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# Using otolith morphometrics to quickly and inexpensively predict age in the gray angelfish (*Pomacanthus arcuatus*)

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#### ABSTRACT

We examined sagittal otoliths from 398 gray angelfish (*Pomacanthus arcuatus*) collected from the lower Florida Keys between September 2000 and September 2003. Fish ranged in size from 78 to 442 mm total length (TL). Males had a mean TL of 329 mm (n = 192), females had a mean TL of 308 mm (n = 166), and an additional 56 fish were unsexed (mean TL = 239 mm). Sectioned otoliths displayed clear increments that were used to estimate fish ages. Marginal increment analysis validated the formation of a single annulus per year.

The relationship between TL and age was described by the von Bertalanffy growth equation  $L_t = 325.1[1 - \exp(-0.0601(t + 0.828))]$  for females and  $L_t = 388.5[1 - \exp(-0.383(t + 0.923))]$  for males. Females and males grew rapidly for the first 5 years of life and eventually reached asymptotic lengths of  $\sim$ 325 and  $\sim$ 388 mm, respectively, with a maximum estimated age of 24 years. Morphometric parameters evaluated for use in the age model for gray angelfish included fish length and weight and otolith length, width, thickness, and weight. As with fish length, otolith length and width became asymptotic between the ages of 4 and 7. Otolith weight increased throughout the life of the fish, but the rate of increase slowed with age. Only otolith thickness was linear with fish age. Stepwise forward regression resulted in the following equation:  $\ln(age + 1) = 1.157 + 2.542 \times \ln(otolith thickness)$  indicating that otolith thickness, which explained 89% of the variation, was the best predictor of age. Additional variables did not improve the regression, nor did dividing the data into subsets based on growth rate. Once the otolith thickness-age relationship was established, the simple process of measuring otolith thickness was as effective for determining the age of gray angelfish as the far more difficult process of sectioning and reading the otoliths. Use of similar models in ageing other species, along with periodic validation to ensure that the otolith parameter-age relationship has not changed over time, could simplify age data collection for population models. This, in turn, could potentially allow fisheries to be better managed at a significantly reduced cost.

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#### 1. Introduction

The age of fish in a population is fundamental to most of the questions fishery biologists attempt to answer about that population. Fish age is essential for constructing an accurate picture of the population dynamics (Shafer, 1989) and for creating modern age structured population models (Fossum et al., 2000). The ability to determine and monitor the age structure of a population is especially important in active, managed fisheries (Boehlert, 1985;

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Campana and Thorrold, 2001). The gray angelfish (*P. arcuatus*) supports such a fishery in Florida, where young fish are harvested for the marine aquarium trade. Current regulations prohibit collection of adult fish without special permits (Fla. Stat. § 370.02 (2006)). Little is known about the life history of the gray angelfish. The present lack of age and growth data for the species hinders researchers from refining strategies for managing this fishery, which focuses on juveniles rather than reproductively mature adults.

The most accurate technique for estimating fish age involves analyzing otolith increments. Gray angelfish otoliths must be sectioned before the increments become visible. The time and cost of such otolith preparation can limit sample sizes and prohibit some researchers from using otoliths to determine ages of fish (Pilling et al., 2003; Worthington et al., 1995). Even after preparation, the age-determination process is very subjective, and ultimately, the designation of annuli is based on the past experience of the reader

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(Ross et al., 2005). Various attempts have been made to simplify the procedure of estimating fish age without reducing accuracy (Butler and Folkvord, 2000). Brander (1974) suggested that perhaps age could be estimated from otolith size. Measuring otolith dimensions requires less skill, time, and equipment than counting otolith increments does. If otolith morphometrics could be used to estimate fish age, the cost of determining age-based population parameters could be significantly reduced. This would allow fisheries in which monitoring is currently underfunded, including small fisheries like the gray angelfish in Florida and fisheries in developing countries, to be better managed (Pilling et al., 2003).

Several studies have demonstrated a significant relationship between otolith size and fish age, suggesting that otolith size can be used as a proxy for otolith ring counts in ageing fish (Boehlert, 1985; Butler and Folkvord, 2000; Choat and Axe, 1996; Choat et al., 1996; Mug-Villanueva et al., 1994; Newman et al., 2000; Reznick et al., 1989; Worthington et al., 1995). The relationship remains significant whether the fish are fast- or slow-growing (Secor and Dean, 1992). Models testing this hypothesis have shown promise (Boehlert, 1985; Francis et al., 2005; Labropoulou and Papaconstantinou, 2000; Lou et al., 2005; Mug-Villanueva et al., 1994; Pilling et al., 2003; Schirripa and Trexler, 2000). Although ontogenetic changes in otolith morphology have been noted (Boehlert, 1985; Fowler, 1990; Maceina et al., 1987; Newman et al., 1996), indicating that some otolith dimensions may be more useful than others for estimating fish age, many studies to date have chosen otolith parameters without consideration of potential species-specific otolith growth patterns. The objective of this study was to investigate the value of using otolith morphometrics and fish-size parameters to predict age in the gray angelfish (P. arcuatus). Four otolith parameters were chosen based on familiarity with the otoliths of this species, and two fish-size parameters were included. Stepwise forward regression was used to determine which parameters would best predict age in this species.

#### 2. Materials and methods

#### 2.1. Sample collection

Gray angelfish were collected from the westernmost 64 km of the Florida Keys, from the Bahia Honda bridge to Key West. A commercial collector using a spear gun and hand nets made monthly collections from September 2000 to September 2003. An average of 10 fish were collected per month, and although there were several collection sites, each month's collections were generally made in a single location. Date of capture, total length (TL,) and weight of each fish were recorded. Fish were sexed by histological examination of the gonads.

#### 2.2. Age and growth

Sagittal otoliths were extracted, cleaned by removing all attached tissue and rinsing with distilled water, and stored dry. A low-speed Isomet saw was used to cut the left otolith into 300- $\mu$ m thick transverse sections through the core on the dorsoventral plane, perpendicular to the sulcus (Figs. 1 and 2). The right otolith was stored dry and used for whole-otolith measurements. If the left otolith was broken, the right otolith was sectioned. Sections were mounted on glass slides using Flo-Texx<sup>®</sup> mounting medium.

Mounted otolith sections were examined under a microscope at  $40 \times$  magnification using either transmitted or reflected light. Increments were counted from the core to the dorsal edge of the sulcus (Fig. 1). When the marginal edge was opaque, that marginal increment was considered complete and counted as an annulus. For each otolith, two readers independently counted the annuli twice, with



**Fig. 1.** Micrograph of section of left sagittal otolith of 17-year-old *gray angelfish*. Diamonds denote annuli counted along dorsal edge of sulcus. Location of first annulus based on young-of-the-year otoliths.

an interval of at least 1 month between counts. The two readings of an otolith by an individual reader were averaged to obtain the age estimated by that reader (Megalofonou, 2006). The mean of all four readings was deemed to be the standard age of the fish and was used in the development and testing of the morphometric age model.

We used marginal increment analysis to evaluate annulus periodicity. We used the width of the marginal increment  $(W_n)$  and the width of the increment just previous to it  $(W_{n-1})$  to obtain an index of completion,  $(W_n/W_{n(1)}) \times 100$ , for each otolith (Tanaka et al., 1981). A mean index of completion was then calculated for each month of the year and examined for trends.

Fish age and TL were used to develop separate von Bertalanffy growth equations for male and female gray angelfish:

$$L_t = L_{\infty}[1 - \exp(-k(t - t_0))],$$

where  $L_t$  is the mean length of fish of age t,  $L_{\infty}$  is the estimated asymptotic mean length, k is a constant that determines the rate at which  $L_t$  approaches  $L_{\infty}$ , and  $t_0$  is the hypothetical age at which the mean length is zero if the fish had always grown in a manner described by the von Bertalanffy growth equation.

#### 2.3. Model development and testing

#### 2.3.1. Model parameters

After ageing the sectioned otoliths, the remaining uncut right otoliths were measured to the nearest 0.1 mm on a dissecting



**Fig. 2.** Schematic diagram of the left sagittal otolith of the gray angelfish, *Pomacanthus arcuatus*. (A) Whole otolith. (B) Transverse section through otolith. Dashed lines represent transverse section through the nucleus (gray zone). L=length, W = width, T = thickness.

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