

Age validation and seasonal growth patterns of a subtropical marsh fish: The Gulf Killifish, *Fundulus grandis*

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Abstract *Fundulus grandis* (Baird and Girard), the Gulf Killifish, is an abundant species throughout the marshes of the northern Gulf of Mexico. Its wide distribution and high site fidelity makes it an ideal indicator species for brackish and salt marshes, which experience a variety of anthropogenic disturbances. Despite the ecological, commercial, and scientific importance of *F. grandis*, age determination methods have not been validated and little is known of its growth pattern. By combining a tag-recapture study with a chemical marker to stain otoliths, we validated an ageing method for *F. grandis* adults (49–128 mm TL) using whole sagittal otoliths and determined growth rates of recaptured individuals in winter ($n = 58$) and summer ($n = 36$) in Louisiana. Mean somatic growth in length was significantly greater during the winter (0.085 mm d^{-1}) than summer (0.054 mm d^{-1}). In contrast, mean otolith growth was significantly greater in summer ($1.37 \text{ } \mu\text{m d}^{-1}$)

than winter ($0.826 \text{ } \mu\text{m d}^{-1}$). The uncoupling of somatic and otolith growth may be primarily attributed to warm summer temperatures, which led to enhanced otolith growth while simultaneously reducing somatic growth. *Fundulus grandis* was aged to a maximum of 2.25 years. The parameters of the von Bertalanffy growth model were estimated as: $L_{\infty} = 87.27 \text{ mm}$, $k = 2.43 \text{ year}^{-1}$, and $t_0 = -0.022$. These findings reveal essential age and growth information for *F. grandis* and provide a benchmark to evaluate responses to environmental disturbances.

Keywords Marsh fish · Otoliths · Age validation · von Bertalanffy · Gulf of Mexico

Introduction

Age and growth data are used in fishery science to inform stock assessments, describe the age structure of a population, help infer the productivity (Beddington and Kirkwood 2005) and dynamics of ecosystems (Frisk et al. 2005; Pardo et al. 2013), and inform chemical analyses (isotopes, trace elements, etc.) of otoliths (Campana 1999). Age is often determined from a fish's calcified structures (e.g., otoliths, scales, and vertebrae) or hard parts (Campana 2001) by counting annuli. These structures can be considered permanent recordings that accumulate information over the lifetime of a fish (Secor et al. 1995; Campana 1999). Otoliths are a preferred ageing structure because they are protected internally, have highly resolvable details, and are one of the first

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tissues to calcify in embryonic and larval fish (McElman and Balon 1979).

Otoliths are also useful for age validation because they can be chemically marked (Fowler 1990; Beamish and McFarlane 2000; Duffy et al. 2012). Chemically marking the otoliths of species that display discernable annular structures allows for the validation of annuli. A mark in the otolith from oxytetracycline (or other fluorescent compound, e.g., calcein, alizarin) can be used to constrain the time of annulus formation because it represents a known date during the life of the individual relative to that of the annulus (Campana 1999; Campana 2001; Choat et al. 2003).

Using otoliths to age fish can be problematic, however, as some species do not exhibit clear annuli or may show false marks due to reproduction, thermal stress, settlement, or food availability (Brothers 1979; Victor 1982). The otoliths of many temperate fish species exhibit a series of translucent and opaque zones due to seasonal differences. Earlier work on subtropical and tropical species concluded reduced environmental seasonality leads to a lack of distinct annular marks in scales and otoliths, making age determination difficult for these species (Pannella 1974; Brothers 1979; Oxenford et al. 1994). Recent work, however, demonstrates that many tropical and subtropical fish species can often be aged successfully based on annular marks (see Morales-Nin and Panfili 2005 for a detailed discussion). Daily increments are often used to age young fish in tropical and subtropical environments; however, this method is both time consuming (Brothers 1979; Ralston and Williams 1989) and of limited value in older fish because of interruptions in growth (Ralston and Miyamoto 1981).

Fundulus grandis, the Gulf Killifish, is a small (maximum length of 180 mm; Smith 1997) fish that inhabits estuaries of the Gulf of Mexico, from northeastern Mexico to Florida (Williams et al. 2008; Galleher et al. 2010). It is ecologically important as a common prey species and an important trophic link in food webs of the northern Gulf of Mexico (Subrahmanyam and Drake 1975; McCann et al. 2017). It is also a commercially important species, commonly harvested as a baitfish (Tatum et al. 1982; Brown et al. 2011; Green 2013).

The salt marshes of the Gulf of Mexico are subject to a variety of stressors, both natural and anthropogenic: hurricanes (Palaseanu-Lovejoy et al. 2013), agricultural runoff, freshwater diversions (Sklar and Browder 1998),

low dissolved oxygen, sea level rise, and oil spills (Chesney et al. 2000; Fodrie et al. 2014). The prevalence and high site fidelity of *F. grandis* positions it as an ideal indicator species for marshes to assess the impacts of the 2010 BP Deepwater Horizon oil spill (e.g. Dubansky et al. 2013; Able et al. 2015; López-Duarte et al. 2016; Martin 2017). *Fundulus grandis* is a closely related congener to the more northerly-distributed *Fundulus heteroclitus* (Galleher et al. 2010; Whitehead et al. 2012). Although both species are considered sentinels of marsh ecosystems (Burnett et al. 2007; Finley et al. 2009), little is known of *F. grandis*' growth patterns or longevity (Able and Hata 1984). Prior studies used scales to age *F. grandis* to a maximum of ~23 months (Lipcius and Subrahmanyam 1986), but this method has not been validated for this species. In addition, scales are frequently damaged and regenerated, and growth patterns may change with scale location (Chilton and Beamish 1982).

The objectives of this study are to elucidate age and growth patterns of *F. grandis*. Specifically we aim to: 1) validate an ageing methodology using sagittal otoliths, 2) construct a von Bertalanffy growth curve, and 3) determine annual and seasonal growth rates.

Materials and methods

Study site

Two salt marsh creeks near the DeFelice Marine Center of the Louisiana Universities Marine Consortium (LUMCON) in Cocodrie, LA were chosen for this study (Fig. 1). The creeks are located 0.4 km apart and near LUMCON's environmental monitoring station (0.1 and 0.5 km away from Creeks A and B, respectively). These creeks range in width from 1 to 10 m and are 70 to 90 m in length. The vegetation along these creeks is dominated by *Spartina alterniflora*. Previous tagging efforts in these creeks have indicated *F. grandis* has high site fidelity (Jensen et al. unpublished), a finding supported by tagging of *F. grandis* elsewhere in the northern Gulf of Mexico (Nelson et al. 2014). These sites, like many on the Gulf coast, experience a low tidal amplitude (~0.2–0.3 m) (Turner 2001), and therefore wind and larger scale meteorological forcing often drive water levels (Rozas 1995). Salinity in adjacent channel water typically ranges from 5 to 25 and water temperature

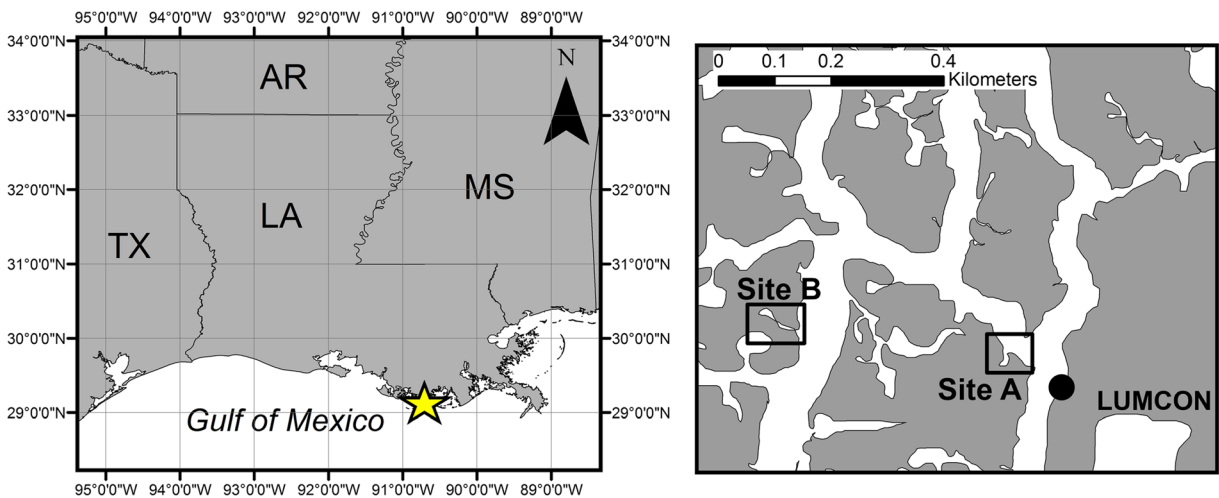


Fig. 1 Map of study area near Louisiana Universities Marine Consortium (LUMCON) with detailed views of tagging sites “A” and “B”. Black circle near LUMCON indicates location of environmental monitoring station

from 5 to 35 °C throughout the year. The LUMCON environmental monitoring station was used to record proxy water temperature and salinity data during the experiment (<http://weatherstations.lumcon.edu/index.html>).

Fish tagging and recapture

A total of 828 *F. grandis* were collected using baited wire mesh traps (41 cm long, 22 cm wide with 3 mm mesh) in November of 2013 ($n = 572$, total length range = 31–131 mm, $\bar{X} = 67.8$ mm, $SD = 17.5$ mm) and May of 2014 ($n = 256$, range = 57–125 mm, $\bar{X} = 79.1$ mm, $SD = 11.9$ mm). Sex was determined using color pattern (Tatum et al. 1982; Boschung and Mayden 2004). All fish were tagged with 1.1 mm long sequentially coded wire tags (Northwest Marine Technologies, Inc. [NMT]), inserted into the dorsal musculature using a tag injector (NMT Handheld Multishot Tag Injector). These tags have been used with a congener (*F. heteroclitus*) and resulted in high survival rates and a 98.9% tag retention rate (Able et al. 2006; Able et al. 2012). After the tag was inserted, fish were immediately placed in a 700 mg L⁻¹ oxytetracycline (OTC) solution with a salinity of 5–10 (after Brown et al. 2002; Fielder 2002). Fish were kept in the solution for 24 h to allow the OTC mark to form before being released into the creek from which they were captured.

Recapture efforts were made with identical wire mesh traps in the same marsh creeks during April and

September of 2014. Recaptured individuals at liberty from 20 November 2013 to 7 April 2014 (138 days) were considered the winter group ($n = 58$) (Table 1). Fish at liberty from 25 May 2014 to 3 September 2014 (101 days) were considered the summer group ($n = 31$). One individual tagged on 20 November 2013 was recaptured during the May tagging effort (187 days at liberty). Four individuals tagged on 20 November 2013 were recaptured on 3 September 2014 (287 days at liberty). All recaptured fish were euthanized, frozen, and then shipped to the Rutgers University Marine Field Station (RUMFS) for processing. Fish were thawed and total length (TL) was measured to the nearest millimeter. Both coded wire tags and sagittal otoliths were removed from all fish. The tag numbers were read by placing the coded wire tags in a magnified viewer (NMT Magniviewer). Sagittal otoliths were rinsed with deionized water and stored dry in the dark to prevent degradation of OTC marks (Howard 2008) until further processing.

Otolith processing

The right sagittal otolith of each recaptured fish was set in Buehler EpoThin 2 Epoxy Resin (Araya et al. 2003), cut and ground (Hillquist Inc. Diamond Saw and Polisher) to ~200–300 μm from the core, and mounted to petrographic slides. Mounted otoliths were cut and ground, and then polished until smooth using a variety of abrasive pads (5" Polishing Discs from Precision Fiber Products; Grits: 30 μm, 9 μm, 3 μm, and buffing

Table 1 Sampling effort for tag (top) and recapture effort (bottom). Top panel shows counts of tagged fish (31–131 mm) during the two tagging efforts (November 2013 and May 2014) and bottom shows two recapture efforts (April 2014 and September 2014, total length ranged from 49 to 128 mm upon recapture), as well as the single fish that was recaptured during the May 2014 tagging effort. Fish counted as “unknown” were missing sex data or were juveniles. See Fig. 1 for location of tag and recapture efforts

	November 2013		May 2014			
Site:	A	B	A	B	Total:	
# Tagged:	302	270	120	136	828	
Female:	105	111	53	71	340	
Male:	68	64	66	45	243	
Unknown:	129	95	1	20	245	
	April 2014		May 2014	September 2014		
Site:	A	B	B	A	B	Total:
# Recaptured:	36	22	1	15	20	94
Female:	17	13	1	12	12	55
Male:	17	9	-	3	7	36
Unknown:	2	-	-	-	1	3

pad) and polishing compound (Buehler Micropolish) to a final thickness of 200–300 μm .

Otolith thin sections were viewed and photographed on an Olympus BX53 microscope at 100X under natural white light to view annuli and then fluorescent light (Lumen Dynamics, X-Cite Series 120 Q) directed through an Olympus U-MWB2 filter cube (excitation filter: 460–490 nm, emission: LP 520, dichromatic mirror: DM 500) to view OTC marks. All thin sections were photographed in 1–3 segments and the images were merged using Adobe Photoshop CS4. Thin sections were measured using ImageJ64 software (Version 1.48). Otolith length was measured along the dorsoventral axis, measuring end to end through the core, perpendicular to the sulcus. Measurements were also taken from the otolith core to the OTC mark, and from the OTC mark to the edge of the otolith (Fig. 2), similar to Fowler (1990). OTC marks were measured on both ends of the thin-sectioned otolith, when visible.

If the right otolith did not have a visible OTC mark ($n = 14$), the left otolith was examined and considered equivalent to the right otolith for the purposes of measuring otolith growth (Araya et al. 2003; Battaglia et al. 2010; Farley et al. 2013). Five of these 14 left otoliths had a visible OTC line. The lack of visible OTC marks in some otoliths is thought to have been due to variations in sectioning thickness rather than physiological differences among tagged fish (Francis et al. 1992).

Growth calculations

Somatic (total) growth was calculated as final TL – initial TL. Growth per day was calculated as total growth

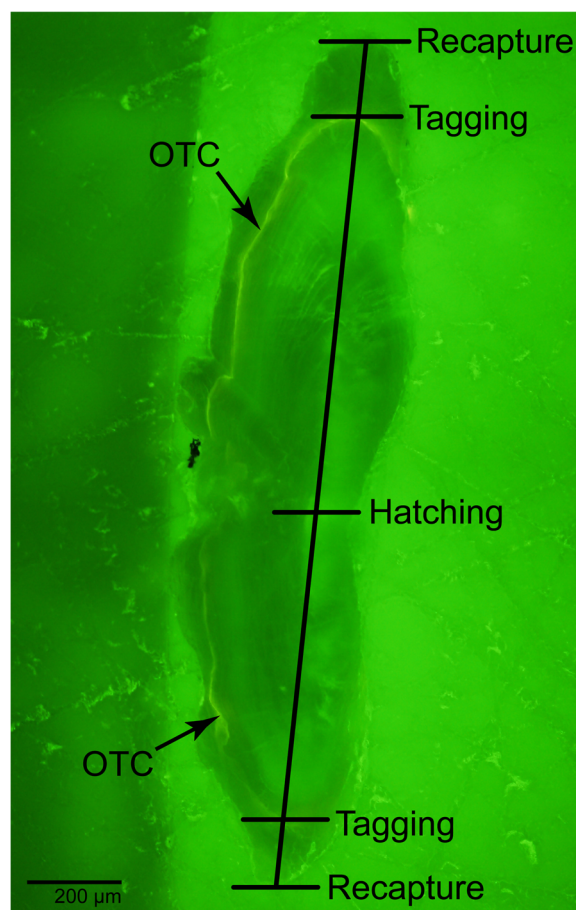


Fig. 2 *Fundulus grandis* (78 mm TL) sagittal otolith thin section photograph showing measurement lines and OTC mark. “Hatching” represents the date of hatching at the core of the otolith. “Tagging” is the location of the OTC mark, which represents the date of tagging. “Recapture” is the edge of the otolith, representing the date of capture

divided by days at liberty. When TL was not available, standard length (SL) was converted to TL using: $TL = (SL)/(0.82)$. This conversion is the mean value from the FishBase *F. grandis* morphometrics webpage (Froese and Pauly 2016) and a comparison of SL and TL values from the data of Vivian (2005) and Vivian et al. (2012).

Ageing

Otoliths were viewed and photographed using three different methods (see Vastano 2016). A subset of whole, right otoliths was photographed under reflected light ($n = 45$) prior to thin sectioning, and a subset of left otoliths was photographed under transmitted light ($n = 74$). Whole otoliths under reflected light were photographed using a Zeiss SMZ-168 Zoom Stereo Microscope with Canon EOS Rebel T3i camera. Whole otoliths viewed using transmitted light (Wild Makroskop Model M420 with PixeLINK Model PL-A662 camera) were immersed in deionized water to increase the contrast of the internal physical structures. Thin-sectioned otoliths ($n = 94$) were photographed while mounted to petrographic slides under transmitted light (Olympus BX53 microscope with Olympus DP80 camera).

Fish were aged by viewing whole otoliths and counting dark, opaque zones that were formed during the winter (see *Otolith age validation*, below). By convention, all fish were given an estimated hatch date of June 1, the midpoint of the reproductive period for *Fundulus grandis* (Waas and Strawn 1983; Greeley et al. 1988; Brown et al. 2011). Since the date of sacrifice was known for all fish, age was back-calculated from the edge of the otolith (representing the date of capture) to the inside of the opaque zone (representing December 1, approximately when the opaque zone starts forming based on the location of the OTC mark). The area of the otolith from the innermost opaque zone to the core was counted as 6 months (June to December). If an additional opaque zone was present, an additional year was added to the age of the individual.

Data analyses

All statistical analyses were performed in RStudio v.0.98.109. Linear regression (lm function) was used to establish the total fish length-otolith width relationship. Analysis of covariance (ANCOVA; lm and anova

functions) was used to determine whether somatic growth differed between seasons, with season set as the categorical variable and initial TL as a covariate. Because initial TL was not found to be a significant covariate in an earlier ANCOVA model, Analysis of variance (ANOVA; lm and anova functions) was used to determine whether otolith growth differed between seasons. To determine whether somatic growth differed between sites A and B or between males and females, t-tests (t.test function) were applied to somatic growth data. Individuals that were at liberty across both seasons were omitted from statistical tests comparing seasonal growth.

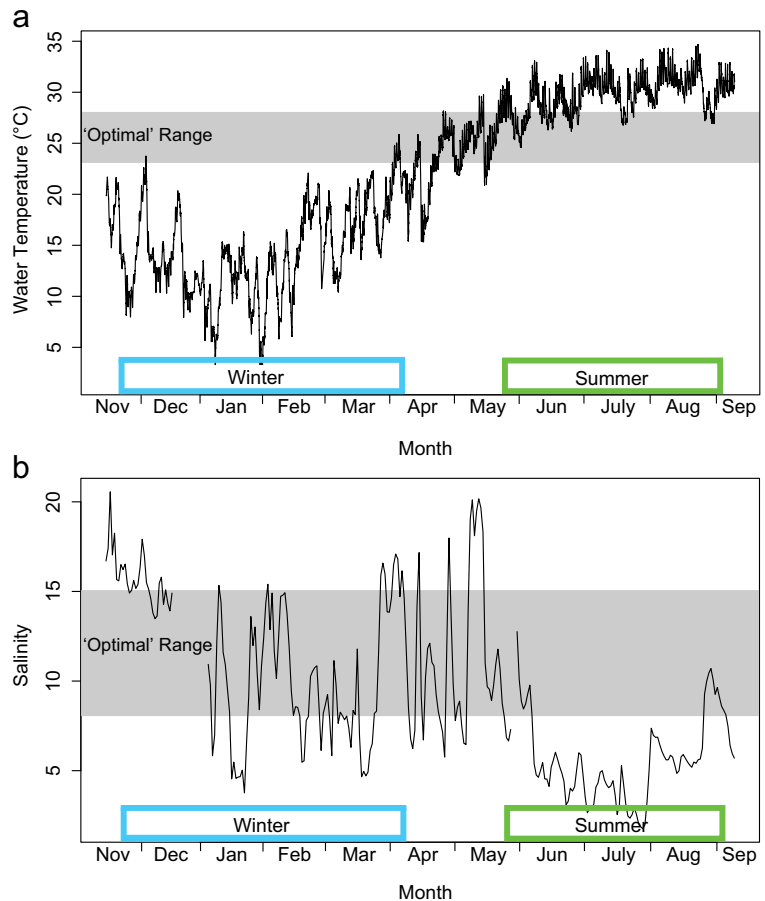
Length at age data from this study were combined with data from Vivian et al. (2012) to provide length at age data for both adult and juvenile fish, respectively. The samples (10–48 mm TL, $\bar{X} = 26$, $n = 42$) of Vivian et al. (2012) were collected from a salt marsh in Pascagoula, MS. The nls (Nonlinear Least Squares) function in RStudio was used to estimate parameters for the von Bertalanffy growth curve of this combined data set. The von Bertalanffy growth equation $L_t = L_\infty (1 - e^{-k(t-t_0)})$, where L_t is the expected TL (mm) at age t (years), L_∞ is the maximum asymptotic TL, k is the Brody growth coefficient, and t_0 is the theoretical age at which TL is zero (Beverton and Holt 1957; Ricker 1979). The fit of the growth curve was investigated by solving for the initial age at tagging for each fish by using the recorded TL upon tagging and then projecting the model TL after the appropriate time at liberty for each recaptured fish. Projected growth was calculated as the difference between final projected TL and initial TL. Linear regression was used to compare measured growth to projected growth from the von Bertalanffy model.

Results

Environmental setting

Water temperature data from a LUMCON environmental monitoring station near the study site indicated temperatures ranged from 1.7–25.9 °C (mean temperature (\bar{T}) = 14.4 °C) in the winter period of November 2013–April 2014 (Fig. 3a). Temperatures ranged from 24.6–34.7 °C ($\bar{T} = 29.8$ °C) in the summer period of May–September 2014. Salinity ranged from 2.5–28.0 ($\bar{S} = 11.4$) in the winter period and 1.4–23.0 ($\bar{S} = 5.51$) in the summer period (Fig. 3b).

Fig. 3 Water temperature and salinity time series from the LUMCON environmental monitoring station near the study site (see Fig. 1) from late November 2013 to early September 2014. **a.** ‘Optimal’ spawning temperature range (23–28 °C) shown in gray (Green et al. 2010; Gothreaux and Green 2012; Green 2013). **b.** Daily mean salinity time series and optimal range (8–15) for culturing *F. grandis* shown in gray (Green 2013)



Tag and recapture

Approximately 11% (94/828) of all tagged fish were recaptured. Fifty-eight fish (10.1%) were recaptured after the winter period and 31 fish (12.1%) after the summer. Recaptured fish ranged in size from 49 to 128 mm TL ($\bar{X} = 76.8$, $SD = 13.6$). Recaptured fish tagged during the November 2013 effort were smaller, on average, than those tagged during the May 2014 effort (winter: $\bar{X} = 62.0$ mm, $SD = 16.2$; summer: $\bar{X} = 75.1$ mm, $SD = 8.6$; $t = 4.69$, $df = 76.92$, $p < 0.001$).

Daily somatic growth (G) did not differ significantly between sites (Site A: $\bar{G} = 0.076$ mm d^{-1} , $SD = 0.028$; Site B: $\bar{G} = 0.078$ mm d^{-1} , $SD = 0.034$; $t = -0.3129$, $df = 72.38$, $p = 0.7552$), or sex (Males: $\bar{G} = 0.075$ mm d^{-1} , $SD = 0.032$; Females: $\bar{G} = 0.077$ mm d^{-1} , $SD = 0.030$; $t = -0.2635$, $df = 68.35$, $p = 0.793$). Controlling for initial size, somatic growth was significantly greater during the winter period ($\bar{G} = 0.085$ mm d^{-1} ,

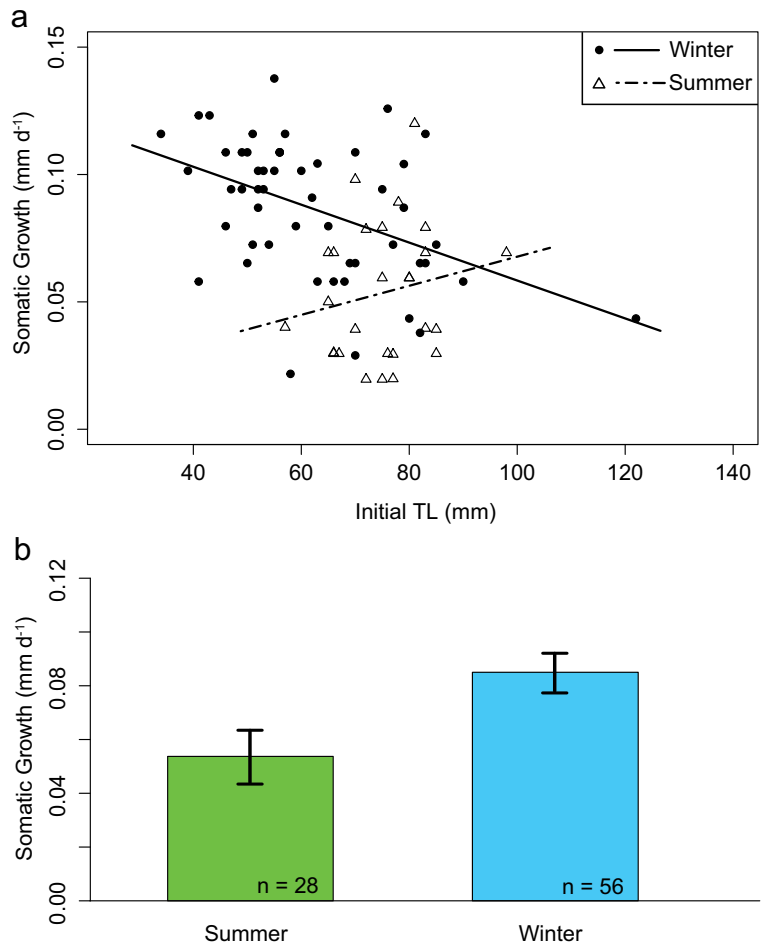
$SD = 0.027$) than during the summer ($\bar{G} = 0.054$ mm d^{-1} , $SD = 0.027$; ANCOVA: $F = 14.65$, $df = 3, 75$, $p < 0.001$; Fig. 4). Otolith width was proportional to fish TL (linear regression: $Y = 0.017X + 0.365$; $r^2 = 0.80$, $p < 0.001$).

Otolith growth rates

Otolith width growth rates (O), as measured from the core to one end of the otolith perpendicular to the sulcus, were less in winter ($\bar{O} = 0.84$ $\mu\text{m d}^{-1}$, $SD = 0.19$) than summer ($\bar{O} = 1.37$ $\mu\text{m d}^{-1}$, $SD = 0.56$; ANOVA: $F = 36.98$, $df = 1, 75$, $p < 0.001$; Fig. 5).

Whole otoliths of fish at liberty during the winter period viewed under transmitted light had a dark, opaque region near the edge of the otolith (hereafter referred to as an opaque zone). This mark appeared more opaque than the surrounding area when viewed with reflected light against a dark background. Fish

Fig. 4 Somatic growth of tagged *F. grandis* over summer versus winter. **a.** Lines are from the fitted ANCOVA model. Winter line: $Y = -0.00074X + 0.1328$; summer line: $Y = 0.00057X + 0.0106$; $r^2 = 0.34$, $p < 0.001$ **b.** Mean somatic growth of tagged *F. grandis* over summer versus winter. Vertical bars represent 95% confidence intervals



at liberty during the summer consistently displayed an opaque zone farther away from the edge of the otolith.

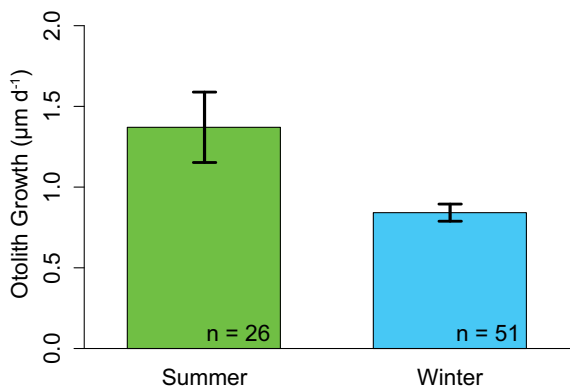


Fig. 5 Mean otolith growth of tagged *F. grandis* during summer versus winter. Vertical bars represent 95% confidence intervals. ANOVA: $F = 36.98$, $df = 1,75$, $p < 0.001$

Thin sections of otoliths marked with OTC in November consistently displayed an OTC ring before the opaque zone. Otoliths marked with OTC in May displayed an OTC ring after the opaque zone. Because winter fish were tagged in late November, and the opaque zone appears almost immediately after the OTC line (Fig. 6), we estimate that the opaque zone begins to form during early winter (around December 1).

Ageing

All fish had an assumed date of birth of June 1, and the date of recapture was factored into the final age estimate. The youngest individuals were tagged in November, and upon recapture were estimated to be 7 months old (2.5 months old upon tagging). The oldest individuals were tagged in May, and upon recapture were estimated to be 27 months old (23 months old upon tagging). The

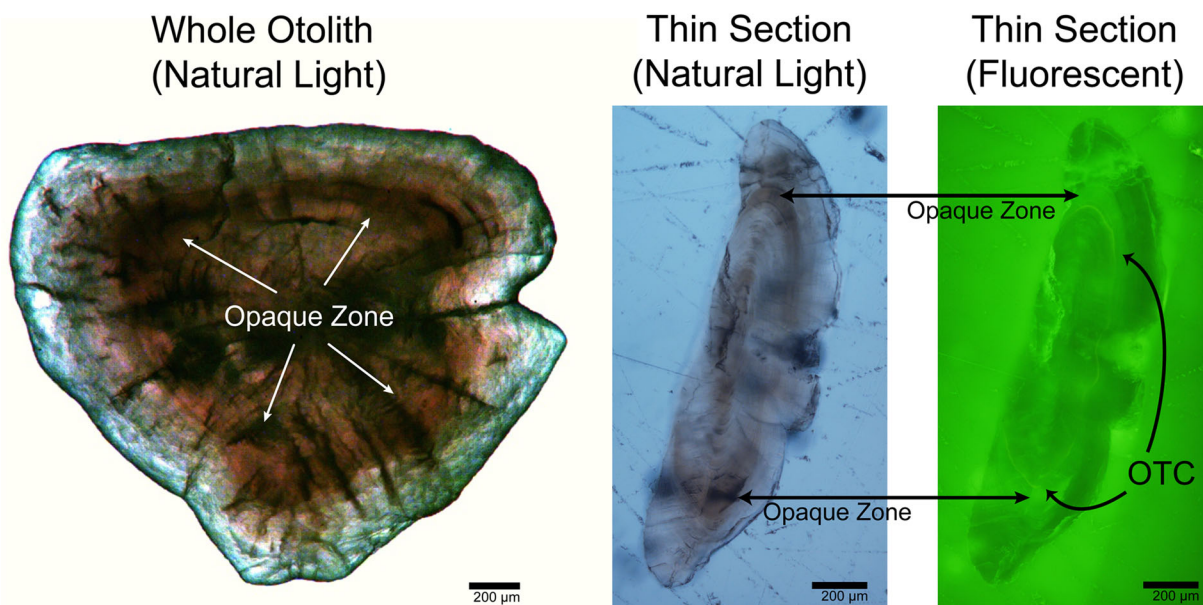


Fig. 6 Images of *F. grandis* otoliths. Left: whole left otolith under transmitted natural light showing dark, opaque zone. Center: thin section of corresponding right otolith under transmitted natural light, also showing opaque zone. Right: same thin section under

fluorescent light, showing the location of the OTC mark relative to the opaque zone. This fish was tagged in November 2013 and recaptured in September 2014

mean age upon recapture was 9 months ($SD = 2.3$) for winter fish and 15.5 months ($SD = 2.9$) for summer fish.

Growth curve

The von Bertalanffy growth model fit to the length at age results of this study and those of Vivian et al. (2012) are estimated to be: $L_{\infty} = 87.27$ mm (95% CI: 82.04, 92.51), $k = 2.433$ year⁻¹ (95% CI: 1.843, 3.023), and $t_0 = -0.022$ (95% CI: -0.055, 0.012) (Fig. 7). The correlation between L_{∞} and k is -0.89 . This model predicts the projected final TL of tagged fish based on their initial TL with an r^2 of 0.93 ($p < 0.001$) (Fig. 8).

Discussion

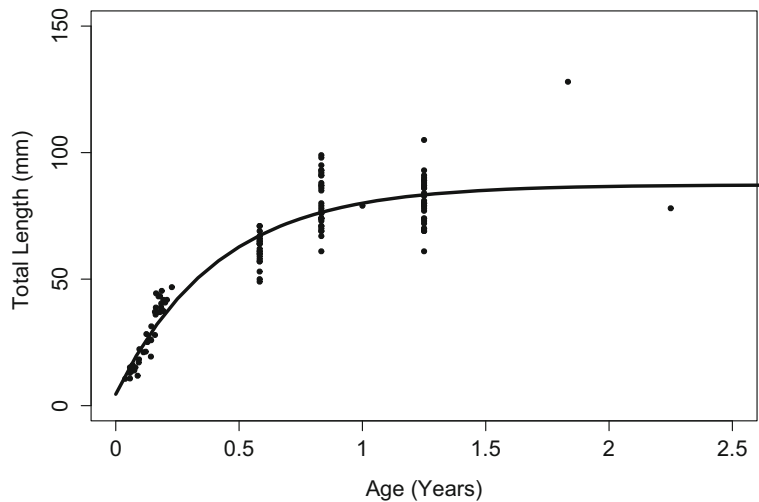
Growth

Our result showing somatic growth of *F. grandis* is greater in winter than summer is contrary to previous work that presumed faster growth in warm months (Lipcius and Subrahmanyam 1986), similar to *F. heteroclitus* (Kneib and Stiven 1978). Unlike *F. heteroclitus*, *F. grandis* inhabits subtropical systems and is therefore exposed to less seasonality. This

reduced seasonality and high summer temperatures appear to lead to different growth patterns. In temperate areas, growth in fish is thought to slow down, or even stop, during cold winter months (Campbell and MacCrimmon 1970; Mann 1971; Able and Fahay 2010) and is expected to be high during warm periods due to increased food availability and high metabolic activity (Warren and Davis 1967; Pauly et al. 1992). In tropical areas, growth is controlled by seasonal rain, reproductive activity, water levels, minor temperature differences, and food availability (Brothers 1979; Ricker 1979; Gibbs et al. 2013).

The optimal temperature range for *F. grandis* is uncertain. Aquaculture studies have identified 23–28 °C as the optimal range for spawning (Green et al. 2010; Gothreaux and Green 2012; Green 2013). Water temperatures remained above this optimal range for most (62%) of the summer liberty period. Temperature swings were likely more extreme in the volumetrically small and shallow marsh creeks and ponds that *F. grandis* inhabited in our study area than in the larger, nearby (0.1–0.5 km away) channel where we collected water temperature and other environmental data. Others found *F. grandis* reproduction was suppressed when temperatures exceeded 28 °C (Green et al. 2010) and ceased above 30 °C (Greeley and MacGregor 1983).

Fig. 7 Fitted von Bertalanffy growth curve for *F. grandis* showing lengths (TL) at age. Data points to the left of the 0.5 year mark are from Vivian et al. (2012). All other points are recaptured lengths at age from our study. Equation of von Bertalanffy growth curve is: $L_t = 87.27 (1 - e^{-2.43(t + 0.022)})$. Fit of growth curve to data is $r^2 = 0.88$



Given these lines of evidence, it appears likely that high creek water temperatures reduced growth during summer.

Fundulus grandis can tolerate a wide range of salinities (0–80) (Perschbacher et al. 1990). However, salinity can have a large impact on juvenile growth, with highest juvenile *F. grandis* growth occurring in salinities of 8–15 (Green 2013). Based on LUMCON environmental monitoring station salinity data, fish at liberty during summer experienced lower

than optimal salinities 81% of the time, while fish at liberty during winter experienced lower than optimal salinities only 19% of the time. Low summer salinities may have contributed to the observed seasonal difference in growth. Additionally, frequent low oxygen levels under warmer conditions (Stierhoff et al. 2003; Martínez et al. 2006) and periods of extended reproduction (Mapstone 1988; Fowler and Doherty 1992; Bonnet et al. 1998; McBride et al. 2013; Patterson 2014) may have influenced growth in the study area during summer.

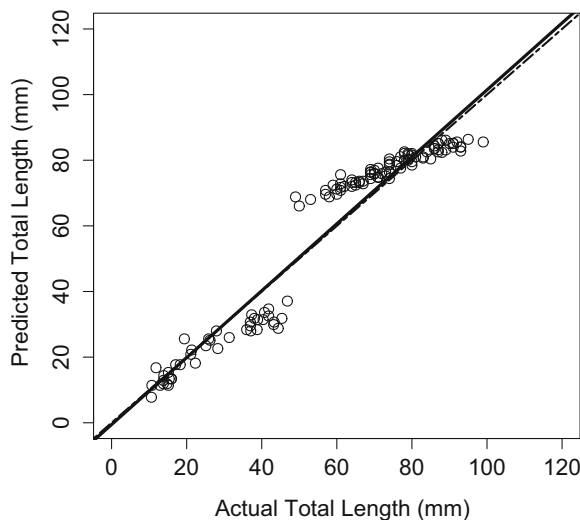


Fig. 8 Predicted versus actual total lengths (TL) of tagged *F. grandis*. X-axis is TL recorded for tagged fish upon recapture. Y-axis is projected final TL based on the von Bertalanffy growth curve. Solid line: $Y = 1.02X - 0.55$, $r^2 = 0.93$, $p < 0.001$. Dashed line represents 1:1

Comparing otolith and somatic growth rates across seasons may better explain controls on *F. grandis* growth. Other studies comparing otolith and somatic growth found that differences in somatic growth are due to food consumption, while otolith growth is controlled by temperature and metabolism (Mosegaard et al. 1988; Barber and Jenkins 2001; Fey 2005). If this pattern holds true for *F. grandis*, the apparent disconnect between somatic and otolith growth could be explained by warm summer temperatures producing high metabolic rates and otolith growth rates (Neat et al. 2008; Takasuka et al. 2008), while these same high temperatures, coupled with allocation of energy to spawning, may decrease somatic growth. Despite an uncoupling of otolith and somatic growth rates, otolith width was still proportional to fish TL in our study because individual fish undergo periods of fast somatic growth with slow otolith growth (winter) and periods of slow somatic growth with fast otolith growth (summer). Integrating asynchronous somatic and otolith growth patterns over the life of a fish would result in the linear relationship

observed between otolith and fish size (see Neat et al. 2008).

Otolith age validation

Fundulus grandis meets the three requirements necessary for age validation of otoliths using annuli: 1) otoliths must have visible internal structures that appear incrementally, 2) these structures must be formed on a regular timescale, and 3) otoliths must continue to grow during the fish's lifetime (Fowler 1990; Fowler and Doherty 1992). The observed dark, opaque zones in whole *F. grandis* otoliths were formed in early winter based on their position relative to the OTC mark. Fowler and Doherty (1992) and Howard (2008) used similar logic for damselfish and parrotfish. Opaque zones were faintly visible in whole otoliths of fish recaptured in April due to their proximity to the otolith margin and were more clearly visible in thin-sectioned otoliths. For fish recaptured in September, opaque zones were more apparent in whole otoliths because additional transparent material formed along the margin. Fish that were aged at 2+ years had two opaque zones, but these were difficult to see in whole otoliths due to their proximity to the edge and thickening of the otolith. For fish that were tagged in November and at liberty across both seasons ($n = 4$), the OTC line was visible prior to the opaque zone, with a large section of translucent otolith growth following both the OTC line and the opaque zone. Similarly, Atlantic croaker in the Gulf of Mexico exhibited opaque zone formation during the period of December through May (Barger 1985). *Fundulus grandis* TL increased linearly with otolith width across the entire size range of fish collected during both seasons, indicating that otoliths grow throughout the life of the fish.

Thus, otoliths can be used to age *F. grandis* by counting opaque zones in whole or thin-sectioned otoliths. Using the approach outlined above (Materials and methods, Ageing), *F. grandis* can be aged to ± 3 months if the date of capture is known (Vastano 2016). More simply, otoliths without an opaque zone are aged as <1, fish with one zone are aged as 1+, and fish with two zones are 2+.

Given these criteria, the oldest fish observed in our study were approximately 2.25 (± 0.25) years old. This is approximately the same age as that (23 months) reported from a previous effort to age *F. grandis* using

scales (Lipcius and Subrahmanyam 1986). *Fundulus grandis* do not appear to live as long as *F. heteroclitus*, which have been aged to four years in the northern portion of their range (Fritz and Garside 1975; Dibble and Meyerson 2012) and have been observed in their fourth season of growth in the southern portion of their range (Kneib and Stiven 1978).

Growth of marked fish

The von Bertalanffy growth curve and the length-at-age approach by Lipcius and Subrahmanyam (1986) agree up to age one. Beyond age one, individuals in their study tended to be slightly larger than those tagged in our study. The majority of the fish they collected in late summer and fall were juveniles and first year adults, which is in agreement with our findings. Their largest fish, reported as 23 months old at approximately 148 mm in TL, was larger than the largest fish observed in our study (131 mm). The method Lipcius and Subrahmanyam (1986) used to age *F. grandis* (counting annular rings in scales) was not validated, but given their results are similar to our study, the use of scales to age *F. grandis* warrants further investigation.

Fundulus grandis approach L_{∞} more rapidly and attain larger sizes than *F. heteroclitus*. *Fundulus heteroclitus*, at least in the southern part of their range, appear to have a much smaller k value, 0.282–0.488 year⁻¹ (Kneib and Stiven 1978), than *F. grandis* ($k = 2.43$ year⁻¹) in coastal Louisiana marshes from our study. In recent work on chemical tracers of oil in the otoliths of *F. grandis*, Nelson et al. (2015) assumed that *F. grandis* measuring 50–90 mm TL were 1 to 4 years of age, as would be expected of *F. heteroclitus* (Fritz and Garside 1975). Although reasonable at the time, the assumption that *F. grandis* exhibit similar age and growth patterns to *F. heteroclitus* is not supported by our study. The supposition that the fish collected by Nelson et al. (2015) were alive at the time of the 2010 BP Deepwater Horizon oil spill most likely does not hold true.

Conclusion

These results provide previously unavailable age and growth information for *F. grandis* that can further solidify its role as an indicator species for marshes

in the northern Gulf of Mexico, an area that is host to a variety of frequent anthropogenic impacts. Future studies of *F. grandis* could use the ageing methodology presented here to determine whether individual fish were present at the time of a stressful event (e.g., an oil spill). Furthermore, the growth curve and seasonal growth data presented could serve as a baseline for measuring the impacts of such disturbances on growth patterns. Finally, this work demonstrates that assuming growth patterns for *F. grandis* are similar to its congener, *F. heteroclitus*, is no longer appropriate.

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Compliance with ethical standards All applicable institutional guidelines for the care and use of animals were followed. Research was conducted under Institutional Animal Care and Use Committee (IACUC) protocol # 88-042 “Determinants of fish habitat quality in stressed and unstressed estuaries.”

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Conflict of interest The authors declare that they have no conflict of interest.

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