

Age and growth assessment of western North Atlantic spiny butterfly ray *Gymnura altavela* (L. 1758) using computed tomography of vertebral centra

K. T. Parsons · J. Maisano · J. Gregg · C. F. Cotton ·
R. J. Latour

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Abstract Life history strategies of batoid fishes have evolved within dynamic marine ecosystems. Adaptations in reproductive and developmental biology are paramount to the survival of species, and therefore knowledge of growth rates to maturity is fundamental for identifying constraints on the conservation of populations. The butterfly rays (Myliobatiformes: Gymnuridae) are highly derived batoids with generally low reproductive potentials for which age and growth information remains unknown. In this study we applied high-resolution X-ray computed tomography (HRXCT) to vertebral centra from a stingray for the first time to estimate age, and used a multimodel approach to investigate growth of spiny butterfly ray, *Gymnura altavela*. Estimated ages of the oldest male and female were 11 and 18 yrs. at disk widths (WD) 1355 mm and 2150 mm, respectively. Disk width-at-age data were analyzed using three growth models (von Bertalanffy, logistic, Gompertz), and the most

parsimonious and empirically supported model was the logistic function with sex treated as a fixed effect on asymptotic disk width (WD_{∞}) and k parameters. Model parameter estimates were (males) $WD_{\infty} = 1285.46 \pm 67.27$ mm, $k = 0.60 \pm 0.10$, and (females) $WD_{\infty} = 2173.51 \pm 129.78$ mm, $k = 0.27 \pm 0.04$. Results indicated sexually dimorphic growth patterns, with males growing faster and reaching asymptotic size at earlier ages than females. These age and growth results are the first reported for the genus, and suggest that *G. altavela* grows at a similar rate as some teleosts and batoids, and relatively fast among chondrichthyans.

Keywords Myliobatiformes · Gymnuridae · HRXCT · Growth coefficient · Logistic growth model

Introduction

Batoids (Chondrichthyes: Batoidea) are a cosmopolitan group of skates and rays for which life history traits remain largely unknown relative to other chondrichthyans and teleosts. Many marine batoids inhabit coastal ecosystems, from shallow estuarine to shelf waters, where their characteristic dorso-ventrally flattened body shapes are adapted to benthic habitats that support diverse prey types such as mollusks, crustaceans, polychaetes, and fishes (McEachran and Dunn 1998; Ebert and Bizzarro 2007; Ebert and Stehmann 2013). Although commercial U.S. fisheries do not target rays, overlapping

K. T. Parsons (✉) · J. Gregg · R. J. Latour
Virginia Institute of Marine Science, College of William & Mary,
Post Office Box 1346, Gloucester Point, VA 23062, USA
e-mail: kparsons@vims.edu

J. Maisano
Department of Geological Sciences, The University of Texas,
2275 Speedway Stop C9000, Austin, TX 78712-1722, USA

C. F. Cotton
Florida State University Coastal and Marine Laboratory, 3618
Coastal Highway 98, St. Teresa, FL 32358-2702, USA

distributions with fishes of economic importance results in their incidental catch (bycatch) in demersal fisheries (Brander 1981; Stevens et al. 2005; Tamini et al. 2006). In general, low value bycatch is unregulated, poorly monitored, and discarded at sea, impeding evaluation of species-specific landings data and the potential impacts on populations. Common effects of fishing include alterations to the size and age structure of populations that may induce compensatory changes in demographic rates (Walker and Hessen 1996; Walker and Hislop 1998; Frisk et al. 2008; Romine et al. 2013). Nearly 20% of batoid fishes are threatened with extinction according to The International Union for the Conservation of Nature Red List of Threatened Species (IUCN Red List; www.iucnredlist.org), and 45% of species are considered 'Data Deficient' due to inadequate life history information, presenting major challenges for the development of effective management strategies (Dulvy et al. 2014). Consequently, reported increases in global batoid landings have generated management and conservation concerns, highlighting the need for improved monitoring of populations and a better understanding of the life history of these taxa worldwide (Simpfendorfer et al. 2011; Mandelman et al. 2012; Dulvy et al. 2014).

In general, large-bodied batoids tend to grow more slowly, live longer, and produce fewer offspring than smaller species, and females grow larger and at a slower rate than males (Frisk 2010; Fisher et al. 2013). Life history traits that are characteristic of most batoids and other chondrichthyans lead to increased vulnerability of populations to depletion from overexploitation (Hoenig and Gruber 1990), particularly species with large maximum sizes (Dulvy et al. 2000, 2014). As both mesopredators and prey that link upper and lower trophic levels, skates and rays may also play important ecological roles in the structure and dynamics of coastal ecosystems (e.g., Murawski 1991; Heithaus et al. 2010; Bornatowski et al. 2014). Thus, perturbations to coastal batoid populations may also impact the stability and productivity of co-occurring species of ecological and economical value. Improved understanding of size-at-age and growth patterns in batoid fishes is prerequisite to assessing the status of populations and evaluating changes in demographics over time.

The spiny butterfly ray *Gymnura altavela* (Linnaeus 1758) is a large coastal stingray

(Myliobatiformes) with an amphi-Atlantic and Mediterranean distribution, inhabiting sandy and muddy substrates of western North Atlantic waters from Massachusetts to Florida (McEachran and Capapé 1984; Robins and Ray 1986; McEachran and Séret 1990; McEachran and de Carvalho 2002). Descriptions of the species in U.S. waters are restricted to spatially- and temporally-limited studies from which few life history parameters have been estimated, despite reported geographical variability in their maximum and maturity sizes, and low ($< 10 \text{ yr}^{-1}$) uterine fecundity (Bigelow and Schroeder 1953; Daiber and Booth 1960; Schwartz 1984; Capapé et al. 1992; Henningsen 1996). While it is not targeted by U.S. fisheries, *G. altavela* may be commonly captured and discarded in demersal trawling operations that occur where densities are high. The impact of fishing pressure on post-release survival of this species remains unknown, which greatly limits assessments and inferences regarding population status. Threats to the U.S. population are classified as Least Concern by the IUCN Red List (2016), although *G. altavela* is considered globally Vulnerable due to population declines observed in the Southwest Atlantic and West Africa, and is Critically Endangered off the coast of Brazil and in the Mediterranean (Vooren et al. 2007; Walls et al. 2016). Reliable life history information including age and growth estimates are needed for improved vulnerability assessments of western North Atlantic populations of *G. altavela*.

Batoid vertebral centra offer a measure of somatic growth through the mineralization of nutrients and deposition of growth bands over time (Ridewood 1921). These structures have been used to estimate age and evaluate growth of several stingray taxa, including the Dasyatidae (Ismen 2003; Jacobsen and Bennett 2010, 2011; O'Shea et al. 2013), Myliobatidae (Martin and Cailliet 1988), Platyrhinidae (Kume et al. 2008), Rhinopteridae (Smith and Merriner 1987; Neer and Thompson 2005; Fisher et al. 2013), Urolophidae (White et al. 2001, 2002), Urotrygonidae (Mejía-Falla et al. 2014), and Rhinobatidae (White et al. 2014). Despite reported disk widths exceeding 2 m (Bigelow and Schroeder 1953; Bini 1967; Schwartz 1984), there is no information on age and growth for any species of butterfly ray (Gymnuridae), presumably due to difficulties interpreting growth bands in the relatively

small and poorly mineralized centra. To provide fundamental age and growth information, there is a need for alternative approaches to the examination of vertebral centra for which conventional methods remain inadequate.

Recent advances in high-resolution X-ray computed tomography (CT) scanning at the microscopic scale (i.e., HRXCT) provide fine-scale three-dimensional models that can be digitally sectioned to reveal the micro-structure of soft and hard tissues, and offer a valid and repeatable method for the analysis of calcified vertebral morphology to estimate age in chondrichthyans (e.g., Geraghty et al. 2012). CT scanning offers a non-destructive alternative to traditional chondrichthyan ageing methods (i.e., serial sectioning of vertebrae). Broadly applied to the study of systematic morphology of vertebrates, CT scanning has become a valuable tool for detailed examination of both fossil (Schultze and Cloutier 1991; Maisey 2001a; Witmer et al. 2008) and extant vertebrates including chondrichthyans (Maisey 2001b, 2004; Hilton et al. 2015; Moyer et al. 2015). The present study applies HRXCT methods to *Gymnura* vertebral centra to determine the age of 49 western North Atlantic *G. altavela*. Age estimates were then used to describe growth patterns and provide key growth parameters for this population. Sex-specific weight-at-length relationships were also examined in 119 specimens collected over a four-year period from multiple fishery-independent surveys along the U.S. Atlantic coast. The novel application of HRXCT methods presented here is broadly applicable to other chondrichthyans with poorly mineralized vertebrae for which age information is needed for stock assessments. Results from this study are intended to augment life history knowledge of *G. altavela* for improved assessment of the western North Atlantic population.

Materials and methods

Sample collection and HRXCT analysis

Specimens of *G. altavela* were collected between 2012 and 2016 from fishery-independent trawl and longline surveys of shelf waters in the U.S. western North Atlantic (Fig. 1). Individuals were sexed, measured, and dissected in the field or stored frozen

for laboratory processing. Disk width (WD) and disk length (LD) were measured to the nearest mm, and individuals were weighed (W) to the nearest 0.1 g. Complete vertebral columns from the synarcual cartilage to the tail tip were excised from specimens and stored frozen for age analysis. Vertebral columns were later thawed and soaked in hot water for 10 to 15 min to enable removal of soft tissues and disarticulation of centra. To identify which centra were ideal for age analysis, a pilot study using a subsample of vertebral columns from seven males ($n = 3$ mature, 4 immature) and eight females ($n = 3$ mature, 5 immature) was conducted. Vertebral columns were completely disarticulated, and each centrum was enumerated and air-dried. Dried whole centrum diameter (C_D) was measured to the nearest 0.1 mm, and the C_D coefficient of variation was calculated for every set of five vertebrae. Variation was smallest among precaudal vertebrae, and centrum numbers 35–40 were the largest across all life stages of both sexes with the exception of one young-of-year female. Based on these observations, one precaudal centrum between numbers 35 and 40 was selected from the posteriormost abdominal region of 49 specimens and preserved in 70% ethanol for age analysis (Fig. 2a).

Whole *G. altavela* centra were air dried and imaged with a Zeiss (formerly Xradia) MicroXCT 400 (<https://www.zeiss.com/microscopy/int/x-ray.html>) at The University of Texas High-Resolution X-ray Computed Tomography Facility. Scans were performed using a Hamamatsu X-ray source set to 70 kV/10 W. Three different protocols were used, yielding resolutions scaled to centrum size and usually accommodating multiple centra within a single scan. The largest centra were scanned using the 0.4X objective, acquiring 1441 views over 360 degrees of rotation at 3 s/view with a 0.35 mm SiO₂ X-ray prefilter, yielding 25.0 μ m resolution. Medium-sized centra were also scanned using the 0.4X objective, at 4 s/view and with distance between the X-ray source and detector set to yield 14.5 μ m resolution. The smallest centra were scanned using the 4X objective and a 0.15 mm SiO₂ X-ray prefilter at 1.75 s/view, yielding 5.5 μ m resolution. Depending on the antero-posterior thickness of the included centra, total number of slices ranged from 491 to 990 per scan (Fig. 2b). Image slices were rendered in three dimensions using the Amira (FEI) software program and visualized using

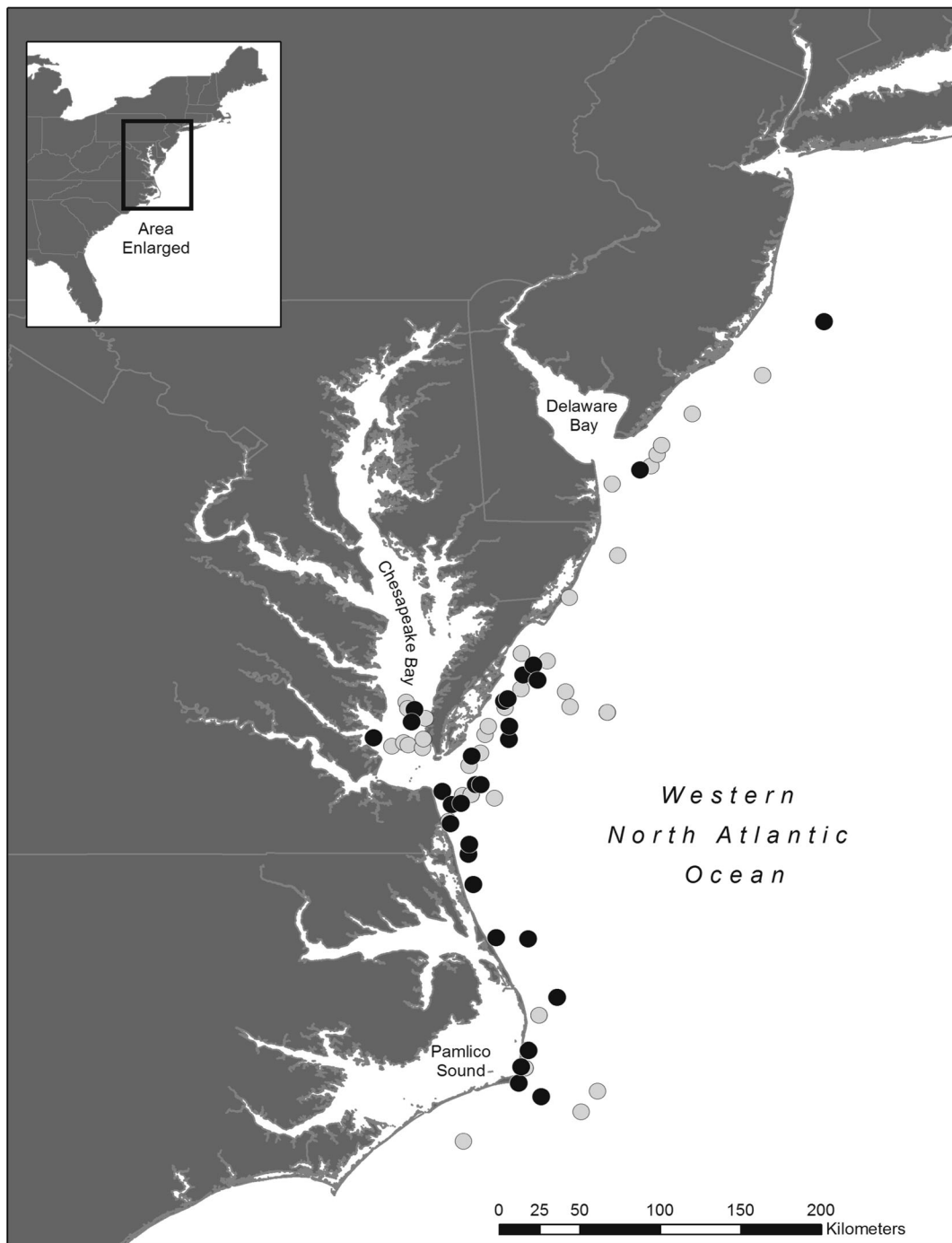
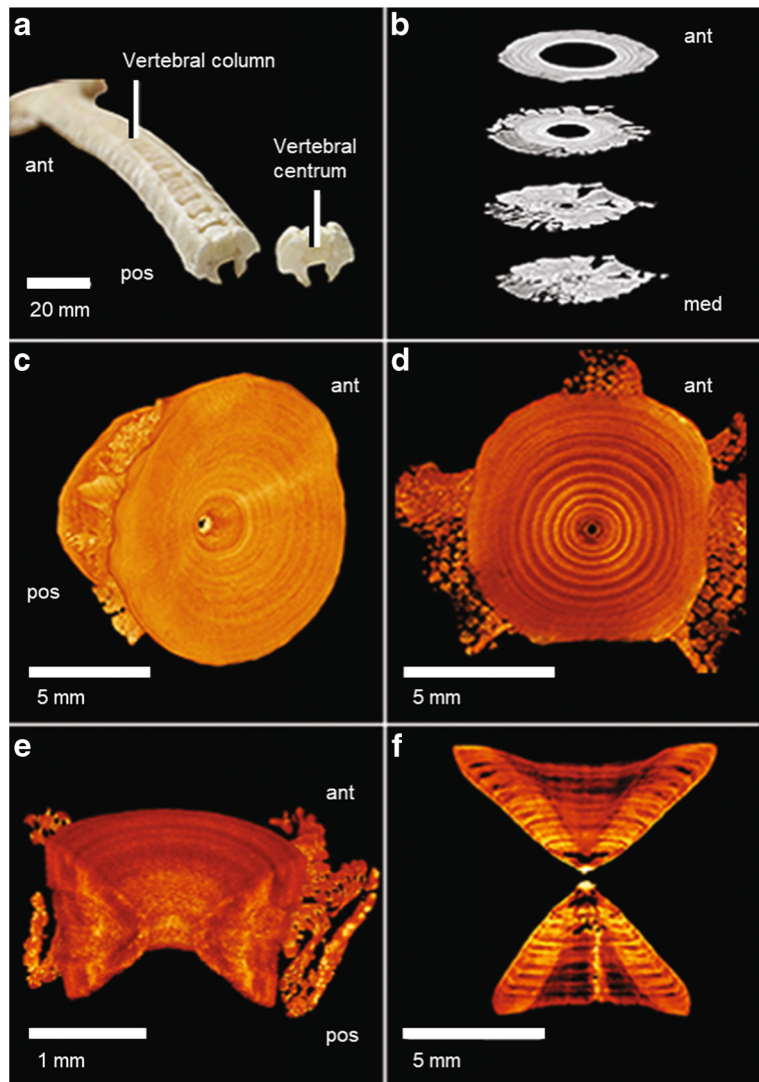


Fig. 1 Map of sampling area and distribution of *Gymnura altavela* specimens collected for the present study. Black circles indicate specimens used for age analysis

false color to enhance centrum density variations (Fig. 2c–f). Virtual models of whole centra were inspected for structural quality using rotation and transparency controls (Fig. 2c–d). Each virtual centrum

was sliced in half along the sagittal plane to assess internal anatomy and calcification patterns (Fig. 2e). A thin virtual section from the sagittal plane was then selected for age determination (Fig. 2f).

Fig. 2 Methods for vertebral centrum processing for HRXCT scanning, reconstruction, and ageing using Amira software. **a** Vertebral centrum (8.4 mm diameter, female 1905 mm WD) excised from *Gymnura altavela* vertebral column, ventral view; **b** examples of slices from reconstructed CT scan of centrum in panel C, anterior (top) to medial (bottom); **c** profile view of 3D reconstructed whole centrum (8.7 mm diameter, female 1737 mm WD), anterior to right; **d** anterior view of whole centrum (9.6 mm diameter, female 1880 mm WD) adjusted for transparency and contrast to enhance growth band visualization; **e** sagittal plane view of centrum (2.1 mm diameter, female 639 mm WD); **f** sagittal section of centrum in panel C used for age analysis. Ant = anterior, pos = posterior, med = medial



Statistical analyses

The relationship between the weight and disk width of 119 individuals ($n_{\text{male}} = 63$, $n_{\text{female}} = 56$) was estimated using the equation:

$$W_i = \alpha_i W D_i^{\beta_i} e^{\varepsilon_i} \quad (1)$$

where for the i^{th} individual ($i = 1, 2, \dots, 119$) α_i is a constant, β_i is the allometric parameter, and ε_i is the multiplicative error term. Sexual dimorphism was examined by assuming:

$$\begin{pmatrix} \alpha_i \\ \beta_i \end{pmatrix} = \begin{pmatrix} \gamma_{0\alpha} + \gamma_{1\alpha} \text{Sex}_i \\ \gamma_{0\beta} + \gamma_{1\beta} \text{Sex}_i \end{pmatrix} \quad (2)$$

where Sex_i is a binary covariate representing the sex of the i^{th} individual (male coded '0', female coded '1'). Eq. (2) implies the parameters $(\gamma_{0\alpha}, \gamma_{0\beta})$ and sums $(\gamma_{0\alpha} + \gamma_{1\alpha}, \gamma_{0\beta} + \gamma_{1\beta})$ define the parameters in Eq. (1) for males and females, respectively (Kimura 2008). Regression assumptions from preliminary model fits were evaluated using histograms, QQ-plots, and visual inspection of residuals, and results supported a multiplicative error structure (Quinn and Deriso 1999). Accordingly, both sides of Eq. (1) were log transformed prior to model fitting. Ordinary least squares was used for estimation and four model parameterizations were considered: (1) no sex effect; (2) effect of sex on α_i ; (3) effect of sex on β_i ; and (4) effect of sex on both α_i and β_i . Model selection was determined by goodness-of-fit mean

squared error (MSE) and Akaike's information criterion (Akaike 1973; Burnham and Anderson 2002) corrected for small sample size (AIC_c ; Zhu et al. 2009). Model-based predictions of weight-at-disk width were back transformed and bias corrected (Sprugel 1983).

Growth band pairs were defined as one opaque and translucent band pair extending through the intermedialia (I) and into the corpus calcareum (CC; Casey et al. 1985; Brown and Gruber 1988). The first opaque band distal to the focus and associated with a change in the angle of the CC was defined as the birth band (BB; age = 0 years), and annual deposition of band pairs in centra was assumed. Age was estimated by counting band pairs distal from the BB and extending from one arm of the CC, through the I and across the opposing CC arm (Fig. 3; Cailliet et al. 2006).

Growth band pairs were counted on HRXCT digital sections prepared independently by two readers using Amira. All centrum images were read twice by each reader for training and fluency in growth band identification, followed by two blind independent readings to assign ages to each specimen, and readings were temporally separated by two weeks to reduce bias. Reproducibility of age determinations between and among readers was examined through age-bias regression analysis (Campana et al. 1995), and systematic differences in age assignments were tested using Evans-Hoenig's and Yates continuity corrected McNemar's χ^2 tests of symmetry (Bowker 1948;

Hoenig et al. 1995; Evans and Hoenig 1998). Age assignment precision was evaluated by calculating within and between reader: 1) percent agreement:

$$PA = \frac{N_a}{N_r} \times 100 \quad (3)$$

where N_a and N_r represent the number of ages agreed upon and read, respectively; 2) average coefficient of variation (ACV; Chang 1982)

$$ACV = \frac{1}{N} \sum_{j=1}^N \left(\frac{\sqrt{\sum_{i=1}^R \frac{(a_{ij} - a_j)^2}{R-1}}}{a_j} \right) \times 100\% \quad (4)$$

and 3) index of average percent error (Beamish and Fournier 1981):

$$IAPE = \frac{1}{N} \sum_{j=1}^N \left(\frac{1}{R} \sum_{i=1}^R \left(\frac{|a_{ij} - a_j|}{a_j} \right) \right) \times 100\% \quad (5)$$

where a_{ij} is the i^{th} age estimate for the j^{th} individual, a_j is the mean age calculated for the j^{th} individual, N is the total number of individuals aged, and the number of times an individual was aged is represented by R .

One HRXCT image slice from each centrum image stack was converted to a two-dimensional 8-bit TIFF file, and linear measurements of the centrum radius (C_R) from the focus to the marginal edge were obtained using the straight line selection tool in ImageJ (<https://imagej.nih.gov/ij>). A linear model was fitted to estimate the relationship between C_R and WD using generalized least squares estimation. Two forms were considered, one with and one without variance modeled as a power function of the mean ($\text{var}(\varepsilon_i) = \sigma^2(f(x_i, \beta))^{2\theta}$) to explore and accommodate heterogeneity (Ritz and Streibig 2008). AIC_c was used for model selection. Due to inadequate seasonal coverage of samples, marginal increment analysis could not be used to assess temporal periodicity in growth band formation.

The relationship between disk width and age of *G. altavela* was investigated using multiple growth models fitted using nonlinear least squares (Thorson and Simpfendorfer 2009). Regression assumptions were evaluated using the graphical methods described above for the weight-at-disk width analysis and diagnostics from preliminary model fits supported an additive error structure. Model classes considered included:

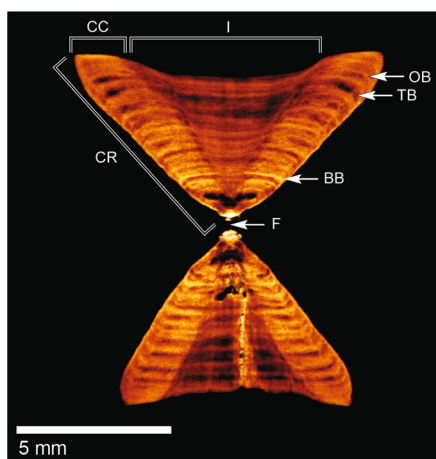


Fig. 3 False-colored HRXCT-reconstructed vertebral centrum section (diameter = 8.8 mm) from female *Gymnura altavela* (1737 mm WD) estimated to be 11 years old. Centrum radius (CR), corpus calcareum (CC), and intermedialia (I) are indicated, and arrows mark the focus (F), birth band (BB), and transparent (TB) and opaque bands (OB)

von Bertalanffy (VBF1; Beverton and Holt 1957)

$$WD_i = WD_{\infty i} \left(1 - e^{-k_i(t_i - t_{0i})} \right) + \varepsilon_i \quad (6)$$

$$\begin{pmatrix} WD_{\infty i} \\ k_i \end{pmatrix} = \begin{pmatrix} \beta_{0WD} + \beta_{1WD_{\infty}} Sex_i \\ \beta_{0k} + \beta_{1k} Sex_i \end{pmatrix} \quad (7)$$

Gompertz (GFF1; Quinn and Deriso 1999)

$$WD_i = WD_{\infty i} e^{-e^{-k_i(t_i - t_{0i})}} + \varepsilon_i \quad (8)$$

$$\begin{pmatrix} WD_{\infty i} \\ k_i \end{pmatrix} = \begin{pmatrix} \beta_{0WD} + \beta_{1WD_{\infty}} Sex_i \\ \beta_{0k} + \beta_{1k} Sex_i \end{pmatrix} \quad (9)$$

and logistic function (LGF1; Ricker 1979)

$$WD_i = \frac{WD_{\infty i}}{1 + e^{b_i - k_i t_i}} + \varepsilon_i \quad (10)$$

$$\begin{pmatrix} WD_{\infty i} \\ k_i \end{pmatrix} = \begin{pmatrix} \beta_{0WD} + \beta_{1WD_{\infty}} Sex_i \\ \beta_{0k} + \beta_{1k} Sex_i \end{pmatrix} \quad (11)$$

where for the i^{th} individual ($i = 1, 2, \dots, 49$), WD_i is disk width, $WD_{\infty i}$ is theoretical asymptotic disk width, k_i is the instantaneous growth coefficient, b_i is a constant (Logistic model), t_i is age, t_{0i} is the theoretical age at zero disk width (von Bertalanffy, Gompertz models), ε_i is an additive error term, and Sex_i is a binary covariate coded as described above for the weight-at-disk width analysis (Kimura 2008). For each growth function, model parameterizations considered included: (1) no sex effect; (2) effect of sex on $WD_{\infty i}$; (3) effect of sex on k_i ; and (4) effect of sex on both $WD_{\infty i}$ and k_i . For the von Bertalanffy and Gompertz models, the latter three parameterizations were intended to explore sexual dimorphism under the parsimonious assumption that the theoretical size at zero disk width did not differ among sexes. For the logistic models, parsimony was again invoked by not including the effect of sex on b_i . Parameter estimates for males were directly estimated, while parameters for females required summation of the baseline (i.e., male) estimate and the coefficient of the sex effect. Accordingly, standard errors for female parameter estimates were obtained using the delta method (Seber 1982). Size-at-birth (WD_0) was calculated from the y-intercept of the model chosen for inference with standard errors estimated from the delta method. Model

selection was based on biological plausibility and concordance of WD_0 , WD_{∞} , and k parameter estimates, goodness-of-fit (MSE), and AIC_c. All statistical analyses were conducted using R (R Development Core Team 2016), and results were considered significant at $\alpha < 0.05$ (where applicable).

Results

Gymnura altavela specimens used for the weight-at-disk width analysis ranged in size from 427 to 2150 mm WD_{Female} (0.56–80.26 kg W_{Female}) and 506–1365 mm WD_{Male} (1.13–25.50 kg W_{Male}) (Table 1). A difference in the weight-at-disk width relationship among sexes was

Table 1 Summary of western North Atlantic *Gymnura altavela* used for age assessment. Age groups not represented in this study were 3–6 and 14–17 for females, and 9–10 for males. Weight (W) summary statistics reported here are only for specimens that were aged. For the full weight-at-disk width (WD) analysis, WD and W ranges were: WD_{female} 427–2150 mm; W_{female} 0.56–80.26 kg; WD_{male} 506–1365 mm; W_{male} 1.13–25.50 kg

	Age Group (n)	Mean Disk Width (mm)	Range Disk Width (mm)	Mean Wet Weight (kg)	Range Wet Weight (kg)
Female	0 (2)	575.0	544–606	1.6	1.2–1.9
	1 (1)	680.0		2.8	
	2 (3)	621.7	600–639	1.8	1.5–2.1
	7 (3)	1387.3	1100–1670	17.7	11.3–24.2
	8 (2)	1602.5	1575–1630	37.3	34.8–39.7
	9 (2)	1892.5	1880–1905	64.0	58.0–70.0
	10 (2)	1862.5	1845–1880	66.5	65.1–68.0
	11 (3)	1592.3	1173–1867	49.8	49.0–50.6
	12 (1)	1780.0		68.0	
	13 (1)	2036.0		76.8	
	18 (1)	2150.0		80.3	
Male	0 (1)	561.0		1.5	
	1 (2)	582.5	527–638	1.7	1.2–2.2
	2 (4)	631.8	565–690	2.1	1.5–2.7
	3 (2)	787.5	770–805	4.3	3.9–4.5
	4 (1)	965.0		14.3	
	5 (5)	1196.8	1080–1330	15.7	11.1–20.8
	6 (3)	1094.0	940–1200	13.5	12.8–14.2
	7 (8)	1225.9	1016–1348	19.3	10.4–25.5
	8 (1)	1110.0		11.8	
	11 (1)	1360.0		20.4	

not empirically supported and was best described by a function of the form $\log W = \log(2.78 \times 10^{-9} WD^{3.17})$ (Table 2; Fig. 4). A significant linear relationship between C_R and WD was described by the equation $C_R = 7.84WD - 2897.07$ ($\alpha = -2897.07 \pm 431.13$; $\beta = 7.84 \pm 0.60$; $\theta = 0.98$; $\Delta AIC_c = 38.82$ between parameterizations with and without the variance function, which provided strong support for inclusion of the power of the mean model), and demonstrated that vertebral growth was proportional to body size (Fig. 5). Therefore, use of *G. altavela* centra for age analysis was appropriate.

Reconstructed HRXCT vertebral centra from *G. altavela* revealed interpretable growth band pairs. Pre-birth bands were observed but not consistent in all centra, and the BB was associated with a change in CC angle, followed by a broad translucent band reflecting the first year of growth. Narrow OB and broad TB pairs were readily distinguishable across the I and both arms of the CC in digital sections, however, these tended to become compacted near the growth margin in larger individuals.

Age estimates from the two readers did not differ systematically (Evans-Hoenig's $\chi^2 = 2.29$, $p = 0.32$; McNemar with Yates continuity correction $\chi^2 = 1.84$, $p = 0.17$). Within reader agreement was 90% (IAPE = 1.29, ACV = 1.82, Fig. 6a) and 92% (IAPE = 1.07, ACV = 1.51) for reader A and B, respectively. Percent agreement between readers increased from 59% (IAPE = 6.00, ACV = 8.48) during the first reading to 78% (IAPE = 3.65, ACV = 5.17) during the second reading (Fig. 6b). Final ages were assigned to all specimens by consensus, and the oldest ages estimated were 18 years for a 2150 mm WD_{female} individual, and 11 years for a 1355 mm WD_{male} individual (Table 1).

Table 2 Number of parameters (p), mean squared error (MSE), AIC_c , ΔAIC_c , and parameter estimates \pm standard errors for models fitted to western North Atlantic *Gymnura altavela* weight-at-disk width data. Model parameterizations were: (1) no covariate; (2) sex covariate on α ; (3) sex covariate on β ; and (4) sex covariate on α and β . The weight-at-disk width relationship was best described by Model 1 with parameter estimates: $\alpha = 2.54 \times 10^{-9} \pm 6.24 \times 10^{-10}$, $\beta = 3.18 \pm 0.04$

Model	p	MSE	AIC_c	ΔAIC_c
1	3	0.0272	-85.23	0.00
2	4	0.0268	-84.43	0.80
3	4	0.0268	-84.51	0.72
4	5	0.0267	-82.61	2.62

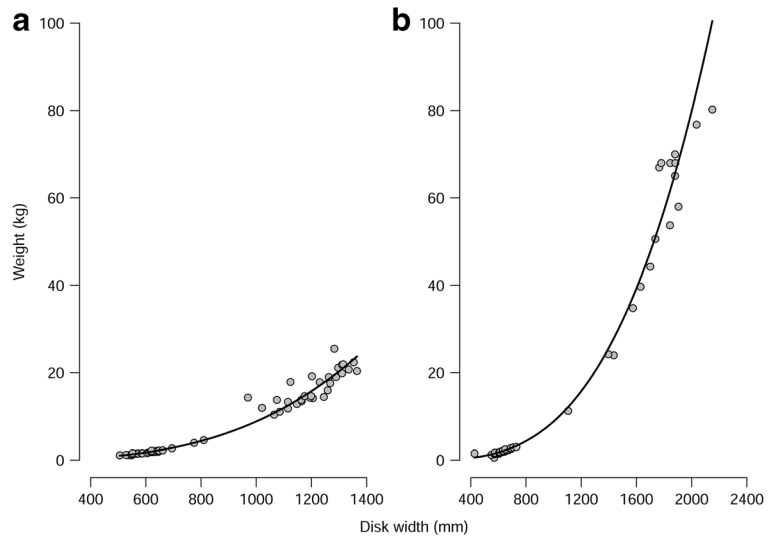
The most empirically supported disk width-at-age model was the logistic parameterized with the Sex covariate in both WD_∞ and k (LGF4), which resulted in biologically plausible estimates of WD_∞ for males (1285.46 ± 67.27 mm) and females (2173.51 ± 129.78 mm) relative to observed sizes (1355 and 2150 mm, respectively) (Tables 3, 4; Fig. 7). Predicted growth coefficients were $k_{\text{male}} = 0.60 \pm 0.10$, $k_{\text{female}} = 0.27 \pm 0.04$, and $b = 1.19 \pm 0.12$ (Table 4). Size-at-birth calculated from the logistic parameter estimates was 300.80 ± 33.81 mm WD_{male} , 508.61 ± 49.63 mm WD_{female} , and averaged 404.70 ± 38.72 mm WD across sexes. The smallest free-swimming individual observed was 496 mm WD.

Discussion

Findings from this study demonstrated the first successful application of HRXCT for ageing a large stingray species with relatively small ($< 1.0\%$ WD), weakly calcified vertebral centra, and offer further support for the utility of this alternative method in chondrichthyan ageing studies. Furthermore, we provided the first known estimates of age and growth parameters in the Gymnuridae, advancing critical life history knowledge necessary for assessment of the U.S. western North Atlantic *G. altavela* population. The use of HRXCT-reconstructed models of vertebral centra for age estimation offers considerable advantages over manual sectioning and these have been reviewed by Geraghty et al. (2012). Most notably, vertebral centra are preserved whole and therefore available for comparative studies as new methods are developed with advances in technology, and users have unconstrained control over the manipulation (e.g., section thickness, transparency, contrast, perspective) of digital three-dimensional sections, improving the ability to identify and interpret growth bands. It is important, however, to establish a standardized protocol for the examination of virtual sections to ensure consistency between readers. The greatest disadvantage of this method is cost, which effectively limited the sample size in the present study. Recent efforts to provide open access to digital libraries of HRXCT-scanned specimens (e.g., Digimorph, <http://digimorph.org>, Accessed: 27 February 2017) may improve future accessibility to this method for use in ageing studies.

Precision of age assignments between and within readers was generally high in the present study, with

Fig. 4 Weight-at-disk width relationship for (a) male ($n = 63$) and (b) female ($n = 56$) western North Atlantic *Gymnura altavela*



percent agreement between readers improving from 60 to 78% between reading trials, and 92% agreement within one year during both trials. Birth band determination was the greatest contributing factor to reader disagreement, followed by the presence of false bands and the compression of marginal bands in the largest individuals. Marginal increment ratios of centrum growth bands (not presented here) were inconclusive because specimens were predominantly collected by surveys operating during autumn months, thus precluding the determination of seasonal periodicity in band pair formation. Consequently, ages reported here

assume an annual deposition of growth bands, which is common in fish ageing studies (Cailliet and Goldman 2004; Okamura et al. 2013), and has been validated in other batoid species (e.g., Natanson 1993; Sulikowski et al. 2003; Jacobsen and Bennett 2010). Nevertheless, validation of annual deposition of growth bands is needed to verify age estimates for *G. altavela*.

Sexually dimorphic body changes are observed during ontogeny in *G. altavela*. Neonates are born at approximately the same size and shape, and increase in mass and width at similar rates during early life stages, irrespective of sex. Later, this species demonstrates sex-specific patterns in growth, with males reaching a smaller asymptotic size as females continue to increase in size over a longer lifespan. These results support previously reported sexual dimorphism for *G. altavela* (Bigelow and Schroeder 1953; Capapé et al. 1992; Alkusaity et al. 2014), other gymnurids (Raje 2003; White and Dharmadi 2007; Jacobsen et al. 2009), and various other batoid species (Isimen 2003; Smith et al. 2007; Sulikowski et al. 2007). Individual mass increased at a greater rate than width during ontogeny, similar to weight-at-length relationships reported for other stingrays, including other species within the genus *Gymnura* (Cailliet and Goldman 2004; Neer and Thompson 2005; White and Dharmadi 2007; Yokota et al. 2012; Teixeira et al. 2016). The allometric parameter estimated for western North Atlantic *G. altavela* ($\beta = 3.17 \pm 0.04$ S.E.) differed from the range of estimates most recently reported for the Mediterranean population ($\beta = 2.795\text{--}3.028$), and may be explained by the smaller size range (300–1650 mm WD) of

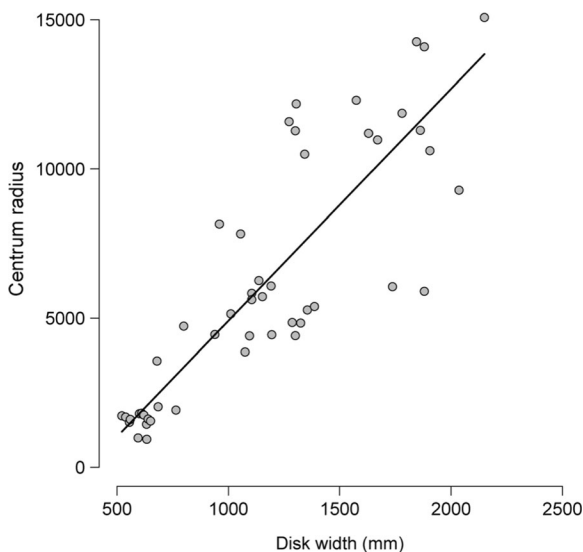


Fig. 5 Centrum radius-at-disk width relationship for western North Atlantic *Gymnura altavela* ($n = 49$)

Fig. 6 Bias plots associated with age assignments from (a) reader A and (b) between readers A and B for western North Atlantic *Gymnura altavela* vertebral centra ($n = 49$). Data points are mean ages (± 2 S.E.) estimated from the first read from reader A and the second read from reader B, respectively, referenced against the second read from reader A. Sample sizes for each age are displayed horizontally and apply to both panels

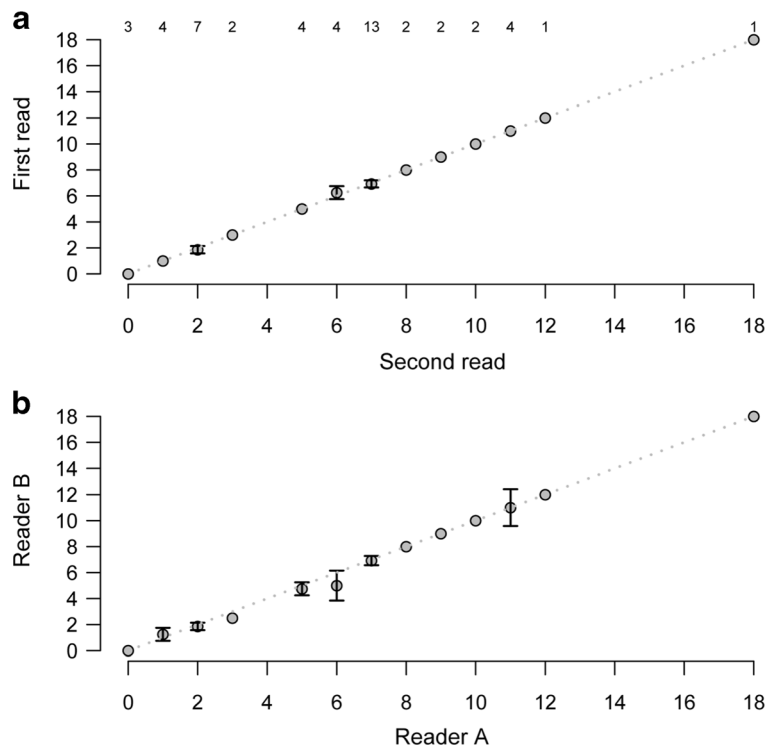


Table 3 Number of model parameters (p), mean squared error ($MSE \times 10^4$), corrected Akaike information criterion (AIC_c), and ΔAIC_c for 12 growth models fitted to western North Atlantic *Gymnura altavela* disk width-at-age data. For each growth function, model parameterizations considered included (1) no sex effect, (2) effect of sex on WD_{x_0} , (3) effect of sex on k_i , and (4) effect of sex on both WD_{x_0} and k_i . Parameters estimated without the sex covariate are reported for pooled sexes. The most empirically supported model was the logistic (LGF4) with sexually dimorphic asymptotic disk width and growth coefficient parameters, and a shape parameter for pooled sexes

Model	p	MSE	AIC_c	ΔAIC_c
VBGF1	4	2.91	651.57	23.91
VBGF2	5	1.95	634.52	6.85
VBGF3	5	2.04	636.67	9.00
VBGF4	6	1.85	634.42	6.75
GGF1	4	2.74	648.67	21.01
GGF2	5	1.85	631.97	4.31
GGF3	5	1.89	633.05	5.38
GGF4	6	1.80	633.14	5.48
LGF1	4	2.66	647.24	19.58
LGF2	5	1.85	631.89	4.23
LGF3	5	1.88	632.79	5.13
LGF4	6	1.61	627.66	0.00

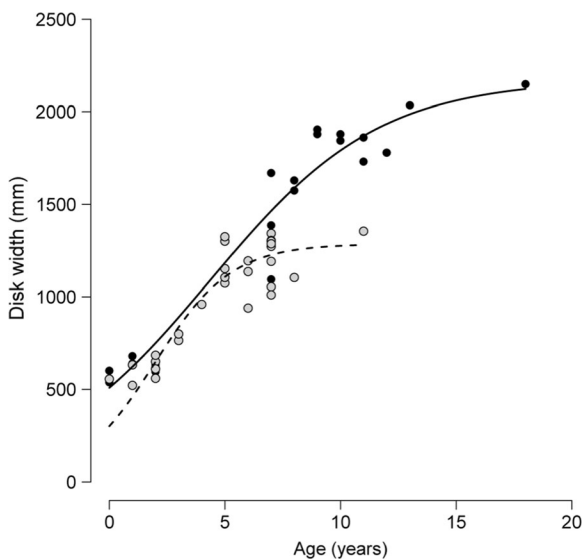
individuals examined from the eastern Atlantic (Başusta et al. 2012; Özbek et al. 2016), differences in maximum size, or variations in rates of growth, among other factors. Understanding intraspecific differences in weight-at-length relationships requires adequate ontogenetic and spatio-temporal coverage of a species (Froese 2006).

To account for changes in rates of batoid growth across juvenile, maturing, and adult life stages, sigmoid functions including logistic and Gompertz growth models have increasingly been utilized (e.g., Mollet et al. 2002; Dale and Holland 2012; White et al. 2014). However, Smart et al. (2016) found little evidence that sigmoid functions consistently perform better than von Bertalanffy growth models for chondrichthyans in general. Growth of *G. altavela* was best described by logistic and Gompertz models, with the former having the greatest statistical support, while less support was associated with von Bertalanffy models that estimated asymptotic size with low precision. The logistic growth model estimated biologically reasonable values of asymptotic size and size-at-birth observed in this and previous studies of western North Atlantic *G. altavela* (Bigelow and Schroeder 1953; Daiber and Booth 1960). Maximum size observed in the present study (2150 mm WD) is

Table 4 Parameter estimates \pm standard errors from the most empirically supported growth model (LGF4) fitted to western North Atlantic *Gymnura altavela* disk width-at-age data. Asymptotic disk width (WD_{∞}) and the growth coefficient (k) parameters

		WD_{∞}	k	b	WD_0
LGF4	Pooled			1.19 ± 0.12	404.70 ± 38.72
	Males	1285.46 ± 67.27	0.60 ± 0.10		300.80 ± 33.81
	Females	2173.51 ± 129.78	0.27 ± 0.04		508.61 ± 49.63

similar to the largest *G. altavela* (2170 mm WD) sampled from the same region prior to 1999 (Wigley et al. 2003), but smaller than 2600 mm WD reported by Schwartz (1984). In the western and eastern Mediterranean, smaller maximum sizes ranging from 1342 to 1650 mm WD are reported for this species (Capapé et al. 1992; Bařusta et al. 2012; Alkusairst et al. 2014; Özbek et al. 2016). Consequently, there is uncertainty in the taxonomic status of *G. altavela* from U.S. and Mediterranean waters, and recent molecular evidence suggests that individuals from the coast of Senegal (type locality) may be genetically distinct from U.S. individuals (Naylor et al. 2012; Alkusairst et al. 2014). Thus, broader spatio-temporal sampling and taxonomic evaluation of eastern and western Atlantic populations are needed to better understand variation in the growth patterns of this species (Goldman 2005; Alkusairst et al. 2014).

**Fig. 7** Logistic growth model fit to disk width-at-age data for male (gray circles, dashed line, $n = 28$) and female (black circles, solid line, $n = 21$) western North Atlantic *Gymnura altavela*

were modeled with a sex covariate, and the shape parameter (b) was modeled for pooled sexes. Disk width-at-birth (WD_0) was derived from the parameter estimates of the LGF4 model

Growth model results from the present study may be widely applied to other batoid taxa to improve understanding of the life history strategies, ecology, and systematic relationships of this diverse group. Sex-specific growth coefficients estimated by the logistic model for *G. altavela* ($k_{\text{male}} = 0.60$; $k_{\text{female}} = 0.27$) were similar to values reported for both large-bodied (> 2400 mm WD) rays, such as spinetail devilray (*Mobula japonica*; pooled sexes $k = 0.28$; Cuevas-Zimbrón et al. 2012), and relatively small-bodied (< 1000 mm WD) species including western North Atlantic cownose ray (*Rhinoptera bonasus*; $k_{\text{male}} = 0.26$ – 0.27 ; $k_{\text{female}} = 0.19$; Fisher et al. 2013), eastern Pacific round stingray (*Urotrygon rogersi*; $k_{\text{male}} = 0.65$; $k_{\text{female}} = 0.22$; Mejía-Falla et al. 2014) and western Pacific fanray (*Platyrrhina sinensis*; $k_{\text{male}} = 0.56$; $k_{\text{female}} = 0.28$; Kume et al. 2008). Female gymnurids have a higher energy demand due to matrotrophy, likely resulting in their slower growth rates compared to males. Furthermore, the large body size of female *G. altavela* may impart an evolutionary advantage (e.g., larger offspring and higher fecundity) compared to most other batoids. Future investigations into stingray life history strategies across broad geographic scales are needed to identify key parameters (e.g., age at maturity and fecundity) for improved assessments of populations.

Maternal provisioning of nutrients in butterfly rays results in extreme increases in organic matter between the egg and term embryo stages (Ranzi 1934), yielding relatively large-bodied neonates. Size-at-birth calculated from the logistic model parameter estimates was 405 mm WD for pooled sexes, 301 mm WD for males, and 509 mm WD for females. The smallest free-swimming *G. altavela* observed during this study was 496 mm WD, while Wigley et al. (2003) reported a 200 mm WD specimen in the western North Atlantic; however, it is possible that this latter individual was an aborted embryo rather than a free-swimming neonate.

Future investigations should focus on trends in energy allocation (including quantifying the magnitude of maternal provisioning over the course of gestation) and physiological responses to environmental influences (e.g., effect of seasonal temperature fluctuations on growth) during ontogeny for improved understanding of growth and longevity in this species.

The present study provides the first known estimates of age and sex-specific growth patterns for any species of *Gymnura*, contributing to the sparse life history data available for western North Atlantic rays. Results from the present study suggest that *G. altavela* displays moderately fast rates of growth and average longevity relative to other ray species for which age and growth information is available, suggesting the potential for reduced vulnerability of the population to depletion from overexploitation relative to slower growing and longer lived taxa. However, certain life history traits are known to increase extinction risk (e.g., large body size, shallow-water residency, and low fecundity) (Dulvy et al. 2014), and may have contributed to the depletion of stocks in the Mediterranean and southern portion of the western Atlantic. These factors emphasize the need for further monitoring of U.S. populations, as well as investigating the effects of non-target fisheries on post-release survival. Equally important initiatives to identify and preserve habitats essential for parturition and survival of early life stages of *G. altavela* should be included in the development of management strategies for the conservation of biodiversity and preservation of healthy ecosystems along the U.S. Atlantic coast. Collectively, such efforts rely on species-specific data, hence a taxonomic re-evaluation of *G. altavela* is recommended to delineate the species' range of distribution and life history parameters, which are essential for predicting the vulnerability of populations.

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

References

- Akaike H (1973) Information theory as an extension of the maximum likelihood. In: Petrov BN, Csaki F (eds) Proceedings of the second international symposium on information theory. Akademiai Kiado, Budapest, pp 267–278
- Alkusaary H, Ali M, Saad A, Reynaud C, Capapé C (2014) Maturity, reproductive cycle, and fecundity of spiny butterfly ray, *Gymnura altavela* (Elasmobranchii: Rajiformes: Gymnuridae), from the coast of Syria (eastern Mediterranean). Acta Ichthyol Piscat 44(3):229–240
- Başusta A, Başusta N, Sulikowski JA, Driggers WB, Demirhan SA, Cicek E (2012) Length-weight relationships for nine species of batoids from the Iskenderun Bay, Turkey. J Appl Ichthyol 28(5):850–851
- Beamish RJ, Fournier DA (1981) A method for comparing the precision of a set of age determinations. Can J Fish Aquat Sci 38:982–983
- Beverton RJH, Holt SJ (1957) On the dynamics of exploited fish populations. U K Minist Agric Fisheries, Fish Investig (Series 2) 19:533
- Bigelow HB, Schroeder WC (1953) Fishes of the Western North Atlantic. Sawfishes, Guitarfishes, Skates, Rays, and Chimaeroids, No. 1, Part 2. Yale University Press. Memoirs Sears Foundation for Marine Research, Yale Univ., New Haven
- Bini G (1967) Atlante dei pesci delle coste italiane. Vol. I. Leptocardi, Ciclostomi, Selaci. Mondo Sommerso Editrice, Milano, pp 206
- Bornatowski H, Wosnick N, Do Carmo WPD, Corrêa MFM, Abilhoa V (2014) Feeding comparisons of four batoids (Elasmobranchii) in coastal waters of southern Brazil. J Mar Biol Assoc UK 94:1491–1499. <https://doi.org/10.1017/S0025315414000472>
- Bowker AH (1948) A test for symmetry in contingency tables. J Am Stat Assoc 43(244):572–574

- Brander K (1981) Disappearance of common skate *Raja batis* from the Irish Sea. *Nature* 290:48–49
- Brown CA, Gruber SH (1988) Age assessment of the lemon shark, *Negaprion brevirostris*, using tetracycline validated vertebral centra. *Copeia* 1988:747–753
- Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information theoretic approach, 2nd edn. Springer, NY
- Cailliet GM, Goldman KJ (2004) Age determination and validation in chondrichthyan fishes. In: Carrier J, Musick JA, Heithaus M (eds) *Biology of sharks and their relatives*. CRC Press, Boca Raton, pp 399–448
- Cailliet GM, Smith WD, Mollet HF, Goldman KJ (2006) Chondrichthyan growth studies: an updated review, stressing terminology, sample size sufficiency, validation, and curve fitting. In: Carlson JK, Goldman KJ (eds) *Age and growth of Chondrichthyan fishes: new methods, techniques and analysis*. Springer, Dordrecht, pp 211–228
- Campana SE, Annand MC, McMillan JI (1995) Graphical and statistical methods for determining the consistency of age determinations. *Trans Am Fish Soc* 124:131–138
- Capapé C, Zaouli J, Tomansini JA, Bouchereau JL (1992) Reproductive biology of the spiny butterfly ray, *Gymnura altavela* (Linnaeus, 1758) (Pisces: Gymnuridae) from off the Tunisian coasts. *Sci Mar* 56:347–355
- Casey JG, Pratt HL, Stillwell C (1985) Age and growth of the sandbar shark (*Carcharhinus plumbeus*) from the western North Atlantic. *Can J Fish Aquat Sci* 42(5):963–975
- Chang WYB (1982) A statistical method for evaluating the reproducibility of age determination. *Can J Fish Aquat Sci* 39: 1208–1210
- Cuevas-Zimbrón E, Sosa-Nishizaki O, Pérez-Jiménez JC, O'Sullivan JB (2012) An analysis of the feasibility of using caudal vertebrae for ageing the spinetail devilray, *Mobula japonica* (Müller and Henle, 1841). *Environ Biol Fish* 96(8): 907–914
- Daiber FC, Booth RA (1960) Notes on the biology of the butterfly rays, *Gymnura altavela* and *Gymnura micrura*. *Copeia* 1960: 137–139
- Dale JJ, Holland KN (2012) Age, growth and maturity of the brown stingray (*Dasyatis lata*) around Oahu, Hawai'i. *Mar Freshw Res* 63:475–484
- Dulvy NK, Metcalfe JD, Glanville J, Pawson MG, Reynolds JD (2000) Fishery stability, local extinctions, and shifts in community structure in skates. *Conserv Biol* 14(1):283–293
- Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM, Harrison LR, Carlson JK, Davidson LN, Fordham SV, Francis MP, Pollock CM, Simpfendorfer CA, Burgess GH, Carpenter KE, Compagno LJ, Ebert DA, Gibson C, Heupel MR, Livingstone SR, Sanciangco JC, Stevens JD, Valenti S, White WT (2014) Extinction risk and conservation of the World's sharks and rays. *Elife*, Cambridge 3:e00590. <https://doi.org/10.7554/eLife.00590>
- Ebert DA, Bizzarro JJ (2007) Standardized diet compositions and trophic levels of skate (Chondrichthyes: Rajiformes: Rajoidei). *Environ Biol Fish* 80(2–3):221–237
- Ebert DA, Stehmann MFW (2013) Sharks, batoids, and chimeras of the North Atlantic. *FAO Species Catalogue for Fishery Purposes*, No. 7. Food and Agriculture Organization, Rome
- Evans GT, Hoenig JM (1998) Testing and viewing symmetry in contingency tables, with application to readers of fish ages. *Biometrics* 54:620–629
- Fisher RA, Call GC, Grubbs RD (2013) Age, growth, and reproductive biology of cownose rays (*Rhinoptera bonasus*) in Chesapeake Bay. *Mar Coast Fish Dynam Manag Ecosys Sci* 5(1):225–234
- Frisk MG (2010) Life history strategies of batoids. In: Carrier JC, Musick JA, Heithaus MR (eds) *Sharks and their relatives II: biodiversity, adaptive physiology, and conservation*. CRC Press, Boca Raton, pp 283–316
- Frisk MG, Miller TJ, Martell SJD, Sosebee K (2008) New hypothesis helps explain elasmobranch 'outburst' on Georges Bank in the 1980s. *Ecol Appl* 18:234–245
- Froese R (2006) Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *J Appl Ichthyol* 22:241–253
- Geraghty PT, Jones AS, Stewart J, MacBeth WG (2012) Micro-computed tomography: an alternative method for shark ageing. *J Fish Biol* 80:1292–1299
- Goldman KJ (2005) Age and growth of elasmobranch fishes. In: Musick JA, Bonfil R (eds) *Management techniques for elasmobranch fisheries*. FAO Fish Tech Pap 474, Rome, pp 97–132
- Heithaus MR, Frid A, Vaudo JJ, Worm B, Wirsing AJ (2010) Unraveling the ecological importance of elasmobranchs. In: Carrier JC, Musick JA, Heithaus MR (eds) *Sharks and their relatives II: biodiversity, adaptive physiology, and conservation*. CRC Press, Boca Raton, pp 607–633
- Henningsen AD (1996) Captive husbandry and bioenergetics of the spiny butterfly ray, *Gymnura altavela* (Linnaeus). *Zoo Biol* 15(2):135–142
- Hilton EJ, Schnell NK, Konstantinidis P (2015) When tradition meets technology: systematic morphology of fishes in the early 21st century. *Copeia* 103(4):858–873
- Hoenig JM, Gruber SH (1990) Life-history patterns in the elasmobranchs: implications for fisheries management. NOAA NMFS Technical Report 90. National Marine Fisheries Service, Washington DC, pp 1–16
- Hoenig JM, Morgan MJ, Brown C (1995) Analyzing differences between two age determination methods by tests of symmetry. *Can J Fish Aquat Sci* 52:364–368
- Ismen A (2003) Age, growth, reproduction and food of common stingray (*Dasyatis pastinaca* L., 1758) in Iskenderun Bay, the eastern Mediterranean. *Fish Res* 60:PII S0165-7836(02): 00058–00059
- Jacobsen IP, Bennett MB (2010) Age and growth of *Neotrygon picta*, *Neotrygon annotata* and *Neotrygon kuhlii* from north-east Australia, with notes on their reproductive biology. *J Fish Biol* 77:2405–2422
- Jacobsen IP, Bennett MB (2011) Life history of the blackspotted whipray *Himantura astra*. *J Fish Biol* 78(4):1249–1268
- Jacobsen IP, Johnson JW, Bennett MB (2009) Diet and reproduction in the Australian butterfly ray *Gymnura australis* from northern and north-eastern Australia. *J Fish Biol* 75:2475–2489
- Kimura DK (2008) Extending the von Bertalanffy growth model using explanatory variables. *Can J Fish Aquat Sci* 65(9): 1879–1891
- Kume G, Furumitsu K, Yamaguchi A (2008) Age, growth and age at sexual maturity of fan ray *Platyrrhina sinensis* (Batoidea: Platyrrhinidae) in Ariake Bay, Japan. *Fish Sci* 74:736–742

- Linnaeus C (1758) *Systema Naturae*, Ed. X. *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Tomus I. Editio decima, reformata. Holmiae 1:1–824
- Maisey JG (2001a) CT-scan reveals new cranial features in Devonian chondrichthyan “*Cladodus*” *wildungensis*. *J Vertebr Paleontol* 21:807–810
- Maisey JG (2001b) Remarks on the inner ear of elasmobranchs and its interpretation from skeletal labyrinth morphology. *J Morphol* 250:236–264
- Maisey JG (2004) Morphology of the braincase in the Broadnose Sevengill shark *Notorynchus* (Elasmoobranchii, Hexanchiformes), based on CT scanning. *Am Mus Novit* 3429:1–52
- Mandelman JW, Cicia AM, Ingram GW, Driggers WB, Coutre KM, Sulikowski JA (2012) Short-term post-release mortality of skates (family Rajidae) discarded in a western North Atlantic commercial otter trawl fishery. *Fish Res* 139:76–84
- Martin LK, Cailliet GM (1988) Age and growth determination of the bat ray, *Myliobatis californica* gill, in Central California. *Copeia* 1988:754–762
- McEachran JD, Capapé C (1984) Gymnuridae. In: Whitehead PJP, Bauchot M-L, Hureau JC, Nielsen J, Tortonese E (eds) *Fishes of the north-eastern Atlantic and the Mediterranean*. Vol 1 Unesco, Paris, pp 203–204
- McEachran JD, de Carvalho MR (2002) Batoid Fishes. In: Carpenter KE (ed) *The living marine resources of the western Central Atlantic*. Vol 1 Introduction, molluscs, crustaceans, hagfishes, sharks, batoid fishes and chimaeras. *FAO Species Identification Guide for Fisheries Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5*, Rome, FAO, pp 508–589
- McEachran JD, Dunn KA (1998) Phylogenetic analysis of skates, a morphologically conservative clade of elasmobranchs (Chondrichthyes: Rajidae). *Copeia* 1998:271–290
- McEachran JD, Séret B (1990) Gymnuridae. In: Quero JC, Hureau JC, Karrer C, Post A, Saldanha L (eds) *Check-list of the fishes of the eastern tropical Atlantic (CLOFETA)*, Vol 1. JNICT, Lisbon; SEI, Paris; and UNESCO, Paris, pp 64–66
- Mejía-Falla PA, Cortés E, Navia AF, Zapata FA (2014) Age and growth of the round stingray *Urotrygon rogersi*, a particularly fast-growing and short-lived elasmobranch. *PLoS One* 9(4):e96077. <https://doi.org/10.1371/journal.pone.0096077>
- Mollet HF, Ezcurra JM, O’Sullivan JB (2002) Captive biology of the pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832). *Mar Freshw Res* 53:531–541
- Moyer JK, Riccio ML, Bemis WE (2015) Development and microstructure of tooth histotypes in the blue shark, *Prionace glauca* (Carcharhiniformes: Carcharhinidae) and the great white shark, *Carcharodon carcharias* (Lamniformes: Lamnidae). *J Morphol* 276:797–817
- Murawski SA (1991) Can we manage our multispecies fisheries? *Fisheries* 16(5):5–13
- Natanson LJ (1993) Effect of temperature on band deposition in the little skate, *Raja erinacea*. *Copeia* 1993:199–206
- Naylor GJP, Caira JN, Jensen K, Rosana K, White W, Last P (2012) A DNA sequence based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bull Am Mus Nat Hist* 367, p 263
- Neer JA, Thompson BA (2005) Life history of the cownose ray, *Rhinoptera bonasus*, in the northern Gulf of Mexico, with comments on geographic variability in life history traits. *Environ Biol Fish* 73:321–331
- O’Shea OR, Braccini M, McAuley R, Speed CW, Meekan MG (2013) Growth of tropical dasytid rays estimated using a multi-analytical approach. *PLoS One* 8(10):e77194. <https://doi.org/10.1371/journal.pone.0077194>
- Okamura H, Punt AE, Semba Y, Ichinokawa M (2013) Marginal increment analysis: a new statistical approach of testing for temporal periodicity in fish age verification. *J Fish Biol* 82: 1239–1249
- Özbek EO, Çardak M, Kebapcıoğlu T (2016) Spatio-temporal patterns of abundance, biomass and length-weight relationships of *Gymnura altavela* (Linnaeus, 1758) (Pisces: Gymnuridae) in the Gulf of Antalya, Turkey (Levantine Sea). *J Black Sea/Mediterr Environ* 21:169–190
- Quinn TJ, Deriso RB (1999) *Quantitative fish dynamics*. Oxford University Press, New York, p 560
- R Development Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Raje SG (2003) Some aspects of biology of four species of rays off Mumbai water. *Indian J Fish* 50(1):89–96
- Ranzi S (1934) Le basi fisio-morfologiche dello sviluppo embrionale dei Selaci. Parti II e III. *Publication Stazione Zoologica di Napoli* 13:331–437
- IUCN Red List (2016) International Union for conservation of nature and natural resources – IUCN red list of threatened species. <http://www.iucnredlist.org/>. Accessed 07 Feb 2017
- Ricker WE (1979) Growth rates and models. In: Hoar WS, Randall DJ (eds) *Fish physiology*. Vol VIII. Academic Press, New York, pp 677–743
- Ridewood WG (1921) On the calcification of the vertebral centra in sharks and rays. *Phil trans R Soc B. Biologicals* 210:311–407
- Ritz C, Streibig JC (2008) *Nonlinear regression with R*. Springer, New York, p 144
- Robins CR, Ray GC (1986) *A field guide to Atlantic coast fishes of North America*. Houghton Mifflin Company, Boston, p 354
- Romine JG, Musick JA, Johnson RA (2013) Compensatory growth of the sandbar shark in the western North Atlantic including the Gulf of Mexico. *Mar Coast Fish* 5:189–199
- Schultze HP, Cloutier R (1991) Computed-tomography and magnetic resonance imaging studies of *Latimeria chalumnae*. *Environ Biol Fish* 32:159–181
- Schwartz FJ (1984) *Sharks, sawfish, skates, and rays of the Carolinas*. Special Publication, Institute of Marine Sciences, University of North Carolina, Morehead City
- Seber GAF (1982) *The estimation of animal abundance and related parameters*, 2nd edn. The Blackburn Press, Caldwell, p 654
- Simpfendorfer CA, Heupel MR, White WT, Dulvy NK (2011) The importance of research and public opinion to conservation management of sharks and rays: a synthesis. *Mar Freshw Res* 62:518–527
- Smart JJ, Chin A, Tobin AJ, Simpfendorfer CA (2016) Multimodel approaches in shark and ray growth studies: strengths, weaknesses and the future. *Fish Fish* 17(4):955–971
- Smith WD, Cailliet GM, Melendez EM (2007) Maturity and growth characteristics of a commercially exploited stingray, *Dasyatis dipterura*. *Aust J Mar Freshwat Res* 58:54–66

- Smith JW, Merriner JV (1987) Age and growth, movements and distribution of the cownose ray, *Rhinoptera bonasus*, in Chesapeake Bay. *Estuaries* 10(2):153–164
- Sprugel DG (1983) Correcting for bias in log-transformed allometric equations. *Ecology* 64:209–210
- Stevens JD, Walker TI, Cook SF, Fordham S (2005) Threats faced by chondrichthyan fishes. In: Fowler SL, Cavanagh R, Camhi M, Burgess GH, Cailliet GM, Fordham S, Simpfendorfer CA, Musick JA (eds) *Sharks, rays and chimaeras: the status of the Chondrichthyan fishes*. IUCN species survival commission shark specialist group, gland and, Cambridge, pp 48–57
- Sulikowski JA, Morin MD, Suk SH, Howell WH (2003) Age and growth of the winter skate, *Leucoraja ocellata*, in the Gulf of Maine. *Fish Bull* 101:405–413
- Sulikowski JA, Elzey S, Kneebone J, Jurek J, Hunting Howell W, Tsang PCW (2007) The reproductive cycle of the smooth skate, *Malacoraja senta*, in the Gulf of Maine. *Aust J Mar Freshwat Res* 58:98–103
- Tamini LL, Chiaramont GE, Perez JE, Cappozzo HL (2006) Batoids in a coastal trawl fishery of Argentina. *Fish Res* 77(3):326–332
- Teixeira EC, Silva VEL, Fabré NN, Batista VS (2016) Length–weight relationships for four stingray species from the tropical Atlantic Ocean. *J Appl Ichthyol* 33:594–596
- Thorson JT, Simpfendorfer CA (2009) Gear selectivity and sample size effects on growth curve selection in shark age and growth studies. *Fish Res* 98:75–84
- Vooren CM, Piercy AN, Snelson Jr. FF, Grubbs RD, Notarbartolo di Sciara G, Serena S (2007) *Gymnura altavela*. The IUCN red list of threatened species 2007: e.T63153A12624290 <https://doi.org/10.2305/IUCN.UK.2007.RLTS.T63153A12624290.en>. Accessed on 07 Feb 2017
- Walker PA, Hessen HJL (1996) Long-term changes in ray populations in the North Sea. *ICES J Mar Sci* 53:1085–1093
- Walker PA, Hislop JRG (1998) Sensitive skates or resilient rays? Spatial and temporal shifts in ray species composition in the central and north-western North Sea between 1930 and the present day. *ICES J Mar Sci* 55:392–402
- Walls RHL, Vacchi M, Notarbartolo di Sciara G, Serena F, Dulvy NK (2016) *Gymnura altavela*. The IUCN red list of threatened species 2016: e.T63153A16527909. Accessed 07 Feb 2017
- White WT, Dharmadi (2007) Species and size compositions and reproductive biology of rays (Chondrichthyes, Batoidea) caught in target and non-target fisheries in eastern Indonesia. *J Fish Biol* 70:1809–1837
- White WT, Platell ME, Potter IC (2001) Relationship between reproductive biology and age composition and growth in *Urolophus lobatus* (Batoidea: Urolophidae). *Mar Biol* 138: 135–147
- White WT, Hall NG, Potter IC (2002) Reproductive biology and growth during pre- and postnatal life of *Trygonoptera personata* and *T. mucosa* (Batoidea: Urolophidae). *Mar Biol* 140:699–712
- White J, Simpfendorfer CA, Tobin AJ, Heupel MR (2014) Age and growth parameters of shark-like batoids. *J Fish Biol* 84: 1340–1353
- Wigley SE, McBride HM, McHugh NJ (2003) Length-weight relationships for 74 fish species collected during NEFSC research vessel bottom trawl surveys, 1992–99. NOAA Tech Memo NMFS NE 171:26
- Witmer LM, Ridgely RC, Dufeu DL, Semones MC (2008) Using CT to peer into the past: 3D visualization of the brain and ear regions of birds, crocodiles, and nonavian dinosaurs. In: Endo H, Frey R (eds) *Anatomical imaging: towards a new morphology*. Springer-Verlag, Tokyo, pp 67–88
- Yokota L, Goitein R, Gianeti MD, Lessa RTP (2012) Reproductive biology of the smooth butterfly ray *Gymnura micrura*. *J Fish Biol* 81(4):1315–1326
- Zhu L, Li L, Liang Z (2009) Comparison of six statistical approaches in the selection of appropriate fish growth models. *Chin J Oceanol Limnol* 27:457–467