Early life history of tarpon (*Megalops atlanticus*) in South Carolina estuaries: Assessment of juvenile recruitment and validity of aging and back-calculation methods

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Early life history of tarpon (*Megalops atlanticus*) in South Carolina estuaries: Assessment of juvenile recruitment and validity of aging and back-calculation methods

By

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Dedication

I dedicate this thesis to my parents, Kim and Jon Elmo, without whom I would not be writing this today. Their love, support, and guidance has given me the perseverance and will to succeed in school and in many other aspects of life. When I was young, still a kid, I was unprepared, never thought before I did, and now as I look back, I can’t believe it. I hope to be able to use their guidance in my future and continue to make them proud.

I would also like to dedicate this thesis to Mike Muglia. Mike gave me many words of encouragement and advised me to follow my passions and dreams during my first year of college. His words have stayed with me throughout the rest of my educational journey, and I often think back on our conversations and how they have impacted my decisions in and out of science.
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Finally, a special thank you to my family, my girlfriend, Lauren, and to all my friends (are here right now). Your love and support have never wavered, and they are seemingly endless. I cannot express my gratitude enough and am truly grateful to have you all in my life. Thank you from the bottom of my heart.
Abstract

I investigated habitat use and age estimation methods for juvenile tarpon (Megalops atlanticus) to better understand use of natural and managed habitats in coastal South Carolina and to provide guidance for estimating life history characteristics that are dependent on age data. Tarpon were sampled during July – November 2019 in natural marsh pools of North Inlet estuary, and in managed impoundments of Kiawah Island and Tom Yawkey Wildlife Center Heritage Preserve to compare arrival duration (duration of ingress) and size structure (standard length, SL) of tarpon in these habitats. The accuracy and precision of age estimation and back-calculation methods were assessed using oxytetracycline to chemically mark juvenile tarpon for an annual increment validation study, a marginal increment analysis study, a controlled back-calculation validation study, and a controlled daily increment periodicity study. One-hundred and two juvenile tarpon (36 – 333 mm SL) were collected during July – November 2019. Tarpon from natural marsh pools (North Inlet estuary; 65.4 ± 20.2 mm) were smaller than those from managed impoundments (Kiawah Island and Yawkey Preserve; 253.9 ± 41.6 mm), with no overlap in size between habitat types. Mean tarpon length was relatively constant throughout the study in natural marsh pools (65 mm SL), but mean tarpon length increased from 180 mm SL in August to 289 mm SL in October in managed impoundments. Peak catch-per-unit-effort occurred from August (natural marsh pools) into September (managed impoundments), and then declined as water temperatures decreased from late October into November. The absence of SL overlap between habitats and increasing tarpon SL over time in marsh impoundments compared to the minimal change in SL over time observed for marsh pools suggests (1) tarpon are transient in
marsh pools early in life, (2) tarpon do not enter impoundments until reaching a certain SL, (3) small juvenile tarpon are cryptic in impoundments and larger juvenile stage tarpon are more susceptible to capture, or (4) a combination of (1), (2), and (3). Oxytetracycline marks were visible on all the otoliths of recaptured tarpon ($n = 22$), prior to a newly formed annulus, validating true age and that one annulus is deposited yearly. However, annuli in scales were more easily identified by readers, thus leading to more accurate and precise estimates of age from scales (100% accuracy of age estimates for age-1 fish) compared to otoliths (88% accuracy). Marginal increment analyses indicated that tarpon annuli are deposited in the spring (March-April) on juvenile tarpon scales and otoliths. Back-calculated lengths were significantly different from measured lengths. Daily increment estimates from otoliths were inaccurate and imprecise, with no correct age estimates and a mean absolute difference of 21 d (37%) from known age. Increments were crowded near the otolith edges, resulting in underestimation of daily increments; therefore, juvenile tarpon daily increment periodicity was unable to be validated. These findings provide valuable information about habitat utilization of juvenile tarpon, provide support for scales as a viable accurate and precise non-lethal age estimation option that will allow researchers to study age, growth, and habitat quality of juvenile tarpon without sacrificing individuals, and validates annual increment periodicity and timing of annulus formation that will aid in future age estimation efforts of juvenile tarpon. Future research should address potential ontogenetic shifts in habitat use by young juvenile tarpon (ages 0-1) and differential growth across habitats, investigate the use of scales for age estimation of older juvenile tarpon ($\geq$age 2), investigate the use of other non-lethal structures for accuracy and precision of age estimation methods, and investigate the
relationship between otolith and somatic growth for young juvenile tarpon (<age 0) to allow for informed management of this species.
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Chapter 1: Juvenile tarpon (Megalops atlanticus) use of natural and managed marsh habitats in coastal South Carolina

Abstract

Historical and current anthropogenic land use in the coastal plain of the southeastern United States has converted natural marsh and swamp land into a mosaic of impoundments managed for waterfowl and mosquito control, remnants of impoundments from historical rice cultivation, and remaining natural habitats. Impoundments are important habitats for fishes and are utilized during early life stages of many fish species. I investigated juvenile tarpon (Megalops atlanticus) use of natural and managed habitats in coastal South Carolina by monitoring recruitment during July – November 2019 in natural marsh pools of the North Inlet estuary, and in managed impoundments of Kiawah Island and Tom Yawkey Wildlife Center Heritage Preserve. One-hundred and two juvenile tarpon (36 – 333 mm standard length, SL) were observed during July – November. Tarpon from natural marsh pools (North Inlet estuary; 65.4 ± 20.2 mm SL) were smaller than those from managed impoundments (Kiawah Island and Yawkey Preserve; 253.9 ± 41.6 mm SL), with no overlap in SL between habitat types throughout the study duration. Mean tarpon SL was relatively constant throughout the study in natural marsh pools (65 mm SL), but mean tarpon SL increased from 180 mm SL in August to 289 mm SL in October in managed impoundments. Peak catch-per-unit-effort occurred during August (natural marsh pools) into September (managed impoundments) across habitat types, and then declined as water temperatures decreased at the end of October into November. The absence of SL overlap between habitats and increasing SL
of tarpon over time in marsh impoundments compared to the minimal change in SL over
time observed for marsh pools suggests (1) tarpon are transient in marsh pools early in
life, (2) tarpon do not enter impoundments until reaching a certain SL, (3) small juvenile
tarpon are cryptic in impoundments and larger juvenile tarpon are more susceptible to
capture, or (4) a combination of (1), (2), and (3). Future research should address potential
ontogenetic shifts in habitat use by young juvenile tarpon (ages 0-1) and differential
growth across habitats.
Introduction

Currently 40% of the United States population lives within coastal counties, and the population in these coastal areas has increased by over 40% (~34.8 million people) since 1978 (NOAA 2020a). A majority of these counties border or have intertidal salt marshes and mangroves, many of which are being altered or lost due to urban development, water control efforts, impounding, and dredging (Dahl 2006). Emergent and intertidal salt marshes have declined in area steadily since 1986, with an annual loss of about 2,240 ha of estuarine habitats across the nation (Dahl 2006). Intertidal marsh and mangrove-dominated areas are impounded for various reasons including mosquito control, waterfowl management, and some aquaculture and erosion control (Harrington and Harrington 1982; DeVoe and Baughman 1986; Montague et al. 1987; Herke et al. 1992). Due to the increasing coastal population, coastal areas have more recently been developed for residential and commercial purposes (i.e., housing developments, golf courses, tourism attractions, infrastructure), resulting in the creation of more managed impoundments (e.g., stormwater retention ponds and golf course ponds).

Both natural and managed estuarine habitats, such as marsh pools and impoundments, are important for a variety organisms, including but not limited to waterfowl, fishes, reptiles, and different types of vegetation (Miglarese and Sandifer 1982; DeVoe and Baughman 1986; McGovern and Wenner 1990, Weber and Haig 1996; Robison and Jennings 2014; Carswell et al. 2015; Mace et al. 2018). Many impoundments within the southeastern United States are currently managed for waterfowl and migrating shorebirds; however, many fish species, such as Atlantic croaker (Micropogonias undulatus), spot (Leiostomus xanthurus), tarpon (Megalops atlanticus),
and inland silverside (*Menidia beryllina*) utilize these habitats at various points throughout their life, but particularly during early life stages (Gilmore et al. 1982; McGovern and Wenner 1990; Stevens et al. 2006; Robinson and Jennings 2014; Carswell et al. 2015). Thus, understanding how and when fishes use impoundments, how use of impoundments compares to natural habitats, and how use of impoundments affects other life history characteristics (i.e., recruitment, mortality, growth) is warranted.

Tarpon use natural and managed marsh habitats as juveniles before emigrating to marine habitats, and are frequently found in impoundments and ponds (Zerbi et al. 2005; Robinson and Jennings 2014; Mace et al. 2018; Cianciotto et al. 2019; Wilson et al. 2019), as well as in pools in natural salt marsh and mangrove habitats (Crabtree et al. 1995; Zerbi et al. 2001; Stein et al. 2016; Mace et al. 2018). Managed marsh impoundments are designed to maintain consistent environmental conditions (e.g., water salinities, depths), with water control structures limiting tidal influence and water flow according to seasonal operation protocols, but on occasion external forces (e.g., severe storms, drought) can combine with internal operations (e.g., low or no water flow) to create extreme water temperature, salinity, or dissolved oxygen conditions (McGovern and Wenner 1990; Robison and Jennings 2014). Tarpon are capable of withstanding these variable and potentially harsh environmental conditions (Robins 1977; Crabtree et al. 1995; Geiger et al. 2000). Marsh impoundments offer juvenile tarpon an abundance of prey (Poulakis et al. 2002; Stevens et al. 2006; Robison and Jennings 2014) and harsh environmental conditions may limit interspecific competition. Similar to marsh impoundments, natural marsh pools are typically shallow (< 0.5 m deep), isolated during
low tide, and also experience extreme water temperatures and salinities (Rickards 1968; Dahlberg 1972; Mace et al. 2019).

In South Carolina, 27% of the population lives within coastal areas (NOAA 2020b), where managed marsh impoundments and natural marsh pools are common estuarine features. These areas were traditionally developed for rice cultivation and mosquito control, converting natural salt marsh and tidal freshwater swamp habitats into impoundments by creating dikes with water control structures (Tiner 1977; DeVoe and Baughman 1986; McGovern and Wenner 1990); however, since the 1990s these areas and other coastal lands have been developed for stormwater retention and irrigation resulting in more than 14,000 storm water ponds along the South Carolina coast (Drescher et al. 2011; Smith 2012; Cotti-Rausch et al. 2019). About 28,000 ha of managed marsh impoundments still exist in South Carolina (Miglarese and Sandifer 1982; DeVoe and Baughman 1986), but the importance of these manmade habitats for juvenile tarpon has received little to no attention. Despite tarpon being documented in several managed marsh impoundments across South Carolina (McGovern and Wenner 1990; Nichols 1994; Robison and Jennings 2014; Mace et al. 2018), only one study has examined their habitat use patterns in detail (Mace et al. 2018). To date, no studies have examined tarpon use of managed and natural estuarine habitats concurrently during their period of estuarine residency in South Carolina; such an approach would allow for a better understanding of the nursery function of these varied habitats. Therefore, my study objectives were to (1) examine juvenile tarpon recruitment and arrival duration to three estuarine locations, one natural and two managed marsh sites, in South Carolina, and (2) compare length structure of tarpon between these habitat types. Information gained from
this study will contribute to our understanding of juvenile tarpon estuarine habitat use and the role of natural and managed marshes as nursery habitats in this region.

Methods

Study sites

Study sites were located within the North Inlet estuary, at the Tom Yawkey Wildlife Center Heritage Preserve, and at Kiawah Island (Figure 1), spanning approximately 125 km of the South Carolina coast. I sampled five natural marsh pools in the North Inlet estuary (hereafter NIE; Figure 1; Table 1), which is a barrier island bounded system (3,300 ha in area) dominated by *Spartina alterniflora* marsh with creeks, intertidal flats, oyster reefs, and open water interspersed throughout (Allen et al. 2014). I sampled four managed marsh impoundments at the Tom Yawkey Wildlife Center Heritage Preserve (hereafter Yawkey; Figure 1; Table 1), a 6,200-ha research area and wildlife refuge maintained by the South Carolina Department of Natural Resources with *Spartina alterniflora* marsh habitat consisting primarily of marsh impoundments managed seasonally for waterfowl migration and reproduction. The managed marsh impoundments are typically shallow (<1 m), except near water control structures, and have salinity profiles that range from fresh to saline. I sampled four managed marsh impoundments located on Kiawah Island (hereafter Kiawah; Figure 1; Table 1), a 3,470-ha coastal barrier island developed and maintained as a private golf resort with a mix of natural *Spartina alterniflora* marsh with creeks, intertidal flats, and managed marsh impoundments that are situated along roads within the highly developed residential communities and along the golf courses on the island. Two impoundments were surrounded by short *Spartina alterniflora* marsh, and two were surrounded by golf course fairways and residential development. The impoundments varied in size, depth (0.5 m - >
3 m), salinity profiles, and shore vegetation. Salinity profiles ranged from fresh to saline; however, most impoundments connect to one another forming a mosaic across the island, and all had direct connections to the bordering salt marsh. Sampling in Kiawah and Yawkey was conducted near metal and wooden water control structures and access points of impoundments because fish are known to congregate near these areas (Kimball et al. 2015, 2017). These locations were chosen because NIE and Yawkey are known to have juvenile tarpon (Mace et al. 2018), there is limited information on tarpon elsewhere in South Carolina, access is limited in other areas where juvenile tarpon have been reported, and fishing guide services have reported juvenile tarpon within managed marsh impoundments on Kiawah Island (M. Kimball, unpublished data).

**Field Collections**

Field collections for juvenile tarpon occurred weekly during July – November 2019 using cast nets (1.8 m diameter, 6 mm mesh). All locations within a study site were sampled on the same day during daylight hours. Sampling at NIE occurred during daytime low tide when marsh pools were disconnected from adjacent habitats and water bodies. Marsh pools in NIE were sampled during multiple high tide events to test the assumption that tarpon were most vulnerable to sampling during low tide, and the tarpon SL structure in marsh pools did not vary based on tides (i.e., larger juveniles were not moving into marsh pools during high tide). Sampling at Yawkey and Kiawah was independent of tidal stage because water levels were held constant, while allowing for some water exchange by the water control structures at each impoundment. Three replicate casts were made at each sampling location. If juvenile tarpon were caught, they were kept in an aerated holding cooler until all casts were thrown at a site and then standard length (SL, mm) was recorded prior to release. Water temperature (WT, °C) and
salinity were recorded using a YSI pro2030 at each sampling location after sampling was completed.

Data Analyses

All juvenile tarpon data were analyzed using Microsoft Excel (Microsoft, Redmond, Washington) and R statistical software within RStudio (2020, version 1.3.1073; R Core Team 2019, version 3.6.1). For statistical purposes, all locations from a study site were considered subsamples and combined to calculate one mean catch-per-unit-effort (CPUE), and one set of water quality measurements for each sampling date. Data from the Yawkey and Kiawah sites were pooled to make comparisons between natural (NIE) and managed (Yawkey and Kiawah pooled) habitats. Descriptive statistics of tarpon lengths (mean ± standard deviation, minimum, maximum) were calculated for both habitat types. Weekly, CPUE was calculated by dividing the number of tarpon caught by the total number of casts thrown per habitat type, per weekly sampling event (e.g., the total number of tarpon caught divided by the total number of casts in high marsh pools in a given week). Analysis of covariance (ANCOVA) was used to determine if tarpon length (mm SL) differed between habitat types over time. The response variable was tarpon SL, the covariate was Julian day, and the independent variables were habitat type (natural or managed) and the interaction between habitat type and Julian day. Residual and Q-Q plots were examined to determine if these data met the assumptions of linear models or if transformations were necessary.

Results

I collected 102 age-0 tarpon, ranging in length from 36 – 333 mm SL (Figure 2). Tarpon SL structure did not vary between tidal stages at NIE during three paired sampling events and the number of tarpon caught at low tide was twice the number
caught at high tide (low tide ($n = 29$) mean SL $\pm$ SD = 68 $\pm$ 24 mm; high tide ($n = 13$) mean SL $\pm$ SD = 80 $\pm$ 30 mm), thus validating that sampling during low tide was sufficient for this study. Tarpon were collected during July – November. However, the timing of tarpon collections differed between habitat types; peak tarpon catches in natural marsh pools occurred about a month earlier than in managed marsh impoundments (Figure 3). Sixty-eight tarpon were collected from July 15 - November 6 within natural marsh pools at NIE, and thirty-four tarpon were collected from August 15 - October 24 within managed marsh impoundments at Yawkey and Kiawah. Tarpon from impoundments (Yawkey and Kiawah; mean $\pm$ SD = 253.9 $\pm$ 41.6 mm SL) were longer than those collected from marsh pools (NIE; mean $\pm$ SD = 65.4 $\pm$ 20.2 mm SL) and differences in length increased over time (interaction of habitat type and Julian day $F = 31.35$, $df = 1$, $p < 0.001$). Mean tarpon length from impoundments increased from 180 mm SL in August to 289 mm SL in October, whereas the mean tarpon length in marsh pools (65 mm SL) was relatively consistent (Figure 4).

Water quality varied among sample locations but followed seasonal patterns typical of the study region (Figure 5). Temperatures at NIE ranged from 10.8 – 37.7 °C, Yawkey from 10.6 – 32.2 °C, and at Kiawah from 19.5 – 33.8 °C (Figure 5). Salinity also varied among study sites, with the highest salinities at NIE (19.9 – 38.2 ppt), lower salinities at Kiawah (13.0 – 25.7 ppt), and the lowest salinities at Yawkey (11.0 – 22.9 ppt; Figure 5). Throughout the study duration, NIE had the highest salinity (~ 38 ppt) during summer, and Yawkey had the lowest (~ 11 ppt) during late spring (Figure 5).
Discussion

Peak densities of juvenile tarpon in both natural marsh pools and managed impoundments were observed during mid-summer through fall (mean natural marsh pool WT ± SD = 25.7 ± 4.5 °C; mean managed impoundment WT ± SD = 25.9 ± 3.6 °C), which is common for this species. The timing of these peak densities matches those observed in an earlier study in some of the same NIE marsh pools and Yawkey impoundments, where tarpon were collected during September 2015 through November 2016, although no tarpon was caught after November or detected after early December (Mace et al. 2018). The peak juvenile tarpon densities observed in this study also coincide with peak densities observed in Puerto Rican (Zerbi et al. 1999) and Louisianan estuaries (Stein et al. 2016); however, recruitment duration in Puerto Rico can be more protracted (June – February; Zerbi et al. 1999). The timing of peak densities appears to be later in the western Atlantic compared to the eastern Atlantic (i.e., Nigeria), where juvenile tarpon recruitment is reported to peak earlier (February – April; Anyanwu and Kusemiju 2008). I did not capture any tarpon during sampling for this study in late November (WT ≈ 10 °C), but tarpon were observed in late November while sampling for other ongoing tarpon work in managed marsh impoundments at Kiawah and Yawkey (M. Kimball, unpublished data). The decrease in juvenile tarpon presence in November in South Carolina is likely due to decreases in water temperature (Mace et al. 2017, 2018), and suggests tarpon leave for waters with more suitable water temperatures, which has also been overserved in Louisiana (Stein et al. 2016).

The juvenile tarpon encountered were most likely age-0 because (1) winter water temperatures in most of South Carolina are below the mean minimum threshold for survival (13 °C) except in unique habitats which provide thermal refuges (e.g., thermally
stratified upland pond [Mace et al. 2020]), (2) no tarpon were collected at any sites until mid- to late-summer (suggesting no overwintering), (3) only one tarpon >age-0 has been collected from a natural marsh pool in NIE or Yawkey (that fish was a tagged fish that migrated from a unique habitat where overwintering does occur), and (4) despite extensive sampling and examination of scales and otoliths from tarpon collected from natural marsh pools in NIE and managed marsh impoundments at Yawkey, a tarpon >age-0 has never been observed in these habitats (excluding the fish mentioned in 3; G. Elmo, unpublished data). However, recent increases in winter water temperatures in coastal South Carolina, corresponding to climate change (Allen et al. 2008), may allow for increased overwintering of age-0 and older juvenile tarpon and in locations outside of rare and unique habitats (e.g., thermal discharges and stratified ponds). For example, Kiawah water temperatures were substantially warmer in November compared to NIE and Yawkey, and never dropped below 19.5 °C during this study (~ 6 °C greater than the mean lower lethal limit for tarpon [13 °C, Mace et al. 2017]). Reports from local guide services, videos, and photographs of juvenile tarpon that are older than age-0 (based on large body size) suggest overwintering of tarpon in golf course impoundments on the southern coast of South Carolina. Tarpon may potentially take advantage of the warmer water temperatures in southern South Carolina compared to northern South Carolina resulting in a year-round northward shift of the species range, which is not a unique phenomenon and has been previously documented for other tropical fish species such as lionfish (Pterois spp.; Grieve et al. 2016) and common snook (Centropomus undecimalis; Purtlebaugh et al. 2020). If tarpon experience range expansion due to warming coastal
waters, the importance of both natural and managed marsh habitats for this species will increase, impacting local estuarine management strategies.

The observed juvenile tarpon length distributions within managed marsh impoundments from this study (Yawkey 155 – 333 mm SL and Kiawah 193 – 280 mm SL) are similar to those observed from previous managed marsh impoundment studies within South Carolina (Nichols 1994; Mace et al. 2018). Outside of South Carolina, juvenile tarpon collected in natural marsh habitats in Louisiana from August 2010 - October 2012 were much larger (55 – <350 mm FL; Stein et al. 2016) than the tarpon observed in natural marsh pools in this study. In Cuba, juvenile tarpon (99 – 850 mm FL) were collected from June 2015 – January 2017 from several natural mangrove habitats with varying characteristics (i.e., water quality, depth, vegetation, inlets; Navarro-Martinez et al. 2020). The smaller observed tarpon (99 – 235 mm FL) from Cuba, which are similar in size to tarpon from this study, were found in habitats similar to NIE marsh pools, had varying water quality ranges, and offered refuge from predation (Navarro-Martinez et al. 2020). In Florida, juvenile tarpon 120 – >300 mm FL were collected in managed ponds September 2012 - February 2014 (Wilson et al. 2019), and in Puerto Rico, juvenile tarpon 27 – 770 mm SL were collected in a managed impoundment from September 1992 – January 1993 (Zerbi et al. 2005). The tarpon collected in Florida impoundments are likely similar in age to tarpon from South Carolina impoundments. However, the tarpon collected in Puerto Rico and the larger tarpon collected in Cuba (>410 mm FL) are likely older juveniles (age 1+), thus potentially explaining the length variation observed in those locations.
I observed no overlap in SL of tarpon collected in natural marsh pools and tarpon collected in managed marsh impoundments in multiple estuarine systems in coastal South Carolina. Similarly, there was little overlap in the SL of tarpon collected in marsh pools and managed impoundments in the North Inlet-Winyah Bay ecosystem (Mace et al. 2018). The absence of SL overlap between habitats and increasing SL of tarpon over time in managed marsh impoundments compared to the minimal change in SL over time observed for natural marsh pools suggests (1) tarpon are transient in marsh pools early in life, (2) tarpon do not enter impoundments until reaching a certain SL, (3) small juvenile tarpon are cryptic in impoundments and larger juvenile tarpon are more susceptible to capture in these habitats, or (4) a combination of (1), (2), and (3). Length differences of juvenile tarpon across habitat types has also been observed outside of South Carolina (Rickards 1968, Zerbi et al. 1999, Navarro-Martinez et al. 2020). Juvenile tarpon utilize natural marsh pools and managed impoundments at different life stages, and future studies should investigate these hypotheses to determine if size differences are due to differential growth or ontogenetic shifts in habitat use. There is a paucity of information about larger juvenile tarpon (>300 mm SL) in South Carolina because natural marsh pools and shallow managed marsh impoundments are likely not suitable habitats for these larger fish. This lack of knowledge highlights the need for specific examination of tarpon emigration from managed marsh impoundments and other nursery areas into deeper estuarine waters where they may potentially migrate to warmer waters and eventually recruit to adult populations. This study demonstrated that multiple habitat types are important for juvenile tarpon. However, there is still a lack in knowledge regarding
habitat use by older juveniles, when tarpon habitat use shifts, and how South Carolina estuaries contribute to the tarpon population.
References


impairment. Paper presented at the South Carolina Water Resources Conference, Columbia SC


### Tables and Figures

**Table 1.** Latitude and longitude of sample locations within the North Inlet estuary (NIE), Tom Yawkey Wildlife Heritage Preserve (Yawkey), and Kiawah Island (Kiawah).

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample Location</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIE</td>
<td>8</td>
<td>33° 19' 21.30&quot;</td>
<td>-79° 12' 16.50&quot;</td>
</tr>
<tr>
<td></td>
<td>8C</td>
<td>33° 19' 23.50&quot;</td>
<td>-79° 12' 16.02&quot;</td>
</tr>
<tr>
<td></td>
<td>2A</td>
<td>33° 20' 03.70&quot;</td>
<td>-79° 11' 44.30&quot;</td>
</tr>
<tr>
<td></td>
<td>2B</td>
<td>33° 20' 04.90&quot;</td>
<td>-79° 11' 45.52&quot;</td>
</tr>
<tr>
<td></td>
<td>3B</td>
<td>33° 19' 56.90&quot;</td>
<td>-79° 11' 53.99&quot;</td>
</tr>
<tr>
<td>Yawkey</td>
<td>Rockfish Bridge</td>
<td>33° 13' 58.81&quot;</td>
<td>-79° 13' 38.43&quot;</td>
</tr>
<tr>
<td></td>
<td>Fort Trunk</td>
<td>33° 14' 57.69&quot;</td>
<td>-79° 13' 41.16&quot;</td>
</tr>
<tr>
<td></td>
<td>Rice Paddy One</td>
<td>33° 12' 32.70&quot;</td>
<td>-79° 16' 05.12&quot;</td>
</tr>
<tr>
<td></td>
<td>Rice Paddy Three</td>
<td>33° 12' 15.94&quot;</td>
<td>-79° 16' 04.42&quot;</td>
</tr>
<tr>
<td>Kiawah</td>
<td>Willet Pond</td>
<td>32° 36' 57.38&quot;</td>
<td>-80° 01' 31.57&quot;</td>
</tr>
<tr>
<td></td>
<td>Ibis Pond</td>
<td>32° 36' 44.85&quot;</td>
<td>-80° 02' 43.40&quot;</td>
</tr>
<tr>
<td></td>
<td>Pintail Pond</td>
<td>32° 36' 57.65&quot;</td>
<td>-80° 03' 00.26&quot;</td>
</tr>
<tr>
<td></td>
<td>Bass Pond</td>
<td>32° 36' 45.67&quot;</td>
<td>-80° 05' 27.43&quot;</td>
</tr>
</tbody>
</table>
**Figure 1.** Study sites (closed circles) at North Inlet estuary (NIE), the Tom Yawkey Wildlife Center Heritage Preserve (Yawkey), and Kiawah Island in South Carolina along the southeastern US Atlantic coast. The cities (closed diamonds) of Georgetown, SC and Charleston, SC are shown for reference.
Figure 2. Length-frequency distributions of juvenile tarpon (*Megalops atlanticus*) collected during July - November 2019 in South Carolina from North Inlet estuary natural marsh pools (Natural, black bars), and from managed marsh impoundments at Tom Yawkey Wildlife Center Heritage Preserve and Kiawah Island (Managed, gray bars).
Figure 3. Mean catch-per-unit-effort of juvenile tarpon (*Megalops atlanticus*) collected from July - November 2019 in South Carolina from North Inlet estuary natural marsh pools (Natural, gray circles), and from managed marsh impoundments at Tom Yawkey Wildlife Center Heritage Preserve and Kiawah Island (Managed, open circles).
Figure 4. Standard lengths of juvenile tarpon (*Megalops atlanticus*) caught during July - November 2019 in South Carolina from natural marsh pools (*n* = 68) in the North Inlet estuary (Natural, gray circles), and from managed marsh impoundments (*n* = 34) at Tom Yawkey Wildlife Center Heritage Preserve and Kiawah Island (Managed, open circles).
Figure 5. Weekly mean water temperatures (°C) and salinities observed during July - November 2019 collections for juvenile tarpon (*Megalops atlanticus*) for North Inlet estuary (NIE, gray circles), Kiawah Island (Kiawah, open circles), and Tom Yawkey Wildlife Center Heritage Preserve (Yawkey, triangles).
Chapter 2: Validity of daily and annual age estimation and back-calculation methods for early life stages of a subtropical-tropical species, the tarpon (*Megalops atlanticus*)

Abstract

Understanding early life history processes (e.g., growth) for fishes is critical because they can affect other life history characteristics (i.e., age at reproduction, migration). Additionally, habitats used by young fishes are often the target of conservation and restoration efforts and growth has been proposed as a metric for evaluating the quality of habitats. Accurate estimates of growth rely on the validation of aging and back-calculation methods, which in turn allow for informed fisheries management decisions. I used oxytetracycline to chemically mark juvenile tarpon (*Megalops atlanticus*) for an annual increment validation study, a controlled back-calculation validation study, and a controlled daily increment periodicity study.

Oxytetracycline marks were realized on all the otoliths of recaptured tarpon (*n* = 22), prior to a newly formed annulus, validating true age and that one annulus is deposited yearly. However, annuli in scales were more easily identified by readers, thus leading to more accurate and precise estimates of age from scales (100% accuracy of age estimates for age-1 fish) compared to otoliths (88% accuracy). Marginal increment analyses indicated that tarpon annuli are deposited in the spring (March-April) on juvenile tarpon scales and otoliths. Back-calculated lengths from otoliths were significantly different compared to measured lengths, and bias of length estimates differed between months. Daily increment estimates from otoliths were relatively accurate during September but
not during October, leading to mean error of 21 d (37% error) across the 56-d study period. Results from this study highlight the importance of validating age estimates for juvenile fishes, particularly for subtropical and tropical species that have protracted spawning seasons.
Introduction

Understanding early life history processes (i.e., growth, mortality, ontogenetic diet shifts) of fishes is critical for interpreting recruitment variability and how changes in these processes may affect other life history characteristics (i.e., migration, reproduction, trophic structure) later in life (Houde 1987; Miller et al. 1988; Ludsin and DeVrois 1997; Hales and Able 2001). Variation in growth rates within the first year of life may provide fish with faster growth or fish with earlier hatch dates advantages over fish with slower growth or fish with later hatch dates (Ludsin and DeVrois 1997; Wilson and Meekan 2002). Having more access or earlier access to food resources or a competitive release based on size because of earlier ontogenetic shifts to piscivorous diets may increase survival (Olson 1996; Ludsin and DeVrois 1997; Wilson and Meekan 2002; Graeb et al. 2005; Stige et al. 2019). Faster first year growth or increased body size has been associated with increased survival and more successful recruitment for several species including largemouth bass (*Micropterus salmoides*; Olson 1996; Ludsin and DeVrois 1997; Garvey and Stein 1998), walleye (*Sander vitreus*; Madenjian et al. 1996), black sea bass (*Centropristis striata*; Hales and Able 2001), smallmouth flounder (*Etropus microstomus*; Hales and Able 2001), and red drum (*Sciaenops ocellatus*; Stewart and Scharf 2008). Differences in first year growth has also been linked to variation in age at maturity, where faster growth can be related to earlier sexual maturation (Hutchings 1993; Morgan and Colbourne 1999; Brophy and Danilowicz 2003; Rowell et al. 2008).

Understanding growth during the early life stages of fishes (i.e., larvae and juveniles) relies on accurate and precise age estimation methods. However, recognizing increments (daily or annual) can be difficult for larval and juvenile fish (particularly the
first annulus), and difficult for tropical species (Victor and Brothers 1982). In some species that are fast growing and short lived, like the European anchovy (*Engraulis encrasicolus*) and the horse mackerel (*Trachurus trachurus*), there may be multiple translucent and opaque zones deposited during the first years of life (i.e., false annuli), resulting in overestimation of age for young fish (Waldron and Kerstan 2001; Basilone et al. 2020). Crane et al. (2020) observed a U-shaped relationship between the coefficient of variation (CV) of age estimates and known age of the moderately long-lived Muskellunge (*Esox masquinongy*), and readers tended to overestimate age of young individuals (ages 1-3) collected from a southern population. Difficulty in discerning increments and subsequent age estimation error may be caused by counting false annuli, missing hidden annuli, or misinterpreting metamorphic marks (Victor and Brothers 1982; Stevenson and Campana 1992). For example, readers commonly overestimated young, known-age walleye because of mistakenly counting the edge of the structure core or lumen as an annulus (Dembowski et al. 2019). Because proportional growth is frequently greatest during the first few years of life, a one-year error in early age estimation can have substantial effects on our understanding of the growth of fishes.

For many temperate and freshwater fishes, young juveniles (i.e., ages 0-1) can be identified and classified into cohorts based on similar length (Staggs and Otis 1996; Hales and Able 2001). However, identifying cohorts based on length of fish with protracted or year-long spawning seasons (e.g., subtropical and tropical marine fishes) may be difficult. The difficulty in identifying cohorts by length for certain subtropical and tropical species highlights the importance of validating age estimates from hard structures (e.g., otoliths and scales), which are used to study growth of early life stages of
fishing. Biologists conducting age validation studies focused on freshwater fishes have been able to take advantage of stocking and recapture of known-age fish because freshwater systems are more closed than marine environments, anadromous fish often return to natal waters, and known age fish are easily obtainable through aquaculture programs (Buckmeier et al. 2017). However, most marine systems are open, and many marine species are long lived and have wide ranges or are considered migratory species, which makes recapture of stocked known-age fish difficult in these systems.

Despite being an iconic sportfish there have been few studies examining the accuracy of age estimates for tarpon (Megalops atlanticus), and most focused on otoliths (Crabtree et al. 1995; Andrews et al. 2001). Only one study examined the accuracy of age estimates for juvenile tarpon (Zerbi et al. 2001) and focused on validating daily increment deposition in sagittal otoliths of age-0 fish. These previous studies on tarpon had high rates of unreadable otoliths, lacked recapture of known-age fish, relied on captively reared fish (Crabtree et al. 1995), or had large age uncertainties (Andrews et al. 2001). Validation of tarpon ages is challenging because they are migratory (Griffen et al. 2018) and they have not been successfully cultured for stocking purposes. Similar to many other tropical and sub-tropical marine fishes, tarpon have an extended spawning period that lasts from April – August, and potentially year-round near the equator (Cyr 1991; Crabtree et al. 1995, 1997). Recent conservation and restoration initiatives have focused on identifying, evaluating, and restoring tarpon nursery habitats (Adams et al. 2014, 2019), and have identified growth as a metric to evaluate habitat quality. However, protracted spawning by tarpon complicates investigations attempting to understand relationships between habitat characteristics and juvenile tarpon growth. For example,
presumed age-0 juvenile tarpon exhibit a large range of lengths during late fall (65 - >300 mm, standard length) throughout the southeastern U.S., which varies across locations and between habitats within locations (Mace et al. 2018; Kurth et al. 2019; P. Stevens, unpublished data). However, limited information on the validity of age estimation methods for young tarpon precludes making definitive conclusions about questions related to discrepancies in length across locations and habitats within locations. For instance, are presumed age-0 truly age-0? Are differences in length a function of hatch date and/or ontogenetic shifts? The objectives of this study were to (1) compare the accuracy and precision of age estimates for juvenile tarpon (ages 0-2) from otoliths and scales to determine which structures provide the most accurate and precise estimates of age, (2) determine the timing of annulus formation, (3) estimate the accuracy of back-calculation of length-at-age methods from hard structures, and (4) compare the accuracy and precision of daily age estimates from known-age fish.

Methods

Annual Age Validation

Juvenile tarpon were collected from the North Inlet - Winyah Bay estuarine system (Georgetown, South Carolina) in fall 2018 with cast nets (1.8 m diameter, 6mm mesh) and hook-and-line (#6 J hook). For each fish, standard length (SL mm) and weight (g) were measured, and scales were collected from below the midpoint of the dorsal fin – three from each of the two rows above the lateral line (Figure 6; Mace et al. 2020). Fish were then tagged (intraperitoneally) with an Oregon RFID 8 mm FDX-B passive integrated transponder (PIT, Oregon RFID, Portland, Oregon, USA) to allow for unique identification of individuals. After PIT-tagging, tarpon were injected (intraperitoneally) with oxytetracycline (OTC, Duramycin-100 containing oxytetracycline hydrochloride at
100 mg OTC/mL, Durvet, Blue Springs, Missouri, USA) at 0.075 mg OTC/g of fish to establish a fluorescent mark in hard structures, which served as a time stamp (Alhossaini and Pitcher 1988). Tarpon were then placed in a holding tank for 24 hours for observation prior to being stocked into an upland pond with suitable temperatures for tarpon to live year-round (Mace et al. 2020). Additionally, six fish were injected with OTC using the same methods as above to evaluate the incorporation of the OTC into tarpon hard structures. Another group of fish ($n = 6$) did not receive OTC to test the assumption that tarpon do not have natural fluorescent marks occurring within their hard structures (Klumb et al. 2001).

The majority of the tarpon marked with OTC and stocked were presumed age-0 because (1) winter water temperatures in the study area are below the minimum threshold for survival (~13 °C) except in unique habitats suitable for overwintering (e.g., thermally stratified pond [Mace et al. 2017, 2020]), (2) tarpon are not present in South Carolina until mid- to late-summer (Mace et al. 2018), (3) despite extensive sampling and examination of scales and otoliths only one tarpon >age-0 has been collected from habitats where the majority of tarpon were sampled (that fish was a tagged fish that migrated from a unique habitat where overwintering does occur). Assuming that most stocked fish were age-0 and had not completed their first growing season at the time of stocking suggested no annuli were formed at the time of capture and stocking. Therefore, these fish were used to validate age and timing of annual increment formation. Some tarpon (6 out of 23) were collected from a unique habitat where overwintering does occur (Mace et al. 2020), so their true age was unknown. Thus, age estimates for these
individuals could not be validated, but I was able to use their structures to investigate annual increment formation.

Tarpon were recaptured from the stocked pond during November 2019, using hook-and-line and a trammel net. Recaptured tarpon were identified using their PIT tag number, measured (SL mm), and then euthanized in an ice bath according to Institutional Animal Care and Use Committee protocols (Coastal Carolina University IACUC protocol number 2018.04) prior to removing sagittal otoliths and scales from the fish’s left side (Figure 6). Otoliths were set in epoxy, sectioned, and fixed to glass slides with Crystalbond (Aremco Products Inc., Valley Cottage, New York, USA), and then sanded and polished for viewing (Fischer and Koch 2017; Long and Grabowski 2017). Annuli in otoliths were defined by crenulation along the ventral ridge of the succal groove (Crabtree et al. 1995) and an associated thin dark line (opaque zone; Figure 7). Scales were cleaned and stored in individual marked envelopes until viewed for age estimation using a zoom stereo microscope (OLYMPUS SZX-10, Olympus Corporation, Tokyo, Japan), Olympus DP-27 camera, and Olympus Cellsens software. Annuli in scales were identified by “cutting over” (Wright et al. 2015), and presence of a “scale within a scale method” (Figure 7; McInerny 2017).

Examination of all hard structures was completed by three independent readers that were only provided the fish’s unique identification number to prevent bias associated with knowing the length of a fish (Geffen 1992; Campana 2001). Ages were assigned based on the number of annuli present. Each reader examined one type of structure at a time, thus eliminating potential bias associated with
inferring age from concurrent examination of multiple structures from an individual. If there were any discrepancies among reader age estimates for a structure, readers examined the structure together to form a concert age estimate, which was then used as the final age estimate for that structure. After ages were estimated, otoliths and scales were viewed using ultra-violet (UV) light to determine if a single annulus was formed during the year tarpon were at-large. Because an OTC mark was not realized in scales, scales collected during initial capture were compared to scales collected one year later to evaluate if a single annulus formed.

Age and length data for juvenile tarpon were analyzed using R (R Core Team 2019, version 3.6.1), and the FSA package (Ogle 2020; Function: agePrecision, version 0.8.30). Descriptive statistics were calculated for (1) lengths of recaptured fish (mean ± standard deviation, minimum, maximum), (2) percentage of samples that had 100% agreement among readers per structure, (3) percentage of samples with consensus agreement per structure (agreement between at least two readers), (4) mean percentage of estimated ages that were correct (calculated per reader and then averaged across readers) per structure, (5) percentage of age-0 fish aged correctly (calculated for final ages based on concert viewing), (6) percentage of age-1 fish aged correctly (calculated for final ages based on concert viewing), (7) percentage of estimates with agreement between structures (calculated for structures pulled at recapture), and (8) percentage of otoliths with a single annulus formed after the OTC mark. Coefficients of variation (100×SD/mean estimated age) were used to estimate precision of age estimates for each structure.

Marginal Increment Analysis
Juvenile tarpon \((n = 30)\) were collected during late summer 2019 using the same methods as described above. Fish were stocked in a holding pen (3 m long x 2 m wide x 1 m deep) within an upland pond in the North Inlet estuary (see Mace et al. 2020 for habitat description). Two to five tarpon were haphazardly sampled monthly from the holding pen between January and June 2020 to evaluate the timing of tarpon annuli formation. Juvenile tarpon were only sampled from January - June because previous research from Florida reported that annuli were formed in tarpon otoliths from April through June, and all otoliths had formed annuli by June (Crabtree et al. 1995). Given that water temperatures in most habitats drop below tarpon lower lethal limits (~13 °C) during late November through early April in northern South Carolina (Mace et al. 2017, 2020), the upland pond where tarpon were stocked is likely representative of the type of unique habitat tarpon might overwinter in at the northern extent of their distribution, but is potentially different than habitats in Florida. Half of the fish died in the pen, so sample size was supplemented by collecting tarpon from the upland pond where the holding pen was located. After tarpon were euthanized, hard structures were removed, processed, and aged as described for annual age validation.

Marginal increments \((\text{MI, mm})\) were calculated by measuring the distance from the most recent annulus to the edge of the structure, if no annulus was present the distance from the core to the edge of the structure was measured. Measurements were taken along the dorsal ridge of the succal groove on otoliths and along the dorsal lateral axis on scales. When a new growth increment begins to form, the measured MI will approach 0, or reach a minimum in MI size, signifying the timing of annulus formation (Buckmeier et al., 2017). The monthly examination (January - June 2020) of hard
structures occurred as tarpon began a new growing season (Mace et al. 2020). Therefore, an annulus should have been deposited and MI measurements should have decreased to a minimum (∼0) during the study period. Descriptive statistics for tarpon lengths (mean ± standard deviation, minimum, maximum) were calculated. Monthly mean MI measurements with 95% confidence intervals were used to visualize annulus formation trends.

**Back-Calculation and Daily Increment Validation**

During summer 2019, age-0 tarpon \(n = 25\) were collected, using the same methods as described for annual age validation, to conduct an 8-week (56 d) controlled back-calculation and daily increment validation study. Tarpon were weighed (g), measured (mm SL), PIT-tagged (8 mm), and marked with OTC (0.075 mg OTC/g of fish), and then placed in holding tanks (>284 L) in a screened (outdoor) seawater laboratory at the University of South Carolina Baruch Marine Field Laboratory, Georgetown, SC, where tanks had a constant flow of ambient seawater from a nearby subtidal creek in the North Inlet estuary. During September 2019, the 25 age-0 tarpon were moved from holding tanks and placed in a holding pen within the upland pond, mentioned above, for 7 days to ensure survival during Hurricane Dorian (which impacted the South Carolina coast in late August and early September). After the hurricane, tarpon were returned to the wet lab holding tanks until the end of the study. Because the holding tanks were in the screened (outdoor) seawater laboratory tarpon experienced a natural photoperiod and environmental conditions. After four weeks (28 d), fish were measured (mm SL), weighed (g), and injected with OTC (0.075 mg OTC/g of fish) again. After the second marking with OTC, fish were placed back in the holding tanks for an additional four weeks (28 d). Then, tarpon were measured (mm SL), euthanized, and hard structures
were removed for back-calculation of length-at-age and daily increment validation. Hard structures were cleaned and prepared using the same methods as in the annual age validation portion of this study.

Images of hard structures were viewed and captured using a compound microscope (OLYMPUS BX51) equipped with a UV light and filter (475 nm excitation, 515 nm emissions barrier), Olympus DP74 camera, and Olympus Cellsens software. Oxytetracycline marks were not realized on scales, therefore, scales were not used for back-calculation purposes. No previous studies have determined the best axes to use for making measurements to back-calculate length-at-age for tarpon otoliths. Therefore, otolith radius measurements (mm) were taken along the dorsal and ventral ridges of the succal groove, then linear regressions were used to determine which axis had the strongest relationship between otolith radius and fish length (mm SL; Figure 8). Back-calculation measurements were taken from the center of the structure (i.e., the structure’s core), to the edge of structure, and to the inside edge of each OTC mark ($n = 2$) incorporated into the structure (Figure 9). Next, back-calculated lengths were estimated with R using the biological intercept model (function: backCalc; RFishBC package, version 0.1.9000; Ogle, 2019) for otoliths. The biological intercept model corrects for the point at which fish and calcified structure growth becomes proportional (Campana 1990), and is defined by:

$$L_i = L_c + [(L_c - L_0)(S_i - S_c)]/(S_c - S_0)$$

where $L_i$ is back-calculated tarpon SL at age $i$, $L_c$ is tarpon SL at capture, $L_0$ is the correction factor for SL, $S_i$ is otolith radius at age $i$, $S_c$ is otolith radius at capture, and $S_0$ is the correction factor for the otolith radius because otolith size is disproportionate to
fish size (Campana 1990). No previous research has looked at otolith growth during and after metamorphosis for Atlantic tarpon. However, somatic and otolith growth becomes uncoupled when leptocephali shrink and otoliths continue to grow during metamorphosis for Pacific tarpon (*Megalops cyprinoides*; Tzeng et al. 1998), and likely does for Atlantic tarpon as well. Therefore, the otolith correction factor ($S_0$) was set to 0.077 mm, which was the mean Atlantic tarpon leptocephalus otolith radius found by Zerbi et al. (2001). The length correction factor ($L_0$) was set to 13 mm, the smallest size tarpon reach as a stage II leptocephalus, when they shrink in size before resuming positive growth as a stage III leptocephalus (Mansueti and Hardy 1967) because the length at which otolith and somatic growth are recouple for Atlantic tarpon is unknown.

To validate daily increment formation in juvenile tarpon otoliths, two independent readers estimated the number of increments between the two OTC marks and the last OTC mark to the structure edge (Figure 9). Otoliths were viewed using the same microscope and software used in the back-calculation validation portion of this study. The readers made three separate counts between the first OTC mark and the second OTC mark (28 d), the second OTC mark and the structure edge (28 d), and from the first OTC mark to the structure edge (56 d). The reader average of the three counts was reported as the number of increments formed per section. All three sections were examined to see if daily increments counts were accurate between markings and if counts were accurate up to the structure edge to evaluate if accuracy changed over time as fish aged.

Descriptive statistics were calculated for (1) lengths of recaptured fish (mean ± standard deviation, minimum, maximum), (2) percentage of samples that had 100% agreement between readers, (3) mean percentage of estimated ages that were correct
(calculated per reader and then averaged between readers) between marking events and for the entire study duration, (4) percentage of samples within a three day range of the correct age (calculated per reader and averaged between readers) between marking events and for the entire study duration, (5) percentage of samples within a one day range of the correct age (calculated per reader and averaged between readers), and (6) the percent mean and percent mean absolute difference in days off from the correct number of days. Coefficient of variation (100×SD/mean estimated age) was used to estimate precision of age estimates.

Back-calculated lengths from each OTC mark were compared to length data from each marking date, and paired t-tests were used to evaluate if back-calculated lengths differed from measured lengths. Finally, the effects of (1) back-calculation error and (2) age estimation error on estimated growth rates were investigated by comparing these estimated growth rates with measured growth rates. Mean daily estimated growth rates were calculated between OTC marking periods and for the entire study duration. Growth rates were calculated using:

\[ G = \frac{(SL_f - SL_i)}{\Delta t}, \]

where \( G \) is growth as a daily rate, \( SL_f \) is the final SL, \( SL_i \) is the initial SL, and \( \Delta t \) is the change in time (time between marking events). Negative growth rates \( (n = 1; 0.4\% \text{ of samples}) \) were not excluded in the calculation of mean daily growth rates, even though they were likely due to measurement error or caudal fin damage, because excluding negative rates may introduce positive bias if positive measurement errors were also made. Paired t-tests were used to make comparisons between estimated and measured growth rates.
Results

Annual Age Validation

Twenty-three OTC-marked juvenile tarpon were recaptured during fall 2019 (Table 2). Final age estimates for the 23 fish ranged from 0-2 y \( (n = 20, 1, 2, \) respectively) from structures pulled at initial capture. Final age estimates from structures pulled at recapture ranged from 1-2 y \( (n = 20, 3, \) respectively) based on scales and 1-3 y \( (n = 17, 4, 1, \) respectively) based on otoliths. One-hundred percent agreement among readers was highest for scales pulled at initial capture (82.6%), followed by scales pulled at recapture (69.6%), and was lowest for otoliths (54.5%; Table 3). Consensus agreement was highest for scales pulled at initial capture (100%), followed by scales pulled at recapture (95.7%), and lowest for otoliths (86.4%; Table 3). One hundred percent of age 0-1 fish were aged correctly using scales. Otoliths were not available for age-0 tarpon, but 88.2% of age-1 individuals were age correctly using otoliths (Table 3). Age estimates based on scales and otoliths were the same for 77.3% of fish. Overall, CV was lowest for scales pulled at initial capture (14.3%), followed by scales pulled at recapture (21%), and then otoliths (27.4%). One otolith was deemed unreadable because a consensus age was unable to be determined and was not included in the analyses.

Six of the 23 captured fish were collected and tagged from a habitat where overwintering is possible (Mace et al. 2017, 2020); therefore, true age of these fish was unknown (because they may have been > age-0 at the time of initial capture). However, for the remaining fish \( (n = 17) \) true age was known because they were captured in areas without suitable overwintering conditions. Post-hoc examination of otoliths indicated OTC marks were visible on all otoliths of recaptured tarpon \( (n = 22) \) prior to a newly formed annulus. Thus, validating true age for the 17 age-0 fish and that the fish with
unknown true age were +1 y at the time of recapture and validating that one annulus is deposited annually. However, readers were unable to age two fish correctly. Because OTC marks were not visible on scales, reader age estimates from scales pulled at initial capture versus recapture were used to validate that all known-age fish ($n = 17$) deposited an annulus and were +1 y at recapture, and that three of the six unknown age fish were +1 y at recapture. No visible fluorescent marks were detected in the six control fish, indicating tarpon do not have any natural fluorescence within their hard structures. Oxytetracycline marks were incorporated and visible after 24 hrs in the six separately marked OTC control fish.

**Marginal Increment Analysis**

Twenty-four age 0-2 tarpon were collected from January through June 2020 for MI analyses (Table 2), and monthly sample size varied from two to five tarpon. A single annulus is formed on scales and otoliths during early spring (March-April) for juvenile tarpon from South Carolina (Figure 10). The smallest scales MIs were observed in April ($22 \, ^\circ C$) while March tended to have the smallest otoliths MIs ($20 \, ^\circ C$; Figure 10). By summer (June; $29 \, ^\circ C$) MIs had increased for both structures, indicating new growth after annulus deposition (Figure 10). Scale MI measurements were similar during March and April (Figure 10). However, one March scale did not yet appear to have an annulus deposited, whereas all April scales had an annulus. Otolith MI measurements were lowest during March ($20 \, ^\circ C$), with a gradual increase in MI size during April - May ($22 – 30 \, ^\circ C$, respectively), but from March ($20 \, ^\circ C$) to June ($29 \, ^\circ C$) there was an overall increase in increment size after annulus deposition (Figure 10).

**Back-Calculation and Daily Increment Validation**
Twenty-one of the original 25 juvenile tarpon survived the 8-week controlled experiment (Table 2). One tarpon died after the first OTC marking event, and three tarpon died after the second OTC marking event. One tarpon showed negative growth between the initial marking and second marking period, which was likely due to measurement error or caudal fin damage. Back-calculated standard lengths were calculated for 17 of the 25 tarpon used in the experiment. One tarpon died after the first marking, therefore no measurements were able to be taken, one tarpon did not have OTC marks incorporated into the otolith, and six otoliths had damage to the core or the core was sanded through during structure preparation. $R^2$ for the relationships between otolith radius and SL were low but similar between axes. The dorsal axis had the stronger relationship with SL ($R^2 = 0.46$; Figure 8). Back-calculated lengths were significantly shorter when compared to measured lengths for the first marking event ($t = 7.22$, $df = 16$, $p = <0.001$; Figure 11) and significantly longer when compared to measured lengths for the second marking event ($t = -3.01$, $df = 13$, $p = 0.009$; Figure 11). Back-calculated growth rates were significantly greater than measured growth rates for the first marking event ($t = -33.1$, $df = 15$, $p = <0.001$; Figure 12) and for the entire study duration ($t = -6.28$, $df = 14$, $p = <0.001$; Figure 12), but were significantly less than measured growth rates for the second marking event ($t = 2.98$, $df = 13$, $p = 0.011$; Figure 12).

Twenty-three of the 25 marked tarpon were used to evaluate daily increment deposition. One tarpon did not have OTC incorporated into the otoliths; therefore, these otoliths were unable to be used. The CV for daily increment estimates was lowest from the first OTC marking event to the second event (11.1%), higher for the entire study duration (14.7%), and highest from the second OTC marking event to the end of the
study (31.1%). The percentage of samples that had 100% agreement between readers was low overall, but highest from both the first OTC mark to the second and from the second OTC mark to the end of the study (12.5%; Table 4). The mean percentage of individual reader daily increment estimates that were correct and within a three day and one day range were also low but were greatest for estimates from the first OTC mark to the second (Table 4). The mean percent difference ± SD (3.7 ± 8.4%) and percent mean percent absolute difference ± SD were lowest between marking events one and two (12.3 ± 8.2%, Table 4). Mean growth rates ± standard errors calculated based upon reader increment estimates were similar to measured growth rates between OTC marking events one and two (≈ 0.05 ± 0.01 mm/d; Table 5), but were greater than measured growth rates between OTC marking event two and the end of the study (> 0.37 ± 0.04 mm/d; Table 5) and from OTC marking event one and the end of the study (> 0.21 ± 0.02 mm/d; Table 5).

Discussion

I conducted a comprehensive examination of age estimation and back-calculation methods for juvenile tarpon collected from a South Carolina estuary. Annual age estimates for juvenile tarpon were relatively accurate and precise from both scales and otoliths; however, age estimates from scales were more accurate and precise. Scales provide a viable non-lethal option for estimation of juvenile tarpon age, which is desirable because tarpon support economically important catch-and-release recreational fisheries. Although otoliths have been the primary structure used for juvenile tarpon daily and annual age estimation, they have long been reported difficult to estimate ages from (Nichols 1994; Crabtree et al. 1995). Crabtree et al. (1995) rejected >20% of otoliths due
to the difficulty of estimating age (difficult to discern annuli) or because of high variation (CV) between readers. Scales are only useful for aging juvenile tarpon because ages can be underestimated using scales from adult tarpon due to difficulty or inability to discern annuli (Breder 1944; Crabtree et al. 1995). I only assessed juvenile tarpon ages 0-2, with a focus on discriminating between age-0 and -1 because these are the most common age tarpon collected within nursery habitats that are currently the target of conservation and restoration (Mace et al. 2018; Wilson et al. 2019). Although scales often result in underestimates of age compared to otoliths for adult tarpon and other species (e.g., striped bass \([\text{Morone saxatilis}]\); Secor et al. 1995), they have been reported to provide accurate age estimates for striped bass up to age-11 (Secor et al. 1995), weakfish \((\text{Cynoscion regalis})\) up to age-6 (Lowerre-Barbieri et al. 1994), and southern bluefin tuna \((\text{Thunnus maccoyii})\) up to age-4 (Gunn et al. 2008). Therefore, a validation study with multiple juvenile age classes is needed to evaluate the limits of age estimates from scales for tarpon.

An annulus formed during early- to mid-spring (\(\sim 20 ^\circ\text{C}\)) on otoliths and scales from juvenile tarpon that overwintered in South Carolina, which coincides with warming water temperatures and increasing light levels at the start of a new growing season (March and April). Similarly, juvenile tarpon from Florida formed an annulus by June; however, timing of formation was much more protracted and occurred during December-May (Crabtree et al. 1995), which may be due to the differences in overwinter water temperatures between the two locations. Timing of annulus formation can be difficult to discern for tropical species when compared to temperate species and cross latitudinally for individuals from the same species, due to lack of seasonal changes (Choat et al.
Timing of annulus formation can vary with latitude within the same species as well, as has been observed for cod (Gadus morhua; Høie et al. 2009) and red throat emperor (Lethrinus miniatus; Williams et al. 2005). Because this study was conducted at the northern edge of tarpon distribution in the western Atlantic Ocean, where environments undergo seasonal changes, the findings observed may not be applicable to more tropical tarpon populations living in thermally stable environments.

Although annuli formed within the majority of scales and otoliths examined by March, the minimum MI for scales lagged behind otoliths. Therefore, differential timing of annulus formation between structures cannot be discounted. Differences in the timing of annulus deposition between structures is not uncommon, and annuli on scales appear at different times compared to other hard structures, which may be linked to a variety of environmental factors, including water temperature, day length, migrations, food sources, and salinity (Simkiss 1974; McInerny 2017). These factors can affect annulus deposition because energy may be allocated to different biological functions (e.g., gonadic growth), and metabolism and diet can shift seasonally. Therefore, somatic and subsequent structure growth can be negatively influenced by reproductive processes, survival in stressful environments (e.g., extreme temperatures), or strenuous activities (e.g., migration). For example, annuli form during April-May in weakfish otoliths, but not until April-August in scales (Lowerre-Barbieri et al. 1994). This likely occurs because of a shift in diet and water temperature as weakfish migrate inshore from offshore winter habitats during April and May and then spawn from April through August, diverting resources to gonadic growth instead of somatic growth, resulting in differential timing of annulus deposition as resources are reallocated (Lowerre-Barbieri et al. 1994). Farmed
red porgy (*Pagrus pagrus*) also display differential annulus formation trends; annuli on scales were deposited in March but annuli on otoliths were deposited from March through August, and both deposition times are thought to be highly influenced by water temperature and food (Machias et al. 1998).

Validation of back-calculation methods is rare (Francis 1990). The biological intercept model worked poorly for back-calculating length and growth rates of juvenile tarpon in this study. Back-calculated lengths were made from measurements along the dorsal ridge of the succal groove in otoliths; however, the relationship between otolith radius and fish length was poor. This suggests that otolith growth and somatic growth are not tightly coupled for juvenile tarpon. For example, mean growth based on measured fish length between the first and second OTC marking events was low (0.05 mm/d), but growth based from back-calculated fish length was higher (0.53 mm/d), suggesting that otoliths were continuing to increase in diameter while fish lengths were not increasing or were increasing slowly. Poor relationships between otolith radii and fish length have been reported for other species including New Zealand snapper (*Pagrus auratus*; Francis et al. 1993), sockeye salmon (*Oncorhynchus nerka*; Marshall and Parker 1982), and Atlantic salmon parr (*Salmo salar*; Wright et al. 1990). The mechanisms between the uncoupling of otolith growth and somatic growth has previously been attributed to large changes in somatic growth, or periods of starvation and dietary changes (Secor et al. 1989). Otolith increment widths and deposition rates have also been linked to temperature fluctuations, circadian rhythm, light cycles, feeding cycles, and metabolic rates (Whitledge 2017). Juvenile tarpon otolith growth and somatic growth could be uncoupled in this study as a result of multiple environmental variables interacting (e.g., temperature change, light
cycle change, end of growing season), stress from transition from a natural setting to laboratory setting, or differences in diet and feeding within the laboratory compared to natural settings. Further research will be needed to address if and when otolith and somatic growth become proportional in young juvenile tarpon (<age 1), or to investigate mechanisms explaining why otolith and somatic growth are not proportional. For example, future research could examine otolith size versus fish length for known age fish sampled at set intervals for post-metamorphic tarpon throughout their period of residency within a region.

Uncoupling of otolith and somatic growth has been reported as a possible explanation for greater error in back-calculations made from otoliths versus scales for hybrid sunfish (Lepomis macrochirus × Lepomis cyanellus; Klumb et al. 2001) and for channel catfish (Ictalurus punctatus; Michaeletz et al. 2009). Scales could not be used to investigate the validity of back-calculation methods in this study because OTC marks were not realized on any scales. However, back-calculating lengths and growth rates from annuli on juvenile tarpon scales will likely provide more accurate results due to the stronger relationship between scale radius and fish length compared to otolith radius and fish length (Mace et al. 2020).

Estimation of daily increments from otoliths proved both inaccurate and imprecise. Readers noted crowding of daily increments near the edge of the otoliths, making identification of increments near the edge difficult. Daily increments have been validated for juvenile tarpon (64 – 113 mm SL) in Puerto Rico (Zerbi et al. 2001), but this study was unable to validate daily increment periodicity for juvenile tarpon from South Carolina. Daily growth increments form by the successive deposition of calcium
and protein rich layers, forming alternating translucent (calcium-rich) and opaque (protein-rich) layers that are deposited throughout a fish’s life, but the layers become narrow and less distinct as fish age (Whitledge 2017). Previous studies on herring (Geffen 1982), summer flounder (Szedlmayer and Able 1992), menhaden (Ahrenholz et al. 1995), and red snapper (Szedlmayer 1998) have reported effects of somatic growth rate on increment formation, where growth rates under a certain threshold resulted in less than daily increment deposition, but when growth rates were above the threshold increments were deposited daily. The variation of growth between marking events and the controlled environment within the outdoor seawater laboratory may have affected daily deposition of increments for tarpon from this study, although water was directly fed from Crab Haul Creek and the light-cycle was controlled by the sun, providing an environmental similar to a natural setting within the laboratory. Further research will be needed to examine the relationship between varying growth rates and daily increment periodicity and deposition to address if this phenomenon is occurring with young juvenile tarpon (<age 1). I observed little to no growth in fish length in September and relatively accurate age estimates but observed lower accuracy when the growth rate was greater in October. The differences in growth rates and deposition rates are conflicting because in a period of slow growth accuracy should be lower as increment width should decrease, and vise versa within periods of greater growth. This is an interesting observation and may potentially be due to accuracy decreasing with age or temporal variation as the tarpon growing season ends in South Carolina resulting in crowding near the structures edge because of slow overall growth. Decreases in accuracy as fish age is common and has been reported for fish >100 d old (Long and Grabowski 2017). For example, daily age
estimates from otoliths of largemouth bass (*Micropterus salmoides*) and white crappie (*Pomoxis annularis*) were found to be accurate up to 100 d, but accuracy decreased in fish >100 d (Miller and Storck 1982; Sweatman and Kohler 1991). A study including tarpon from multiple geographic regions and in a more natural setting will further our understanding of the limits of daily age estimates to estimate growth and hatch dates for tarpon.

The observed difference in daily increment estimates compared to the known number of increments from this study highlights an important issue when calculating growth rates for juvenile fish. Growth rates calculated from reader increment estimates were on average greater than measured growth rates (Table 5). The overestimation of growth rates calculated from inaccurate daily increment estimates could result in inflated estimates of growth rates for specific habitat types, resulting in misguided conservation efforts and incorrect management strategies. Therefore, growth rates should be used with caution when based off of daily increment counts from juvenile tarpon otoliths until further research has examined possible growth, age, or length thresholds for increment deposition rates in more natural settings.

Otoliths and scales are both acceptable structures to estimate age for juvenile tarpon (ages 0-2). Using scales offers an accurate and precise non-lethal method for estimating ages of tarpon up to age 2. Having a validated non-lethal method should allow future researchers to address habitat and growth questions more readily, and aid in the conservation efforts for juvenile tarpon. Additional research will be needed to validate use of scales for older juveniles (≥ age-3); however, the validation of the annual deposition of one annulus should allow for easier and more reliable future interpretations
of juvenile tarpon structures. Based on previously identified relationships between scale radius and tarpon length, scales may offer a more accurate method for back-calculating length-at-age and may provide more accurate back-calculated growth data. Therefore, continued efforts should be made to investigate the validity of back-calculation of length-at-age from scales. Finally, tarpon from more tropical latitudes may experience different annulus deposition and differences in timing of annulus formation relative to temperate populations; therefore, further assessment of tropical populations is recommended to gain a broader geographic understanding of the life history of juvenile tarpon and allow for informed management of this migratory species.
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Tables and Figures

**Table 2.** Standard length (SL, mm) data by study section for juvenile tarpon (*Megalops atlanticus*) collected during 2019 and 2020 from North Inlet estuary, South Carolina. SL data is reported by collection event (annual age validation) and oxytetracycline (OTC) marking event (back-calculation and daily increment validation) within respective study sections.

<table>
<thead>
<tr>
<th>Study Section</th>
<th>Mean ± SD (mm)</th>
<th>Minimum (mm)</th>
<th>Maximum (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Annual Age Validation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial capture</td>
<td>23</td>
<td>189 ± 25</td>
<td>139</td>
</tr>
<tr>
<td>Recapture</td>
<td>23</td>
<td>222 ± 18</td>
<td>185</td>
</tr>
<tr>
<td><strong>Marginal Increment Analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>197 ± 39</td>
<td>145</td>
</tr>
<tr>
<td><strong>Back-Calculation and Daily Increment Validation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First OTC marking</td>
<td>25</td>
<td>95 ± 16</td>
<td>68</td>
</tr>
<tr>
<td>Second OTC marking</td>
<td>24</td>
<td>96 ± 17</td>
<td>69</td>
</tr>
<tr>
<td>End of study</td>
<td>21</td>
<td>108 ± 20</td>
<td>72</td>
</tr>
</tbody>
</table>
Table 3. Descriptive statistics for accuracy and precision of age estimates from juvenile tarpon (*Megalops atlanticus*) hard structures: 100% agreement among all readers (all readers had the same estimate), consensus agreement (agreement between at least two of three readers), percent correct based on individual reader estimates (percentage of samples that readers correctly estimated averaged across readers), percent of age-0 fish aged correctly (based on concert estimates), and percent of age-1 fish aged correctly (based on concert estimates).

<table>
<thead>
<tr>
<th>Structure</th>
<th>n</th>
<th>100% agreement (%)</th>
<th>Consensus (%)</th>
<th>Age-0 (%)</th>
<th>Age-1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otoliths</td>
<td>22</td>
<td>54.54%</td>
<td>86.36%</td>
<td>-</td>
<td>88.24%</td>
</tr>
<tr>
<td>Recapture scales</td>
<td>23</td>
<td>69.56%</td>
<td>95.65%</td>
<td>-</td>
<td>100%</td>
</tr>
<tr>
<td>Initial scales</td>
<td>23</td>
<td>82.61%</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: missing numbers, denoted by hyphens, are representative of values that could not be calculated due to differences in timing of structure removal.
Table 4. Descriptive statistics of accuracy and precision of daily increments estimates from juvenile tarpon (*Megalops atlanticus*) otoliths: 100% agreement among all readers (all readers had the same estimate), percent correct (mean percent of samples that readers correctly estimated), percent of estimates within ± 3 days of the correct number of increments, percent of estimates within 1 day of the correct number of increments, percent mean difference ± standard deviation (SD) from the correct number of increments (calculated per reader than averaged across readers), and the percent mean absolute difference ± SD (MAD) from the correct number of increments (calculated per reader than averaged across readers). Increment estimates were made from the first oxytetracycline (OTC) mark (OTC 1) to the second OTC mark (OTC 2; 28 d), from the second OTC mark to the otolith edge (Edge; 28 d), and from the total estimate from the first OTC mark to the otolith edge (56 d).

<table>
<thead>
<tr>
<th>Estimate location</th>
<th>100% agreement</th>
<th>Correct (%)</th>
<th>± 3 days (%)</th>
<th>± 1 day (%)</th>
<th>Mean difference (%)</th>
<th>MAD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC 1 – OTC 2</td>
<td>12.50%</td>
<td>12.50%</td>
<td>66.67%</td>
<td>33.33%</td>
<td>3.73 ± 8.36%</td>
<td>12.27 ± 8.21%</td>
</tr>
<tr>
<td>OTC 2 - Edge</td>
<td>12.50%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>-64.94 ± 9.96%</td>
<td>64.94 ± 9.96%</td>
</tr>
<tr>
<td>OTC 1 - Edge</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>-36.65 ± 8.12%</td>
<td>36.65 ± 8.12%</td>
</tr>
</tbody>
</table>
Table 5. Growth data calculated from oxytetracycline (OTC) marking events for juvenile tarpon (*Megalops atlanticus*) that were used in a 56 d controlled back-calculation and daily increment validation study. Mean growth ± standard error (G, SE, mm/d) was estimated by subtracting size at final capture by size at the previous OTC marking event or size at the initial OTC marking event and then dividing by the number of days in between. True growth (TG) was calculated using the number of days between OTC marking events one (OTC 1) and two (OTC 2; 28 d), the number of days between OTC marking event two and the end of the study (F; 28 d), and the number of days between OTC marking event one and the end of the study (56 d). Reader growth (R1 and R2) was calculated using the readers’ estimated number of increments per sample between marking events one and two, between OTC marking event two and the end of the study, and between OTC marking event one and the end of the study. The range of growth rates is included in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>TG</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTC 1 – OTC 2</td>
<td>0.05 ± 0.01 (-0.04 – 0.14)</td>
<td>0.05 ± 0.01 (-0.04 – 0.13)</td>
<td>0.06 ± 0.01 (-0.04 – 0.18)</td>
</tr>
<tr>
<td>OTC 2 - F</td>
<td>0.37 ± 0.04 (0.11 – 0.68)</td>
<td>1.11 ± 0.23 (0.23 – 4.75)</td>
<td>1.81 ± 0.38-0.04 (0.20 – 6.33)</td>
</tr>
<tr>
<td>OTC 1 - F</td>
<td>0.21 ± 0.02 (0.05 – 0.36)</td>
<td>0.32 ± 0.03 (0.08 – 0.57)</td>
<td>0.42 ± 0.06 (0.07 – 1.07)</td>
</tr>
</tbody>
</table>
Figure 6. Location of scale collection (open circle) for juvenile tarpon (*Megalops atlanticus*). At least six scales were pulled from below the posterior end of the dorsal fin, three from each of the two rows above the lateral line. Scales from the right side of the tarpon were pulled at capture and oxytetracycline marking, and scales from the left side were pulled at recapture.
Figure 7. Example of an annulus from an age-1 juvenile tarpon (*Megalops atlanticus*). (a) A scale with “cutting over” and a scale within a scale shown (yellow arrows), and (b) an otolith with the crenulation and associated annulus shown (yellow arrows). The scale bar in (a) represents one mm, and the scale bar in (b) represents 200 μm.
Figure 8. Relationship between juvenile tarpon (*Megalops atlanticus*) otolith radius and standard length along the (a) dorsal ridge of the succal groove ($R^2 = 0.46$, $n = 17$) and (b) ventral ridge of the succal groove ($R^2 = 0.40$, $n = 17$). Otolith radius scale differs between measurement locations.
Figure 9. Cross sections from a juvenile tarpon (*Megalops atlanticus*) otolith. (a) The dorsal (1) and ventral (2) ridges of the succal groove where the otolith radius was measured, (b) two oxytetracycline marks highlighted under ultraviolet light, and (c) a zoomed in view of tarpon otolith increments along the ventral ridge of the succal groove where daily increments were estimated. Pictures (a) and (b) are at the same scale (200 μm), and the scale bar in (c) represents 20 μm.
Figure 10. Mean marginal increment measurements for juvenile tarpon (*Megalops atlanticus*). (a) Scales and (b) otoliths during January-June 2020. Bars are 95% confidence intervals, and sample sizes are given above each point.
Figure 11. Juvenile tarpon (*Megalops atlanticus*) measured standard length (mm) plotted against back-calculated standard length (mm) from (a) oxytetracycline marking event one and (b) oxytetracycline marking event two. The black line represents a 1:1 line plotted to show the relationship between measured and back-calculated lengths.
Figure 12. Juvenile tarpon (Megalops atlanticus) measured growth (SL mm/d) plotted against back-calculated growth (mm/d) from (a) the first oxytetracycline (OTC) marking event to the second marking event (28 d), (b) the second OTC marking event to the end of the study (28 d), and (c) the first OTC marking event to the end of the study (56 d). The black line represents a 1:1 line to show the relationship between measured and back-calculated growth rates.