fish. Researchers at the NOAA Beaufort Lab are working to build a larger catalogue of paired spine and otolith samples, the analysis of which will create a more complete picture of gray triggerfish ageing.

KEYWORDS: Gray triggerfish, *Balistes capriscus*, age validation, otoliths, dorsal spines

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