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Multi-decadal variation in cohort specific sex ratios and otolith increment growth characteristics of juvenile blue grenadier (*Macruronus novaezelandiae*)

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ABSTRACT

This study presents inter-annual variability of otolith growth from 1993 to 2011 and cohort specific sex ratios from 1976 to 2011 in blue grenadier *Macruronus novaezelandiae* on the west coast of Tasmania. Length-at-age data highlighted that *M. novaezelandiae* females grow larger than males with mean length-at-age statistically diverging by three years of age and remaining separated thereafter. Statistical differences in both cumulative, and age specific otolith increment widths-at-age between sexes were detected at earlier ages, but these effects were variable and not consistent with the sexual dimorphism observed for mean length at age. No differences between sexes were evident for the ages 8–14 or 7–11 for cumulative increment widths or age specific otolith increment widths-at-age respectively. Inter-annual variation in mean otolith increment widths for the first five increment zones (juvenile zones) showed a trend of declining increment widths from 2006 to 2010. Inter-annual cohort sex ratio variation across year-of-birth was also significant displaying a similar trend to the first-year otolith increment growth profiles. Regression analysis of birth year sex ratio with mean otolith increment widths indicated a significant negative relationship for first year growth with increasing numbers of male offspring. The inter-annual variation in cohort specific sex ratio suggests that environmental factors may be involved with sex determination. When coupled with climate change these influences could have long term consequences for the fitness of the stock and corresponding flow-on effects into the stock assessment process and future management of the stock.

1. Introduction

Blue grenadier *Macruronus novaezelandiae* (Hector 1871) is an important commercial fish species for both the Australian and New Zealand fishing industries (Gunn et al., 1989). In Australian waters, they are found on the upper continental slopes from New South Wales to Tasmania and across the Great Australian Bight (Kenchington and Augustine, 1987; Kailola et al., 1993). The species is targeted by both trawl and long-line fishers in the Southern and Eastern Scalefish and Shark Fishery, with the Commonwealth Trawl Sector accounting for most of the catch (Department of Sustainability, 2013; Georgeson et al., 2014). The Australian 2012/13 annual production was valued at $15.5 million with a total landing of 3954 t (Georgeson et al., 2014). The fishery consists of two sub-fisheries, a non-spawning fishery operating throughout the year and a winter spawning fishery. The spawning fishery provides much of the Australian product (Kloser et al., 2007; Tuck, 2013) by targeting mature adults in the winter spawning aggregation from July through to September off the western Tasmanian coast. Targeted winter trawling at depths of 300 m–600 m occurs over a slender 100 NM area between 41° 20′ S and 42° 40′ S. Trawls typically occur on the bathymetric features of the Pieman and Strahan Canyons and the Sandy Cape region with additional catches from the mid-slope regions within the area (Kloser et al., 2007; Ryan and Kloser, 2012; Tuck, 2013). Studies into the early larval history of *M. novaezelandiae* from the Tasmanian spawning ground suggest that larvae are passively advected by longshore currents to southern and eastern Tasmanian coastal waters, but also that the advection route is inter-annually variable (Thresher et al., 1988). Larvae disappear from the water column at about 55 days on the East coast of Tasmania (Lyne and Thresher, 1994). There are contradictory reports on where juveniles inhabit, some juveniles have been reported from near shore coastal environments and large estuaries (Wilson, 1981; Yearsley et al., 1999). Other studies suggest they inhabit the mid shelf depth ranges of 200–400 m (Kuo and Tanaka, 1984). How or when juveniles move

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between coastal or mid shelf habitats back to spawning areas in the species is poorly understood (Thresher et al., 1988). The lack of significant numbers of juveniles being captured by recreational fishers in near shore environments (Lyle et al., 2014), compared to the large mode of juveniles in trawl captures reported by Kuo and Tanaka (1984) suggests they may prefer to inhabit offshore environments.

Fish growth is a basic parameter underlying population characteristics such as stock biomass, population density and size frequencies (Jobling, 2002). Many investigators have found that growth rates are dynamic and vary across years, sometimes exhibiting distinct temporal trends (Thresher et al., 2007; Neuheimer et al., 2011). Variable growth rates in fish are influenced by intrinsic and extrinsic factors. Intrinsic influences on fish growth are species or individually explicit, such as age, sex and/or genetics (Weisberg et al., 2010; Morrongiello et al., 2012). For example, *M. novaezelandiae* are a sexually dimorphic species with females reaching larger sizes than males (Kailola et al., 1993; Smith, 1994; Froese and Pauly, 2014). Extrinsic influences differ because they exert external pressure on a fish's ability to grow and include aspects such as temperature, competition, food availability, habitat, and anthropogenic pressures for example, fishing effort (Thresher et al., 2007; Neuheimer et al., 2011; Morrongiello et al., 2012). Temperate fish species are very reactive to seasonal environmental factors (Gillanders et al., 2012) with growth variations being reflected in the width of individual growth zones within the otolith. The annual zones in *M. novaezelandiae* otoliths have been validated to form annually for ages two through 21 by modal progressions of age-classes (Robertson, unpublished). This technique has been previously used to validate ageing of this species in New Zealand waters (Horn and Sullivan, 1996). Wide translucent increments represent fast growth and narrow opaque increments represent slow growth (Francis and Campana, 2004; Bone and Moore, 2008; Green et al., 2009; Stocks et al., 2011). A combination of one translucent zone and one opaque zone represent one year of otosomatic growth. Increment widths near the otolith core are wider as juvenile otosomatic growth is relatively faster, but as the individual ages and matures, increment widths become progressively narrower. Variations in otolith increment widths have been previously used to provide insights into the factors influencing growth (Gillanders et al., 2012; Morrongiello et al., 2012). By examining variations in juvenile otolith increment widths, many of the confounding influences reproduction exerts on growth, such as reduced growth due to the attainment of maturity or growth effects influenced by inter-annual variations in reproductive activity and investment (Massou et al., 2004) can be eliminated. Few studies have been conducted on understanding inter-annual variations in otolith growth patterns in *M. novaezelandiae*. Therefore, the aim of this study was to investigate inter-annual variation in otolith growth patterns of *M. novaezelandiae* with a focus on juvenile otolith growth patterns from increment measurements. We also develop a 35 year time series of cohort specific sex ratios to test the hypothesis that they vary over time and present the correlation between juvenile otolith growth and cohort specific sex ratios.

2. Materials and methods

2.1. Data collection and growth modelling

Biological data and samples were collected by observers on-board commercial vessels while at sea, or at the ports of landing. Catches were randomly sub-sampled, with observers recording standard length, sex and collecting otoliths for each individual as part of the routine stock assessment and monitoring activities. Otoliths were aged from transverse sections using transmitted light at a magnification of 12.5× using a Leica MZ80 dissecting microscope using the established ageing protocols (see Kalish et al., 1996; Morison et al., 1998). The first 15 annual increments were marked along a ventral region adjacent to the sulcus acusticus and saved on each otolith image using custom software. Annual increments were composed of one translucent 'summer' and one opaque "winter' zone with marks placed on the outside edge of the opaque zone. To test the utility of the increments marked on the otolith image, pre-marked zone locations were regressed against a linear transect using the image analysis software package ImageJ (Rasband, 2013) and the ObjectJ plugin, (Vischer and Nastase, 2014). Otolith increment measurements precision and linearity was validated ($F = 1037$, df 1744, $P < 0.001$) with the re-analyzed object J otolith increment data after log–log transformation of the data. Otolith increment data were recorded from the otoliths of *M. novaezelandiae* captured over a five-year period between 2009 and 2013 with a total of 7334 sectioned otolith samples being processed.

Standard length-at-age for 34860 individual fish caught between the years 1984–2013 were used for regional and sex based von Bertalanffy growth model development, Eq. (1). Where L_t is the length of a fish at age/time t; L_∞ the asymptotic length; t_0 is the theoretical age/time when length is equal to zero. The growth coefficient K is a measure of the rate at which the theoretical maximum size is attained.

$$L_t = L_\infty\left[1 - e^{-K(t-t_0)}\right]$$

Growth curves were generated for fish caught in four Tasmanian fishing zones, eastern Bass Strait (EBS), eastern Tasmania (ETAS), western Bass Strait (WBS) and western Tasmania (WTAS). Due to limited age ranges in some regions and to allow direct comparisons of models, data for modelling were restricted to fish 15 years of age and less.

The von Bertalanffy growth models were tested for significant differences in sex and capture zone using Analysis of Residual Sums of Squares (AoRSS) (Haddon, 2011). Following these analyses, all further tests were restricted to data from the WTAS fishing zone (standard lengths: n = 21759; otolith sections: n = 4049). Pair wise comparisons between sexes using T-tests were conducted on mean standard length, mean cumulative otolith increment widths- and age specific otolith increment widths. Effect size and direction was tested using Cohen D values, where values ≤ 0.2 are small/trivial, values between 0.2–0.8 are medium effect sizes and values above 0.8 are large effect sizes (Cohen, 1988). To further clarify between biological relevance and statistical effects due to large sample sizes, length-at-age, cumulative otolith increment widths and age specific otolith increment widths data were bootstrapped with replacement using a visual basic program in Excel (Haddon, 2011). The program generates a random sex specific subsample of the data with sample sizes of n = 1000, 100, 50 and 30 and conducts a T-test for each pair wise sex comparison at age. The output of each bootstrapped T-test was saved for each sample size grouping at age and repeated for 1000 cycles. Significant bootstrapped T-tests findings for each age bracket up to the age of 15 years were reported as a percentage of the total number of runs conducted. Due to the outcomes of the Cohen's D values and bootstrapping tests all remaining analyses on otolith increment measurements were conducted on pooled data.

2.2. Juvenile otolith growth and sex ratio

Inter-annual variation in juvenile cohort specific otolith growth patterns were investigated for the first five growth zones. Year-of-birth (YOB) was assigned by subtraction of the assigned age from the capture date for each individual. Specific sample descriptions for each YOB are detailed in Table 1. Inter-annual variability in mean otolith increment widths for each zone against YOB were analysed using one-way analysis of variance. Inter-annual variation in the sex ratio across capture years and YOB were also examined. Chi-squared tests were performed on the ratio of males to females based on capture year and cohort YOB. The influence of male offspring ratio per cohort on inter-annual otolith growth patterns was investigated using linear regression analysis for the first five growth zones. These statistical analyses were done using the statistical software 'R'(R Core Team, 2016).

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