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Age and growth of the gulf toadfish *Opsanus beta* based on otolith increment analysis

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In the present study, sagittal otoliths of confirmed male and female specimens of the gulf toadfish *Opsanus beta* that were collected monthly over the course of a year from Biscayne Bay, Florida, U.S.A. were analysed. The timing and frequency of *O. beta* spawning seasons are reported by examination of the gonado-somatic index. The estimated ages of males and females ranged from <1 year to 6 and 5 years, respectively. Strong sexual dimorphism in growth was apparent with von Bertalanffy parameter estimates for males of $L_{\infty} = 393.8$ mm, $K = 0.30$, $t_0 = 0.36$ and females of $L_{\infty} = 201.1$ mm, $K = 0.79$, $t_0 = 0.47$. Comparison with previously published growth trajectories of the more northerly distributed conspecific *Opsanus tau* showed that *O. beta* males had a higher growth rate. Female *O. beta* and *O. tau* growth trajectories appear similar, with an indication that the former becomes asymptotic at least a year before the latter. Results are discussed in the context of temperature regimes, reproductive energy allocation and waste urea excretion in the two species.

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Key words: age determination; ureotely; von Bertalanffy growth.

INTRODUCTION

Two congeners of the family Batrachoididae inhabit the shallow coastal waters of the eastern seaboard of the continental U.S.A. (Walters & Robbins, 1961; Avise *et al.*, 1987). The oyster toadfish *Opsanus tau* (L.) resides in estuarine habitats from Cape Cod, Massachusetts south to northern Florida (Robins *et al.*, 1986) and has been the focus of detailed age and growth studies (Schwartz & Dutcher, 1963; Wilson *et al.*, 1982; Fine *et al.*, 1984; Radtke *et al.*, 1985). Less is known about the age and growth of the gulf toadfish *Opsanus beta* (Goode & Bean), which occurs in warmer embayments along the Atlantic Coast from Cape Canaveral, Florida (*c.* 28.5° N) south to the Florida Keys (*c.* 25.0° N) and along the coastline of the Gulf of Mexico

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to Campeche, Mexico (Robins *et al.*, 1986). Both species of *Opsanus* have similar life histories in that they are cryptic, sedentary and seek refuge by burrowing or hiding inside shells and crevices (Ryder, 1886; Gill, 1907; Robins *et al.*, 1986). During spawning season, *Opsanus* males occupy nesting sites and produce courtship calls to attract potential mates (Tavolga, 1958; Breder, 1968; Fine & Thorson, 2008), and, if successful, the males then defend embryos and benthic larvae for several weeks (Barimo & Fine, 1998; Barimo *et al.*, 2007).

Unlike *O. tau*, the age and growth characteristics of *O. beta* have not been the subject of direct study. Based on length-frequency distributions and assuming a single spawning season (Breder, 1941), Serafy *et al.* (1997a) inferred growth trajectories for *O. beta* (gender not distinguished) and concluded that its growth was slower than that of *O. tau*. These authors speculated that this difference was partially due to the higher energy demands associated with the more ureotelic *O. beta* (Hopkins *et al.*, 1997; Wang & Walsh, 2000). Serafy *et al.* (1997a), however, stressed the tentativeness of their growth curves and emphasized the need for more direct age and growth studies on *O. beta*, especially investigation of hardparts, such as otolith annuli.

Therefore, the main objectives of the present study on *O. beta* were to: (1) determine the timing and frequency of spawning seasons by examination of monthly variation in the female gonado-somatic index (I_G), (2) characterize the age-length relationships by direct analysis of sagittal otoliths and (3) test for sex differences in growth trajectories. Finally, the role of ureogenesis underlying the differential growth rates of *O. beta* *v.* *O. tau* was reconsidered.

MATERIALS AND METHODS

Opsanus beta were collected monthly (April 2001 to May 2002) from Biscayne Bay, Florida (25° 37' N; 80° 15' W) from the by-catch of the local commercial live bait shrimp fishery, which uses rollerframe trawling gear. The trawls measured 3 m wide and 1 m high; the attached netting was 7 m long with a mesh-size of 10 mm. Trawls had fibreglass bars, known as finger bars, spaced 40 mm apart and extending vertically across the mouth, except for a 100 mm gap above the bottom edge of the frame. All live bait trawlers employ finger bars to: (1) make trawling possible in areas where unattached benthic macroalgae is dense and (2) prevent entry of large, heavy objects (*e.g.* wood, rubble and turtles) from entering the net and damaging both gear and catch. In the present study, trawling was conducted exclusively at night. *Opsanus beta* ($n = 355$) were held for not more than 1 week in 80 l tanks with flow-through sea water (salinity 30–35) at a daily temperature averaging 2° C cooler than the source in Biscayne Bay (19–30° C). Photoperiod was kept consistent with natural day length (25° 44' N).

To examine early growth, *O. beta* embryos with known spawning dates from nearby Florida Bay were collected from field nesting sites and maintained in the laboratory with flowing sea water and gentle aeration (Barimo & Walsh, 2005). A sub-set was maintained to full yolk-sac absorption ($n = 14$) and killed for subsequent total length (L_T) values. The remainder of these individuals was maintained in a holding tank (300 l) with flow-through sea water, naturally recruiting algae and small invertebrates, and no supplemental food. At 9 months of age, two specimens were captured from the tank and measured for L_T and otoliths aged. After 5 months, an additional specimen was recovered from the tank, measured and its otoliths removed and processed to validate annuli. An exponential regression model was fitted to the L_T -at-age data, thereby generating an estimate of the early growth trajectory of these captive fish.

The bulk of otoliths were extracted from field-collected individuals ranging from 65 to 329 mm L_T . These fish were killed with an overdose of 0.5 g l⁻¹ of MS-222 (tricaine methane

sulphonate; Sigma-Aldrich; www.sigmaaldrich.com). L_T was measured to the nearest mm and wet mass for whole fish (M_T) as well as ovaries (M_G) were determined (± 0.1 g). Fish sex was determined by a combination of microscopic ($\times 1000$) and macroscopic examinations. Ovaries were readily identified by large (4 mm diameter) eggs (Breder, 1941) during the spawning season or by a grainy opaque appearance at other times. Testes had a milky white translucent string or ribbon-like appearance. If there was uncertainty as to sex, the fish was excluded from subsequent analyses.

Sagittal otoliths were processed following standard protocols (Secor *et al.*, 1991). Each otolith was weighed to the nearest 0.0001 g (Mettler AE163 analytical balance; <http://www.mt.com/lab-weighing>), embedded in epoxy and sectioned with a Buehler Isomet low speed circular saw (<http://www.buehler.com/productinfo/saws.htm>). Two diamond blades spaced 1–2 mm apart were used to cut a transverse section on either side of the core. Previous studies (Faunce *et al.*, 2002) suggest that multiple sections are useful in determining annuli by showing different planes near and around the core. Thus, a second section was cut for several otoliths, and the section most visually suitable for reading was used for ageing. The core section was then glued to a microscope slide with thermoplastic adhesive (Crystal Bond™ 509; www.crystalbond.com) and polished using 800 and 1200 grit sandpaper until annuli were clearly visible. Otoliths ($n = 44$) were read four times using a compound microscope with transmitted light at $\times 40$ and $\times 100$ with a polarizing filter at intervals > 1 day to avoid reader recall or bias. If three of the four reads did not agree, the otolith was removed from the sample. The precision of ageing was calculated using the coefficient of variation (c.v.) (Chang, 1982).

Spawning events were observed in Florida Bay during the months of February and March (Barimo *et al.*, 2007); thus, each fish was assigned an estimated hatching date of 1 March. To gauge how often females spawn per annum, a gonado-somatic index (I_G) for each female was calculated from $I_G = 100 M_G M_T^{-1}$ and mean I_G values plotted with month following Hopkins *et al.* (1997). Relationships between L_T (mm) and M_T (g) were generated following the power function $M_T = aL_T^b$, where a and b are regression coefficients, and sex-specific differences tested using ANCOVA. Non-linear least-squares procedures (SAS; www.sas.com) on the age at L_T data were used to produce the parameters for the von Bertalanffy growth equation (Ricker, 1975): $L_{Tt} = L_{T\infty}(1 - e^{-K(t-t_0)})$, where $L_{Tt} = L_T$ (mm) at time t , $L_{T\infty}$ = asymptotic L_T , K = Brody growth coefficient, t = age (years) and t_0 = age at $L_T = 0$. To determine whether L_T -at-age curves differed significantly by sex, the likelihood ratio method of Kimura (1980) was used. To reveal general patterns of growth between *O. beta* and *O. tau*, estimated growth trajectories from this study were plotted with those reported by Radtke *et al.* (1985) and Schwartz & Dutcher (1963). One-way ANOVA was used to examine differences in I_G among monthly samples. All data were tested for equal variance using the Levene median test and statistical procedures followed recommendations of Zar (1996). Results are presented as means \pm S.E. Throughout, statistical significance was accepted at $P = 0.05$.

RESULTS

ANNUAL SPAWNING CYCLE AND EARLY GROWTH

Female *O. beta* had a single peak in I_G plots during March (Fig. 1), and significant differences were found in females when plotting I_G and month of the year (ANOVA, $n = 128$, $P < 0.001$). The March I_G peak marked the height of spawning season with ovaries accounting for 5.6% of M_T among all females ($n = 12$) and 9.34% among gravid females ($n = 7$). March was reaffirmed as the birth month of *O. beta* for the purposes of ageing otoliths in this study.

Young *O. beta* of known age that were maintained in captivity were aged along with the other specimens to avoid bias. Two of them of the same L_T (25.5 mm) lacked a yearly annulus and were determined to be 9 months old based on the assigned

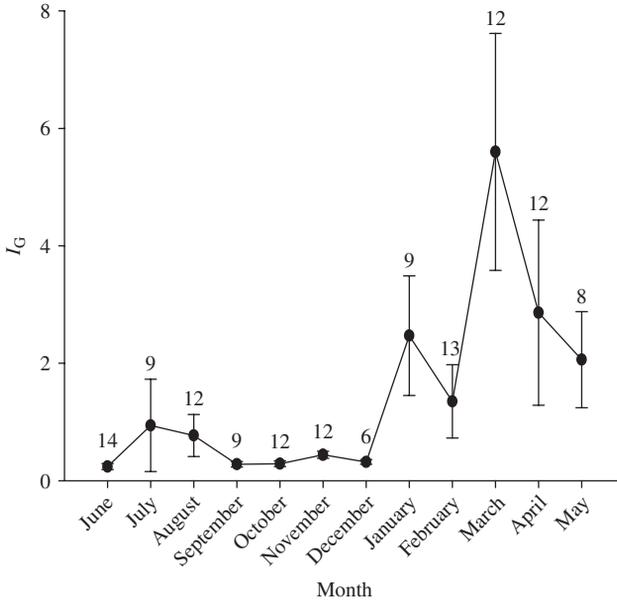


FIG. 1. Mean \pm s.e. gonado-somatic index (I_G) value at each sampling month for *Opsanus beta* females (●) for the months of June 2001 to May 2002 from Biscayne Bay, Florida. The number examined at each interval is shown.

1 March hatching date, the third fish showed an annulus [Fig. 2(a), (b)] and was aged 14 months. Regression of L_T and age for the above very young captive *O. beta* yielded the exponential relationship $L_T = 8.9^{1.7275X}$ ($r^2 = 0.50$; $P < 0.001$), where x = age (years).

GROWTH CHARACTERISTICS

A plot of all *O. beta* L_T and M_T data collected ($n = 373$) is shown in Fig. 3. While male specimens attained larger sizes than female, the difference between sexes in their L_T and M_T relationships was not statistically significant (ANCOVA, $n = 373$, $P > 0.05$).

Increments deposited in the otolith matrix varied in width and contrast making it difficult to distinguish each annulus in all samples processed; thus, 17 otoliths were discarded because fewer than three of the four reads agreed. The criteria for determining annuli were selected by observing continuous alternating opaque and translucent zones that made their way around the otolith. Annuli were counted as the opaque rings when viewed under transmitted light. Based on otolith data [see Fig. 2(c)], the specimens ranged in age from <1 to 6 years ($n = 44$; Fig. 4) with 20 females, 24 males and six of unknown sex. Maximum age for aged females and males were 5 and 6 years, respectively. The precision (c.v.) of our ageing estimates was 3.2%.

The growth trajectories for male and female *O. beta* appear similar for 0–2 years; however, growth curves diverged past 2 years (Fig. 4). Application of the Kimura (1980) method indicated that sex-specific von Bertalanffy growth curves were statistically justified (Fig. 4).

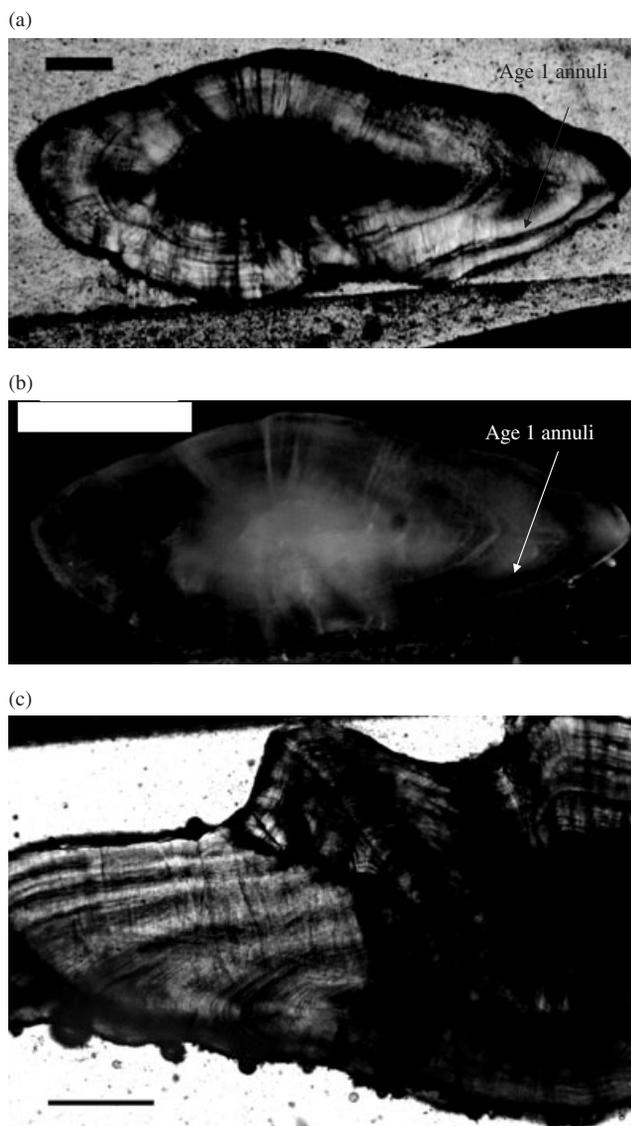


FIG. 2. Micrographs of a transverse cross-section of sagittal otoliths of *Opsanus beta* sampled in Biscayne Bay, Florida: (a), (b) 1 year-old fish [77 mm total length (L_T) and 4.3 g total wet mass (M_T)] of undetermined sex from mesocosm holding tank; annuli is shown (\leftarrow) in (a) transmitted light with polarized filter $\times 40$ magnification and, (b) reflected light $\times 6.3$ magnification (scale bar = 500 μm) and (c) 6 year-old male (329 mm L_T and 515 g M_T).

SPECIES COMPARISON

Sex-specific growth trajectory plots were generated (Fig. 5) for *O. beta* (this study) and *O. tau* (Schwartz & Dutcher, 1963; Radtke *et al.*, 1985). The *O. tau* trajectories of Schwartz & Dutcher (1963) and Radtke *et al.* (1985) generally tracked one another; growth curve differences between these two studies can probably be

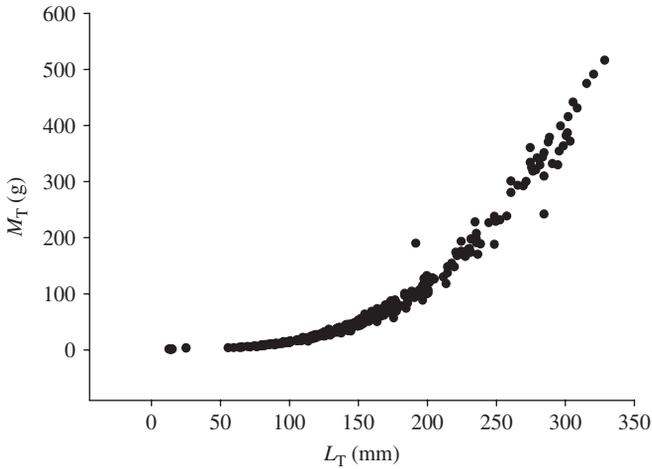


FIG. 3. (a) Relationship of total length (L_T) and wet total mass (M_T) ($n = 373$) for *Opsanus beta* sampled in Biscayne Bay, Florida. Immature fish of undetermined sex ($n = 47$). The curve was fitted by: $y = 0.0000033x^{3.28}$ ($r^2 = 0.99$).

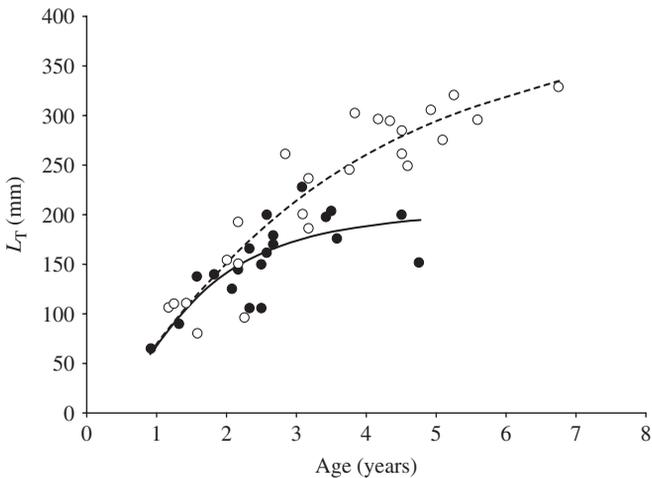


FIG. 4. Relationship of age and total length (L_T) of *Opsanus beta* ($n = 44$) males (○) and females (●). Von Bertalanffy parameters: $L_{T\infty} = 393.8$ mm, $K = 0.30$, $t_0 = 0.36$ for males, $L_{T\infty} = 201.1$ mm, $K = 0.79$, $t_0 = 0.47$ for females. Aged fish of undetermined sex were not included ($n = 6$).

attributed to differences in methodology (vertebrae *v.* otoliths were examined), rather than geography (Maryland and Virginia are adjacent). Comparison of the otolith-based *O. beta* growth trajectory to that estimated from *O. tau* otoliths by Radtke *et al.* (1985) indicates that *O. beta* males grow much more rapidly than *O. tau*. In contrast, female *O. beta* and *O. tau* growth trajectories appear very similar until *c.* 3 years of age. Beyond this age, female *O. beta* and *O. tau* trajectories diverge with the former slowing (towards its $L_{T\infty}$) at least 2 years before the latter (Fig. 5).

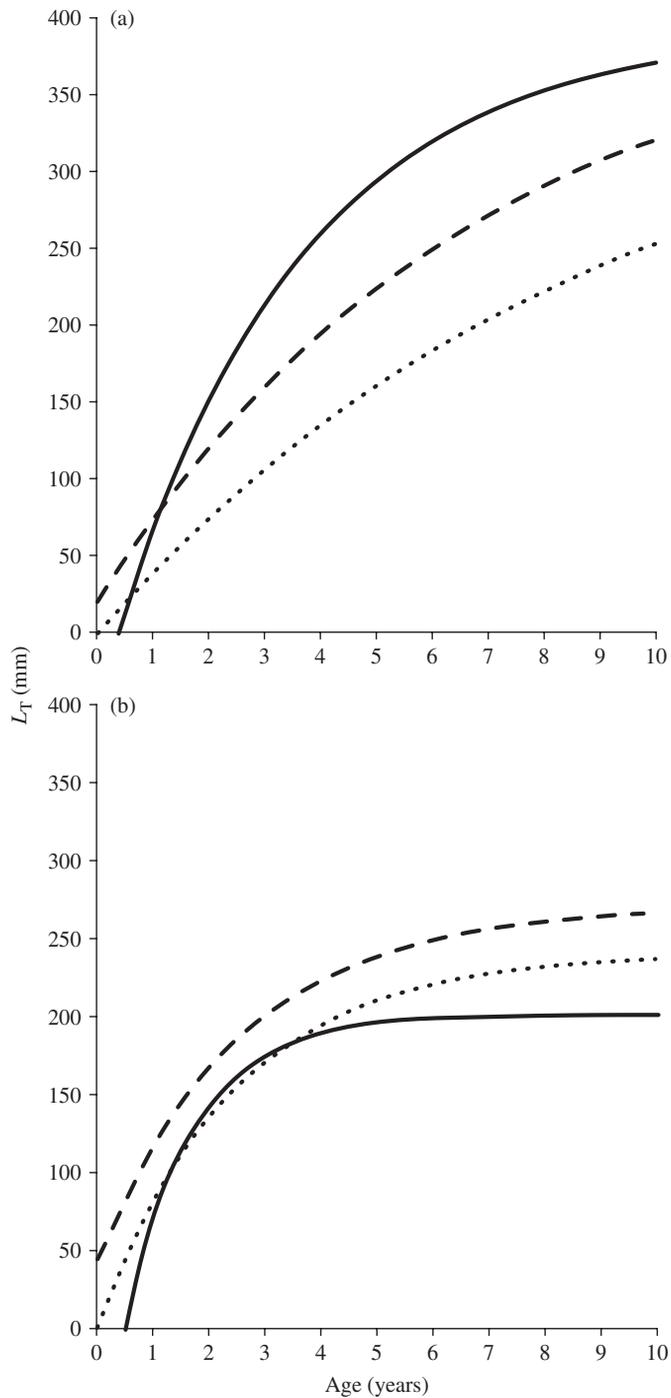


FIG. 5. Comparison of the von Bertalanffy curves for *Opsanus beta* (present study; —) and *Opsanus tau* (Radtke *et al.*, 1985; --- and Schwartz & Dutcher, 1963;) for (a) male and (b) female fishes.

DISCUSSION

ANNUAL SPAWNING

Analysis of 12 months of I_G data indicates that there is only one spawning per year for *O. beta* in Biscayne Bay. The number of annual spawnings was previously considered a point of uncertainty with modelling the age and growth relationship for *O. beta* (Serafy *et al.*, 1997a). The present I_G data are consistent with field observations in Biscayne Bay (Serafy *et al.*, 1997b) and nearby Florida Bay (Barimo *et al.*, 2007) and fail to support the hypothesis of Breder (1941) that *O. beta* spawn twice annually in southern Florida waters. It should be noted, however, that the present data span only a single year and are limited to Biscayne Bay.

AGEING AND GROWTH

This study is the first direct attempt to age *O. beta* by examination of otoliths. It is also the first to provide estimates of early growth and validation of annuli *via* maintenance in captivity. The first annulus was clearly observed in the sole fish maintained in captivity for over a year, suggesting that only one annulus is laid down per year [Fig 2(a)]. Attempts using light microscopy to observe daily increments were unsuccessful. Although the number of individuals held in captivity was small, the growth trajectory that estimated for their first year of life is more realistic than that obtained by fitting the von Bertalanffy curve. It is possible, however, that the early growth observed in this study differs from that of *O. beta* in their natural environment. Therefore, future validation of the first annulus is needed for *O. beta* otoliths. Laboratory rearing (Campana, 2001) and ageing based on other structures is recommended such as opercula, fin rays and vertebrae, since scales are absent on *O. beta*. In general, ageing of the first two annuli were difficult to identify; however, annual rings at 3–6 years of age were easier to distinguish. These observations are perhaps because growth in *O. beta* <2 years of age is less influenced by seasonal environmental fluctuations and by the seasonal bioenergetic demands of oogenesis in females, and fasting while nest brooding in males (Barimo *et al.*, 2007).

DIFFERENCES IN GROWTH AND AGES BY SEX

Sex-specific growth trajectories were similar at ≤ 3 years of age, but female growth declined rapidly thereafter. The observed differences in male and female growth patterns are probably linked to reproduction and the bioenergetic cost of oogenesis where I_G values among gravid females approach 10%. Furthermore, *O. beta* eggs average 4.1 mm in diameter (Breder, 1941) compared with an average of 1 mm in egg diameter for most marine fishes (Richards, 2006). There is considerable maternal investment in offspring where *O. beta* embryos and cling larvae are sustained by yolk proteins until they emerge from paternal brooding after *c.* 3 weeks (Barimo *et al.*, 2004, 2007).

The largest female collected in this study was 232 mm L_T . This maximum could reflect several factors including: sex-specific habitat utilization or spatial distributions within Biscayne Bay, females being better able to avoid sampling gear or that females indeed attaining a smaller $L_{T\infty}$ than their male counterparts. The selective effect of the trawl's finger bars may have limited the collection of larger specimens and thus

may have affected the growth curves (Ricker, 1975). The largest *O. beta* female documented had an M_T of 400 g (Walsh *et al.*, 1989) with an estimated L_T of 298 mm based on the M_T and L_T relationship from this study.

Reporting both the M_T and L_T and L_T and age relationships in the current study provides important information for physiological studies of *O. beta*. Nearly all studies of nitrogen excretion physiology in *O. beta* report values on a per mass basis, as well as report mass ranges for the animals used (Wood *et al.*, 2003). The data in the current study allow conversion of these mass data to L_T , and then to age, such that age of fish used may be estimated in past and future physiological studies where only M_T is determined.

COMPARISONS WITH *O. TAU*

The differences in sex-specific growth rates for *O. beta* parallel the sexual dimorphism noted with *O. tau* (Wilson *et al.*, 1982; Radtke *et al.*, 1985). The diameter of *O. tau* eggs is *c.* 5 mm (Dovel, 1960) and mean female I_G values of *c.* 10 have been reported (Fine *et al.*, 2004). Although *O. tau* eggs are larger than *O. beta*, the similarity in I_G values between species suggests comparable bioenergetic demands with regards to oogenesis. It is also suspected that the bioenergetic cost of oogenesis in *O. tau* may suppress growth rates, although there may also be a genetic basis to this dimorphic pattern in both species. It is possible that the more benign temperature regimes at southern latitudes may result in earlier maturity in *Opsanus* females and faster growth rates in males. Mature gonads were observed in this study in female *O. beta* as small as 76 mm L_T , which corresponds to year 1 based on the von Bertalanffy growth curve. Fine (1975) found that *O. tau* females were immature at 106 mm. Temperature-controlled growth trials with both species are warranted to quantify how water temperature mediates maturity schedules of *Opsanus* females and the somatic growth rates of males.

The present finding that male *O. beta* were generally larger than *O. tau* at any given age appears contrary to Bergmann's (1847) rule. The rule states that in endotherms increased body size is inversely proportional with ambient temperature, which allows for increased conservation of heat with a reduced surface area to mass ratio (Bergmann, 1847). Although there is some question as to the taxonomic level at which Bergmann's rule applies (Blackburn *et al.*, 1999), it is also thought to generally apply to ectotherms (Ray, 1960). A more recent study of 18 species of freshwater fishes across latitudinal gradients, however, suggests that ectothermic fishes do not follow Bergman's rule and, to the contrary, body size is often found to be directly proportional to temperature (Belk & Houston, 2002). The current data on *Opsanus* appear to support these more recent observations.

The present data for these estuarine teleost congeners also fits well with the general effects of temperature on fish metabolic rates. While there is some compensation to temperature such that metabolic rates at respective habitat temperatures are usually similar, species at warmer climes tend to have slightly higher metabolic rates (Hochachka & Somero, 2002). This pattern appears also to be the case for the two species of *Opsanus*. The oxygen consumption rate of *O. beta* under laboratory conditions at 25°C is *c.* 67 $\mu\text{l g}^{-1} \text{h}^{-1}$ (Gilmour *et al.*, 1998), whereas that of *O. tau* at 22°C ranges from 21 (in spring animals) to 31 (in autumn animals) $\mu\text{l g}^{-1} \text{h}^{-1}$

(Ultsch *et al.*, 1981). Thus, the higher growth rates of male *O. beta* appear to follow from their higher metabolic rates. The positive effect of elevated temperature appears also to apply to growth within *O. tau* in that Mensinger & Tubbs (2006) have found, for example, much higher growth rates at 29 *v.* 20° C in controlled laboratory growth studies.

When previous estimates of growth rates appeared to suggest that *O. beta* grew more slowly than *O. tau*, it was speculated to be a result of higher rates of urea production in the former species (Serafy *et al.*, 1997a). In the intervening years between that study and current estimates based on otolith data, much research has been conducted on ureotely in these species (Wood *et al.*, 2003). Indeed, recent measurements indicate that *O. beta* excretes roughly a 50:50 mixture of ammonia and urea in its natural habitat, including juvenile stages (Barimo *et al.*, 2004, 2007), and that this mixture appears to be critical to a chemosensory cloaking mechanism to limit predation (Barimo & Walsh, 2006). Furthermore, laboratory studies subsequent to the Serafy *et al.* (1997a) study have demonstrated that *O. tau* is itself capable of substantial rates of urea synthesis and excretion (Wang & Walsh, 2000), although it is not known if the proportion excreted by *O. tau* in the wild is close to the 50:50 urea : ammonia ratio seen in captive *O. beta*. Thus, it is more likely that any growth rate differences between these two congeners are not due to differences in energy investment in nitrogenous waste production. Of interest would be further comparisons of their growth rates to confamilial (Family: Batrachoididae) members that do not excrete urea.

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