Validation of Annular Increments and Formation Timing in Otoliths of Red Snapper (*Lutjanus campechanus*)

Validación de Incrementos Anulares y Tiempos de Formación en Otolitos de Pargo Rojo (*Lutjanus campechanus*)

Validation des Incréments Annulaires du Temps de Formation dans les Otolithes du Vivaneau Rouge (*Lutjanus campechanus*)

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EXTENDED ABSTRACT

Introduction

Life history parameters such as age and growth are necessary for stock assessments and management of commercially and recreationally important species such as Red Snapper *Lutjanus campechanus*. Perhaps the most widely accepted method to establish age in Red Snapper is by counting the number of opaque bands on otoliths (one increment consists of a translucent and an opaque band). This method assumes the periodicity of opaque band formation (representing a full increment) is an annual occurrence that is synchronous for all individuals in a species for a geographical area (Campana 2001). If timing of opaque band formation varies, individuals may be assigned incorrect year classes, leading to mismanagement. Therefore, accurate aging from Red Snapper otolith relies on validation of both the periodicity and the timing of opaque band formation. Mark-recapture studies of oxytetracycline (OTC) marked fish and marginal increment analysis (MIA) are common validation methods

A winter seasonal formation of the opaque band by Red Snapper in the Gulf of Mexico has been reported in previous MIA validation studies (Render 1995, Wilson and Nieland 2001, Patterson et al. 2001). The opaque bands are hypothesized to represent seasonal events such as decreased water temperature or seasonal feeding patterns (Campana 1999). However, several MIA studies have also reported a summer season opaque band formation (Moseley 1966, Futch and Bruger 1976, Nelson and Manooch 1982). Similarly, two previous OTC mark-recapture studies reported a summer to fall formation of opaque bands in Red Snapper (Szedlmayer and Beyer 2011, Szedlmayer et al. 2020). These differences in the timing of opaque band formation between MIA and OTC mark-recapture studies indicated a need for further examination of MIA. The present study was a reexamination of MIA analysis in Red Snapper. The purpose of the present study was to evaluate the MIA method and determine if this reexamination would support previous MIA studies or support previous OTC studies.

Methodologies

The present study examined transverse otolith sections from a stratified random sample of 514 from total of 1,753 Red Snapper that were previously prepared and analyzed in Szedlmayer and Grundy (2020). All Red Snapper were collected in the northern Gulf of Mexico during all months of the year except February and ranged from age-2 to age-16.

Otolith sections were examined under a Leica MZ6 microscope at 0.63x to 4x magnification with reflected and transmitted light. The marginal increment along the dorsal side of the sulcus was recorded as either opaque or translucent based only on direct observations under the microscope (i.e., the reader was afforded a 3-D view of the otolith edge). Examples of otoliths with marginal increments identified as opaque or translucent are shown in Figure 1. The proportions of otoliths with opaque or translucent margins by month of capture were compared to determine the timing of opaque band formation.

Results and Discussion

The present MIA of Red Snapper otoliths indicated that opaque bands formed during most months of the year, except for November and March when only translucent bands were present (Figure 2). However most (>15 % per month) opaque bands were formed from April to October with higher percentages (>50 %) from June to August. The highest percent (66 %) of opaque bands occurred in June (Figure 2). There were lower (< 5 %) percentages of opaque bands from November to March (Figure 2).

The present MIA analysis supports the previous OTC studies except that MIA analysis indicated an earlier start of opaque bands in April and an earlier ending in October, compared to August to December formation (Szedlmayer and Beyer 2011) and a June to November formation (Szedlmayer et al. 2020). In contrast, previous MIA studies concluded winter to spring opaque band formation (Wilson and Nieland 2001, Patterson et al. 2001, Allman et al. 2005), and these formation periods have been widely accepted as the standard protocol for Red Snapper aging (VanderKooy and Guindon-Tisdel 2003, VanderKooy 2009).

Differences between the present MIA analysis and previous studies may be attributed to differences to otolith counting

methods. Difficulties in correct opaque band identification on the otolith edge from light diffraction are a known source of error (Campana 2001). The present study corrected for this difficulty by only examining otoliths directly under a microscope. In contrast, identification of marginal increments through the use of projected images usually only provide 2D views and some loss of resolution might be expected that may cause difficulties in edge separations. The present direct views allowed readers better separation of marginal increment translucent and opaque bands, compared to 2-D projected images. Further, edge condition was better defined as focus, magnification, and light were altered and evaluated for each otolith under 3-D views.

Slowed growth during summer into fall can be attributed to spawning stress occurring from May to October (Collins et al. 2001). A similar conclusion was suggested by earlier MIA studies (Moseley 1966; Nelson and Manooch 1982) and in a more recent study on a congeneric species humpback red snapper, *Lutjanus gibbus*, in New Caledonia (Morre 2019). Thus, it is likely that slower growth as a result of spawning is the mechanism of opaque band formation occurring later in the year for Red Snapper rather than reduced winter temperatures.

KEYWORDS: MIA, OTC, aging, validation, edge band

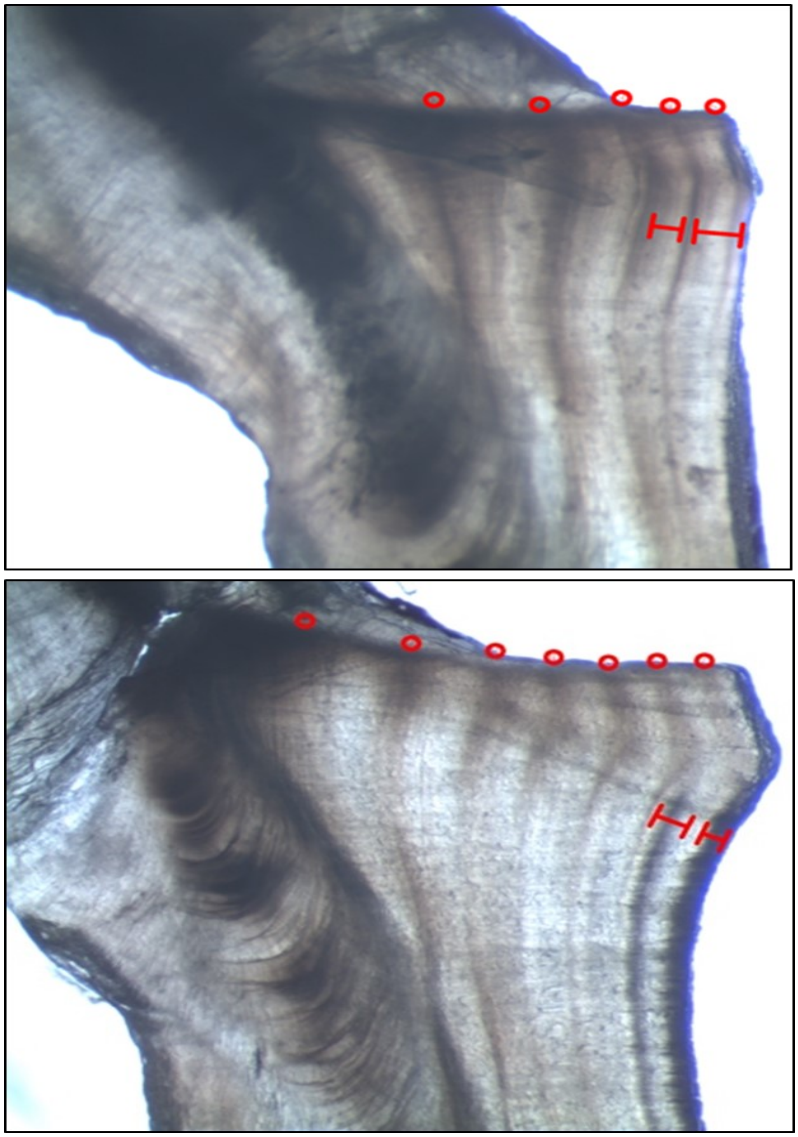

Figure 1. Two transverse otolith sections of Red Snapper, *Lutjanus campechanus*, examined at 3.2x magnification. A) Fish was captured in August and the edge was classified as opaque (upper right red circle). B) Fish was captured in November and the edge was identified as translucent (upper right red circle). The right edge in B marked with the red transect indicates the vertical edge that may be mistaken as an opaque band.

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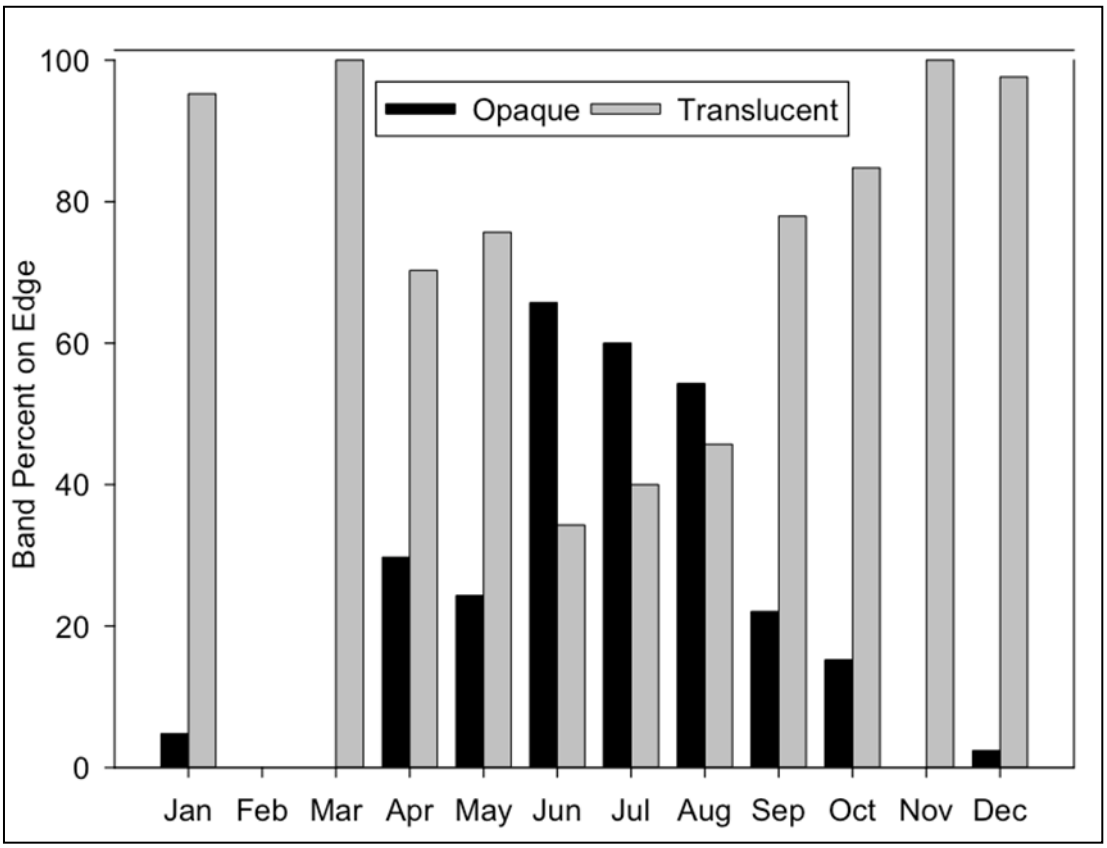

Figure 2. Percent frequency of opaque and translucent bands on the edge of otoliths by month for Red Snapper *Lutjanus campechanus* (n = 514).