

ANALYSIS OF BAND PAIR FORMATION IN  
ELASMOBRANCH VERTEBRAE WITH IMPLICATIONS  
FOR FISHERIES MANAGEMENT

BY  
KELSEY JAMES

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY  
IN  
BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2018

DOCTOR OF PHILOSOPHY DISSERTATION  
OF  
KELSEY JAMES

APPROVED:

Dissertation Committee:

Major Professor      David Bengtson

Brooke Flammang

Jeremy Collie

Nasser H. Zawia

DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND  
2018

## ABSTRACT

Sharks, skates, and rays are particularly sensitive to over-exploitation due to their life history traits (slow growth, late age at maturity, small litter size, and extended longevity). It is important to know the age of sharks, skates, and rays because it has implications for our ability to assess the status of populations and to manage fisheries. They are aged most often using the concentric bands that alternate in appearance: opaque and translucent in their vertebral centra. An opaque and translucent band together (a band pair) is assumed to represent one year of growth and is counted to estimate an individual's age. However, counts of these bands are being shown to underestimate age in a growing number of instances.

An alternate explanation to annual band-pair deposition suggests that the number of band pairs may vary with body size and vertebral centrum morphology and not age. I examined centrum morphology and band-pair counts along the vertebral column within and among species. I measured the morphology of 80 centra from various-sized individuals of both sexes of five batoid species and counted the band pairs in every fifth centrum along the vertebral column from a subset of these individuals. Centrum morphology and band-pair count both varied along the vertebral column in all individuals of all species except young of the year.

This evidence that the number of opaque and translucent bands does not reflect age reinforces the need to understand the differences between these two band types. In the second study, the bulk chemical composition of opaque and translucent bands was examined using energy-dispersive X-ray spectrometry, focusing on 11 elements across 12 elasmobranch species. I found that there was no difference in chemical composition

between opaque and translucent bands in the little skate, *Leucoraja erinacea*, ( $p = 0.954$ ) or across the 12 species ( $p = 0.532$ ). Vertebral centra are composed mostly of oxygen, calcium, and phosphorus. The evolutionary significance of optical differences between opaque and translucent band types requires further research.

Validation of age estimates from band-pair counts has only been successful for individuals at or prior to sexual maturity. Therefore, I investigated the rate of formation of band pairs in mature individuals. Mature male and female little skates were injected with oxytetracycline and maintained in captivity for 13 months to assess centrum growth and the frequency of band-pair deposition. Of 41 individuals analyzed, 63% did not deposit a full band pair over the 13-month period, meaning that a majority of individuals did not exhibit deposition of an annual band pair.

Such potentially inaccurate age estimates are still used in the construction of stock-assessment models that dictate how elasmobranch fisheries are managed. To reconcile the fact that the data for stock assessment models is biased I examined the effect of intentionally biased age data on stock assessment model output in the final part of the study. Length-at-age data for little skate and winter skate were biased  $\pm 10\%$  and  $\pm 25\%$  of the lifespan for (1) all ages and (2) mature ages only. For each species, these eight scenarios and an unbiased (normal) scenario were modeled with the von Bertalanffy growth model and applied to a statistical catch-at-age model. The effects of biased age data were subtle and had the largest effect on estimating spawning stock biomass. As age underestimation is identified in more elasmobranch species, research on the implications of biased age estimates that are incorporated into stock assessment results will be crucial until an alternate method to estimate elasmobranch age is found.

## **ACKNOWLEDGMENTS**

I would like to first thank my major professor, Dr. David Bengtson. I next must thank Dr. Lisa Natanson. You have been an exceptional mentor and friend. I wouldn't be here if I hadn't bombarded your house one fateful winter with Megan Winton and then bombarded you again at that ageing workshop in La Jolla, CA. I would like to thank my committee Dr. Brooke Flammang and Dr. Jeremy Collie for making sure my science was sound, well-thought out, and properly executed. I would like to thank my dissertation defense chair Dr. Jacqueline Webb for extensive edits of all manuscripts and pushing me to become a better writer, I hope.

I would like to thank Rodman Sykes and the crew of the F/V Virginia Marise, and the crew of the Cap'n Bert for the collection of skates. I would like to thank Dr. Kady Lyons and Dr. Doug Adams for stingray samples. I would like to thank the soon-to-be Dr. Megan Winton for statistical advice and R code. I would like to thank Dr. Vanessa Trijoulet and Dr. Kathy Sosebee for modeling guidance and all the skate data I could ever want. I would like to thank Dr. Michael Frisk for digging up and sharing his data with me. I would like to thank Ed Baker for husbandry assistance. I would like to thank Dr. Chris O'Brien at the University of New Haven and Louie Kerr at the Marine Biological Laboratory for assistance and use of the SEM and EDS equipment.

I would like to thank the friends and colleagues I've made at URI, the list is long and I'll never forget how we all got through this together. Perhaps unusually I would like to thank everyone I've met through volleyball: Newport Volleyball Club and Yankee. You brought balance to my life. Lastly, I would like to thank my parents. You are an

unending supply of support – financial and otherwise. I have never had a doubt I could do whatever I wanted with my life and that's thanks to you. I love you.

## **PREFACE**

This dissertation is presented in manuscript format with four chapters each representing a unique manuscript. Chapter 1 is in preparation to be submitted to Environmental Biology of Fishes. Chapter 2 is in preparation to be submitted to Journal of Morphology. Chapter 3 in review with the Journal of Fish Biology. Chapter 4 is in preparation to be submitted to Fisheries Research.

## TABLE OF CONTENTS

<b>ABSTRACT .....</b>	<b>ii</b>
<b>ACKNOWLEDGMENTS .....</b>	<b>iv</b>
<b>PREFACE.....</b>	<b>vi</b>
<b>TABLE OF CONTENTS.....</b>	<b>vii</b>
<b>LIST OF TABLES .....</b>	<b>viii</b>
<b>LIST OF FIGURES .....</b>	<b>x</b>
<b>CHAPTER 1 .....</b>	<b>1</b>
<b>CHAPTER 2 .....</b>	<b>35</b>
<b>CHAPTER 3 .....</b>	<b>62</b>
<b>CHAPTER 4 .....</b>	<b>95</b>
<b>CONCLUSIONS .....</b>	<b>120</b>

## LIST OF TABLES

### **Chapter 1**

Table 1.1 Individuals of five batoid species used in this study.....	6
Table 1.2 Generalized additive model results for 10 different models to best describe centrum morphology along the vertebral column for five batoid species. k is the number of knots used in each model.....	18
Table 1.3 Bias and precision of band-pair counts within and between readers. Asterisk indicates significant bias .....	19
Table 1.4 Linear mixed-effects model comparing band-pair counts with the three centrum measurements with individual included in the model as a random effect. Asterisk indicates significant correlation .....	22

### **Chapter 2**

Table 2.1 Percentage composition for opaque and translucent bands of each of eleven elements for 32 opaque bands and 32 translucent bands .....	44
Table 2.2 Pairwise correlation coefficients (r) for eleven elements.....	45

### **Chapter 3**

Table 3.1 Differences in ten growth variables in males versus females at the end of the 13-month experimental period for <i>Leucoraja erinacea</i> analyzed using t-tests. LT is total length and WT is total weight. Asterisk indicates significant difference. ....	72
Table 3.2 Sex, location of oxytetracycline mark, size, and weight of captive <i>Leucoraja erinacea</i> used in this study. ....	78
Table 3.3 Differences between the number of band pairs present distal to the	

oxytetracycline mark (0.5, 1) and seven growth variables in males and females at the end of 13-month experimental period for *Leucoraja erinacea* analyzed using logistic regressions. Asterisk indicates significant difference. .... 80

## **Chapter 4**

Table 4.1 Bias scenarios and corresponding age at maturity, maximum age, and von Bertalanffy parameters used as input into the statistical catch-at-age model for little and winter skates ..... 100

## LIST OF FIGURES

### **Chapter 1**

Figure 1.1 Band-pair count and standardized centrum measurements along the vertebral column for nine little skates analyzed for band pairs .....	11
Figure 1.2 Band-pair count and standardized centrum measurements along the vertebral column for six winter skates analyzed for band pairs.....	12
Figure 1.3 Band-pair count and standardized centrum measurements along the vertebral column for barndoor skates analyzed for band pairs .....	13
Figure 1.4 Band-pair count and standardized centrum measurements along the vertebral column for six Atlantic stingrays analyzed for band pairs .....	15
Figure 1.5 Band-pair count and standardized centrum measurements along the vertebral column for six round rays analyzed for band pairs.....	16
Figure 1.6 Example of generalized additive model variations with (a) all data pooled, data pooled (b) by sex, (c) by size class, and (d) by individual fit to barndoor skate data .....	17

### **Chapter 2**

Figure 2.1 Pin indentations identified with black arrows in (a) the intermedialia of <i>Alopias vulpinus</i> marking an opaque band at the top of the photo and a translucent band in the middle of the photo and (b) the corpus calcareum of <i>Leucoraja ocellata</i> marking opaque bands.....	41
Figure 2.2 Observed percentage of (a) oxygen, (b) calcium, (c) phosphorus, and (d) sulfur for 12 elasmobranch species separated by opaque and translucent bands.....	43

Figure 2.3 First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae between opaque and translucent bands using eleven elements. Each polygon represents a convex hull. Grey lines represent the distance to the centroid .....	47
Figure 2.4 First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae among 12 species using eleven elements. Each polygon represents a convex hull. Grey lines represents the distance to the centroid.....	48
Figure 2.5 First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae among 12 species and band type (O = opaque; T = translucent) using eleven elements. Each colored line connects each data point with its centroid. Each polygon represents a convex hull. Grey lines represent the distance to the centroid .....	49
Figure 2.6 First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae among shark and batoid body plan using eleven elements. Each polygon represents a convex hull. Grey lines represent the distance to the centroid.....	50
Figure 2.7 First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae among shark and batoid body plan and band type using eleven elements. Each polygon represents a convex hull. Grey lines represent the distance to the centroid .....	51

### **Chapter 3**

Figure 3.1 (a) Diagram of a typical *Leucoraja erinacea* indicating the position of

different vertebrae (1, ~24, and 80). The transition between abdominal and caudal vertebrae varies among individuals but occurs at vertebra 24 on average. The dotted box indicates where vertebrae were sampled. (b) Parts of a vertebra and (c) resulting horizontal section used for ageing. The rectangle in (b) is represented in (c). (c) is a section from a Pacific starry skate, <i>Raja stellulata</i> .....	65
Figure 3.2 Sample histological sections with oxytetracycline (indicated by white arrow) superimposed. (a) Oxytetracycline in the ultimate band. (b) Oxytetracycline with a band pair formed distally. Oxytetracycline was deposited in a translucent band in both images. Light grey line indicates the edge of the corpus calcareum. Orientation is from left to right: rostral (R) to caudal (C) .....	70
Figure 3.3 Monthly total number of eggs laid by 20 <i>Leucoraja erinacea</i> by mean monthly water temperature (°C) in 2015 and 2016. Error bars represent standard error .....	73
Figure 3.4 (a) Total consumption over 13 months by sex (Females = 21; Males = 20). (b) Mean monthly growth rate over 13 months by sex (Females = 21; Males = 20). (c) Mean total centrum growth over 13 months by sex (Females = 20; Males = 15) .....	75
Figure 3.5 Locations of oxytetracycline in <i>Leucoraja erinacea</i> centra. Oxytetracycline was expected to be across the corpus calcareum (A), but also occurred on the outer edge of the corpus calcareum (B), diffuse in the corpus calcareum (C), diffuse in the intermedia (D), at the focus (E), and in the arch tissue surrounding the centrum (F). Centra belong to specimens #23 and #99.....	77
Figure 3.6 (a) Monthly growth rate for males (black circles, n=20) and females (grey circles, n= 21). Male linear regression: $y = 0.021x - 33.775$ ; $p < 0.01$ ; adj $r^2 = 0.42$ .	

Female linear regression:  $y = -0.0015x + 8.746$ ;  $p > 0.05$ ; adj  $r^2 = -0.04$ . (b) Total centrum growth by individuals in males (black circles, n=15) and females (grey circles, n=20). Male linear regression:  $y = 5.29 \times 10^{-5}x - 0.048$ ;  $p > 0.05$ ; adj  $r^2 = 0.22$ . Female linear regression:  $y = -3.23 \times 10^{-5}x + 0.136$ ;  $p > 0.05$ ; adj  $r^2 = 0.03$  ..... 81

## Chapter 4

Figure 4.1 Von Bertalanffy parameter estimates for the no-bias and eight bias scenarios for (a) <i>L. erinacea</i> and (b) <i>L. ocellata</i> .....	102
Figure 4.2 Observed and estimated total catch data for seven scenarios for (a) little skate and (b) winter skate.....	106
Figure 4.3 Observed and estimated survey catch data for seven scenarios for (a) little skate and (b) winter skate.....	107
Figure 4.4 Estimated spawning stock biomass (SSB) for seven scenarios for (a) little skate and (b) winter skate. The 95% confidence interval around the no-bias scenario is shown in grey .....	108
Figure 4.5 Estimated fishing mortality (F) for seven scenarios for (a) little skate and (b) winter skate.....	110

## **CHAPTER 1**

### **Morphological Variation in Centra Along the Vertebral Column and Through Ontogeny in Five Batoids**

Kelsey C James<sup>1</sup> and Lisa J Natanson<sup>2</sup>

<sup>1</sup>Department of Fisheries, Animal and Veterinary Sciences. University of Rhode Island, Rhode Island 02881, USA

<sup>2</sup>National Marine Fisheries Service, Northeast Fisheries Science Center, NOAA, Narragansett, Rhode Island 02882, USA

*in preparation for submission to Environmental Biology of Fishes*

## **Abstract**

The ageing of elasmobranchs has traditionally relied on the analysis of annual band-pair deposition in vertebral centra. An increasing number of studies show exceptions, in which band-pair counts do not accurately reflect age, particularly for older individuals. An alternate explanation besides annual band-pair deposition suggests that the number of band pairs may vary with body size and vertebral centrum morphology and not age. We measured the morphology (dorso-ventral diameter, lateral diameter, and rostro-caudal length) of 80 centra from various-sized individuals of both sexes of five batoid species (little skate, *Leucoraja erinacea*, winter skate, *Leucoraja ocellata*, barndoor skate, *Dipturus laevis*, Atlantic stingray, *Dasyatis sabina*, and round ray, *Urotrygon halleri*) and counted the band pairs in every fifth centrum from a subset of these individuals. Centrum morphology and band-pair count both varied along the vertebral column in all individuals of all species except young of the year. Centrum morphology, while roughly similar among shark and batoid species, was best described by individual variation rather than trends by sex, size, or species. Variation in band-pair counts among centra within individuals supports the hypothesis that band-pair formation is related to somatic growth and body shape rather than to an annual cycle.

## **Introduction**

The vertebral centra of elasmobranchs have characteristic concentric bands that alternate in appearance: opaque and translucent (Ridewood 1921; Cailliet et al. 2006; Dean and Summers 2006). A band pair composed of one opaque and one translucent

band has been assumed to represent one year of growth and has been used to estimate age in elasmobranch fishes (Ridewood 1921; Haskell 1948; Ishiyama 1951). The basis of this method was the fact that more band pairs in the vertebral centra are formed in larger individuals (Ridewood 1921). Later studies showed a positive relationship between somatic growth and centrum size and seemingly seasonal alternation of opaque and translucent bands (Ishiyama 1951; Jones and Geen 1977; Cailliet and Goldman 2004). However, a growing body of research shows that band-pair deposition slows and/or stops in older animals, so that the number of band pairs does not necessarily accurately reflect age throughout the entire lifespan of an individual (Kalish and Johnston 2001; Francis et al. 2007; Andrews et al. 2011; Hamady et al. 2014; Natanson et al. 2014; Passerotti et al. 2014; Natanson et al. 2015; Harry 2017; Natanson et al. 2018). In some species a direct relationship between the number of band pairs and somatic growth has been suggested (Natanson and Cailliet 1990; Tanaka 1990; Natanson et al. 2008), and this has recently been demonstrated in a study of seven species of sharks (Atlantic angel shark, *Squatina dumeril* Lesueur 1818, white shark, *Carcharodon carcharias* (Linnaeus 1758), porbeagle, *Lamna nasus* (Bonnaterre 1788), shortfin mako, *Isurus oxyrinchus* Rafinesque 1810, common thresher shark, *Alopias vulpinus* (Bonnaterre 1788), blue shark, *Prionace glauca* (Linnaeus 1758), and dusky shark, *Carcharhinus obscurus* (Lesueur 1818)) (Natanson et al. 2018). The results of this study have led to the conclusion that band-pair deposition is related to growth in girth of the fish, such that band-pair deposition is only coincidentally related to age, and only at some life stages (Natanson et al. 2018).

It has been further suggested that differences in deposition patterns are correlated with body type and swimming mode among species (Natanson et al. 2018).

An additional assumption critical for ageing elasmobranchs using vertebral centra is that all centra along the vertebral column have the same number of band pairs at a given point in time. If this assumption is true, then band-pair deposition could be related to age; if it is false, then band-pair deposition cannot be related to age. Several studies (Natanson and Cailliet 1990; Natanson et al. 2008; Huveneers et al. 2013; Natanson et al. 2018) have shown that small (young of the year [YOY]) individuals have the same number of band pairs throughout their vertebral column. However, they also showed that band-pair counts vary for medium (juvenile) and large (mature) individuals of several species in different families (these include Pacific angel shark, *S. californica* Ayres 1859, basking shark, *Cetorhinus maximus* (Gunnerus 1765), ornate wobbegong, *Orectolobus ornatus* (De Vis 1883), Atlantic angel shark, white shark, porbeagle, shortfin mako, common thresher shark, blue shark, and dusky shark). Variation in the number of band pairs among centra along the column of larger fish has been found to be directly correlated with the girth of the fish where the centra were taken. As the sharks grow in girth (which is most pronounced in the abdominal region), the variation in the number of band pairs among centra along the column increase (Natanson et al. 2018). Regardless of species, larger centra have more band pairs indicating that band-pair deposition is a structural requirement of the individual and not related to time (Natanson et al. 2018).

The goal of the present study was to investigate whether centrum morphology and the number of band pairs in a centrum varies along the vertebral column in several

batoid species. To accomplish this, we measured centrum dimensions and counted the band pairs in individual centra along the columns of various-sized individuals of both sexes of five batoid species (little skate, *Leucoraja erinacea* (Mitchill 1825), winter skate, *Leucoraja ocellata* (Mitchill 1815), barndoor skate, *Dipturus laevis* (Mitchill 1818), Atlantic stingray, *Dasyatis sabina* (Lesueur 1824), and round ray, *Urobatis halleri* (Cooper 1863)).

## Methods

Species were chosen based on geographic and life history differences, their identity as local species important for conservation and fisheries management, and their availability. Batoid specimens were obtained opportunistically from commercial fishermen off the coast of Rhode Island and Massachusetts, USA. Forty-two little skates (14 immature [small], 15 near size-at-maturity [medium], and 13 mature [large]) six winter skates and six barndoor skates (two small, two medium, and two large of each), nine Atlantic stingrays (three small, three medium, and three large), and ten round rays (two small, four medium, and four large) were collected for analysis of centrum morphology (Table 1).

For analysis of the number of band pairs among central along the vertebral column, a subset of little skate individuals (one male and two females of similar sizes; three small, three medium, and three large), a subset of Atlantic stingray individuals (two females of similar sizes; two small, two medium, and two large) and a subset of round ray individuals (one male and one female of similar sizes; two small, two medium, and two large) were used from the analysis of centrum morphology. All

Table 1. Individuals of five batoid species used in this study.

Species	TL (cm)	DW (cm)	Size	Sex	Maturity	Transition <sup>a</sup>	Centra Counted <sup>b</sup>	Individual ID
<b>Little Skate</b>	49.0	29.0	L	F	Mature	22	13	LE03
	48.5	29.5	L	M	Mature	22	12	LE01
	48.0	27.0	L	F	Mature	24	17	LE07
	42.8	25.2	M	F	Immature	27	17	LE06
	41.5	23.8	M	M	Mature	26	17	LE05
	39.6	24.0	M	F	Immature	27	17	LE19
	26.1	14.5	S	M	Immature	26	17	LE04
	25.6	15.5	S	F	Immature	21	14	LE02
	23.4	13.7	S	F	Immature	22	15	LE20
	43.0-47.5	24.6-27.3	L	5 F, 5 M	1 Immature, 9 Mature	22-25	N/A	Large
	38.3-42.4	22.8-25.4	M	5 F, 7 M	11 Immature, 2 Mature	23-26	N/A	Medium
	25.1-32.6	15.2-19.8	S	5 F, 6 M	Immature	21-25	N/A	Small
	80.0	49.9	L	F	Mature	27	17	LO04
<b>Winter Skate</b>	75.3	47.8	L	M	Mature	30	17	LO07
	63.3	39.1	M	F	Immature	26	17	LO08
	61.7	40.5	M	M	Immature	27	16	LO09
	43.2	26.9	S	M	Immature	31	17	LO06
	37.1	22.0	S	F	Immature	29	16	LO05
<b>Barndoor Skate</b>	130.0	92.6 <sup>c</sup>	L	M	Mature	25	17	DL04
	117.4	80.9	L	F	Mature	25	17	DL16
	107.5	76.7 <sup>c</sup>	M	F	Mature	27	14	DL01
	90.0	64.3 <sup>c</sup>	M	M	Immature	27	16	DL03
	52.3	37.6	S	F	Immature	31	17	DL05
	49.0	36.3	S	M	Immature	24	17	DL06
<b>Atlantic Stingray</b>	48.9	26.5	L	F	Mature		15	DS24
	44.8	26.9	L	F	Mature	36	17	DS26
	40.0	18.0	M	F	Immature	49	16	DS32
	36.6	17.8	M	F	Immature	46	17	DS30
	34.2	13.4	S	F	Immature	46	16	DS27
	25.0	13.3	S	F	Immature	47	15	DS33
	59.8	30.3	L	F	Mature	31	N/A	DS31
	42.6	16.0	M	F	Immature	46	N/A	DS29
<b>Round Ray</b>	30.0	11.0	S	M	Immature	46	N/A	DS25
	36.7	20.4	L	M	Mature	31	17	UH01
	21.3	20.0	L	F	Mature	32	11	UH08
	30.5	16.7	M	F	Mature	32	17	UH05
	30.0	16.5	M	M	Mature	34	16	UH10
	24.0	13.3	S	M	Immature	34	17	UH09
	22.8	13.1	S	F	Immature	41	17	UH07
	34.2	19.8	L	M	Mature	34	N/A	UH02
	33.4	18.6	L	M	Mature	30	N/A	UH03
	29.9	17.8	M	F	Mature	31	N/A	UH04
	27.2	16.1	M	F	Mature	31	N/A	UH06

<sup>a</sup>Vertebral number where the transition between abdominal and caudal vertebrae occurs.

<sup>b</sup>Number of centra counted for each individual. N/A means band-pair counts were not determined.

<sup>c</sup>Disc Width was estimated using TL to Disc Width relationship from Gedamke 2006.

individuals (one male and one female of similar sizes; two small, two medium, and two large) of winter skate and barndoor skate were analyzed for the number of band pairs.

Total length (TL), measured as the straight-line distance from snout tip to tail tip, and disc width (DW), measured as the straight-line distance from wing tip to wing tip, were measured to the nearest 0.1 cm. Sex and maturity status were determined by visually inspecting gonad condition (Ebert 2005).

### *Centrum Morphology*

The vertebral column was extracted from fish starting with the first vertebra behind the synarcual cartilage and ending at the 80<sup>th</sup> vertebra. Centra posterior to the 80<sup>th</sup> centrum were too small to successfully separate without damage, especially in small individuals in all species. The 80<sup>th</sup> centrum was underneath the first dorsal fin in little skate, just rostral to first dorsal fin in both barndoor and winter skates, beneath the barb in the round ray, and just caudal to the barb in Atlantic stingray. Caudal centra were particularly difficult to separate in little skate, and only centra through approximately the 60<sup>th</sup> centrum from two individuals could be accurately measured. One round ray sustained tail damage and no centra beyond the 48<sup>th</sup> centrum were available.

Each vertebral centrum was measured to the nearest 0.1 mm in three dimensions: dorso-ventral diameter (DVD), medio-lateral diameter (LD) and rostro-caudal length (LEN), using Vernier calipers following Natanson et al. (2018). Each measurement was divided by TL for the skate species and DW for the stingray species

to standardize data across sizes. By convention, TL is used to measure size of skates, while DW is used to measure the size of stingrays. These standardized data were plotted against centrum number for each individual, noting the centrum number at the transition from abdominal cavity to tail.

To assess if centrum morphology was similar within a species, by sex, by size, or varied by individual it was analyzed using multiple generalized additive models (GAMs) fit to each species using mgcv package in R (Wood 2011; R Core Team 2017). Four GAM variations were run for each species: all data pooled, data grouped by sex, by size class, and by individual. For each of the sex, size class, and individual scenarios, three GAM iterations were run: different intercepts only, different smoothing functions only, and different intercepts and smoothing functions. For each GAM variation the number of knots was specified to be larger than the estimated degrees of freedom using the gam.check function of mgcv in R. Model fit was assessed with Akaike's Information Criterion (AIC) (Haddon 2001).

### *Band-pair Counts*

To determine if band-pair number varied along the vertebral column of an individual, every fifth centrum was assigned a random ID number and processed histologically to visually enhance the band pairs (as per Natanson et al. 2007). Every fifth centrum was chosen for analysis to evenly sample the vertebral column. This analysis included the centrum attached to the synarcual, 4-9 abdominal centra, and 7-12 caudal centra, depending on species. Centrum sections were viewed under a dissecting microscope (Nikon SMZ1500®, Melville, NY, USA) using reflected light

and images were captured with a digital camera (Nikon DSR12, Tokyo, Japan) and image processing software (NIS Elements, v. 4.40, Nikon, Tokyo, Japan). The birth band was identified as the first fully-formed band beyond the focus and was associated with an angle change in the corpus calcareum of the centrum (Cailliet and Goldman 2004). Two band-pair counts were made for each individual by a primary (KCJ) and a secondary reader. To assess repeatability of counts, precision was determined using the coefficient of variation (CV) within and between readers (Chang 1982), with a target value of <10%. Bias as a result of either systematic or random error was assessed using the Evans-Hoenig's (1998) test of symmetry. Within reader precision and bias were compared between the first and second count of each reader while between reader precision and bias was compared between the second band-pair counts of both readers. If the second band-pair counts differed by three or more band pairs between primary and the secondary reader, the centrum was examined together and a consensus count was reached. Final band-pair counts were assigned from the primary reader's second count or the consensus count if appropriate. The final band-pair count was plotted by centrum number for each individual. The mean band-pair count and 95% confidence interval (CI) of the mean was calculated among vertebral centra for each individual to test if band-pair count varied significantly among centra along the vertebral column. Band-pair counts that fell outside of the 95% CI indicated significantly different counts within an individual.

A mixed-effects model was used to determine if there was a correlation between band-pair counts and the three centrum measurements (DVD, LD, and LEN) for each species with individual included as a random effect.

## **Results**

### *Centrum Morphology*

Centrum morphology varied along the vertebral column in all species. The transition between abdominal and caudal centra occurred at the 24<sup>th</sup> to the 47<sup>th</sup> centra in the study species (Table 1). Dorso-ventral diameter (DVD) and medio-lateral diameter (LD) for the little skate and winter skate increased from the head and peaked at the level of the mid-abdominal cavity (approximately in line with the pectoral fin tips) then decreased through the transition from abdominal to caudal centra and continued to decrease in the caudal centra (Figures 1 & 2). Dorsal to the abdominal cavity, the centra were wider than they were tall (ovoid), while the more caudal centra were circular. Rostro-caudal length (LEN) in little skate and winter skate increased from the head to the transition from abdominal to caudal centra where the LEN decreased sharply; LEN was constant among the caudal centra (Figures 1 & 2).

Dorso-ventral diameter and medio-lateral diameter along the vertebral column in the barndoor skate demonstrated similar trends as in winter skate and little skate. However, barndoor skate centra were circular throughout most of the vertebral column (Figure 3), except near the caudal end of the abdominal cavity the centra were ovoid. In the barndoor skate LEN followed a similar trend to that in little and winter skate except that, starting at approximately the 45<sup>th</sup> centrum, LEN was greater than DVD or LD (Figure 3). The centra in Atlantic stingray and round ray had similar morphologies, which differed from the skate species examined. In Atlantic stingray and round ray DVD and LD increased from the head, were constant along the

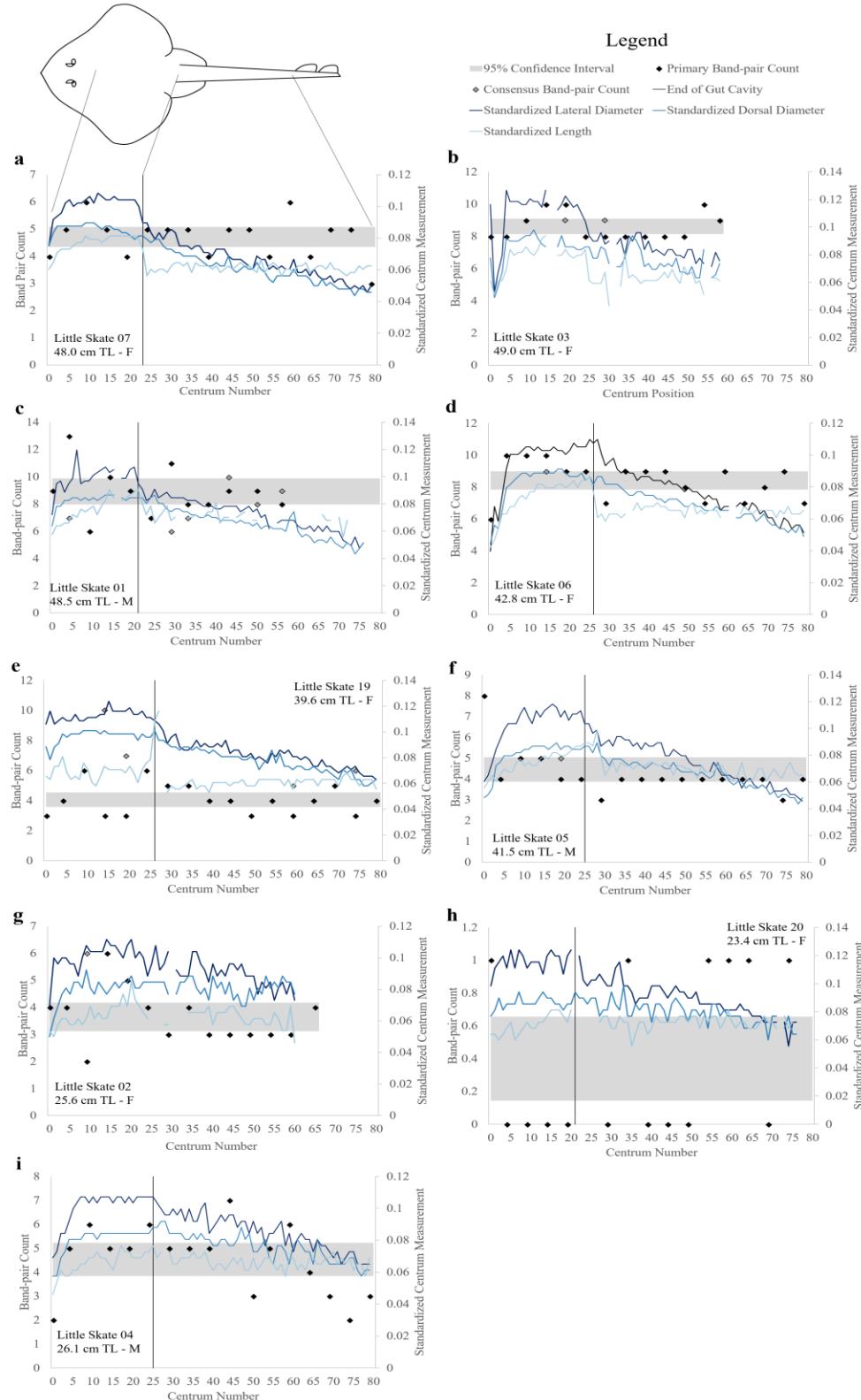


Figure 1 Band-pair count and standardized centrum measurements along the vertebral column for nine little skates analyzed for band pairs.

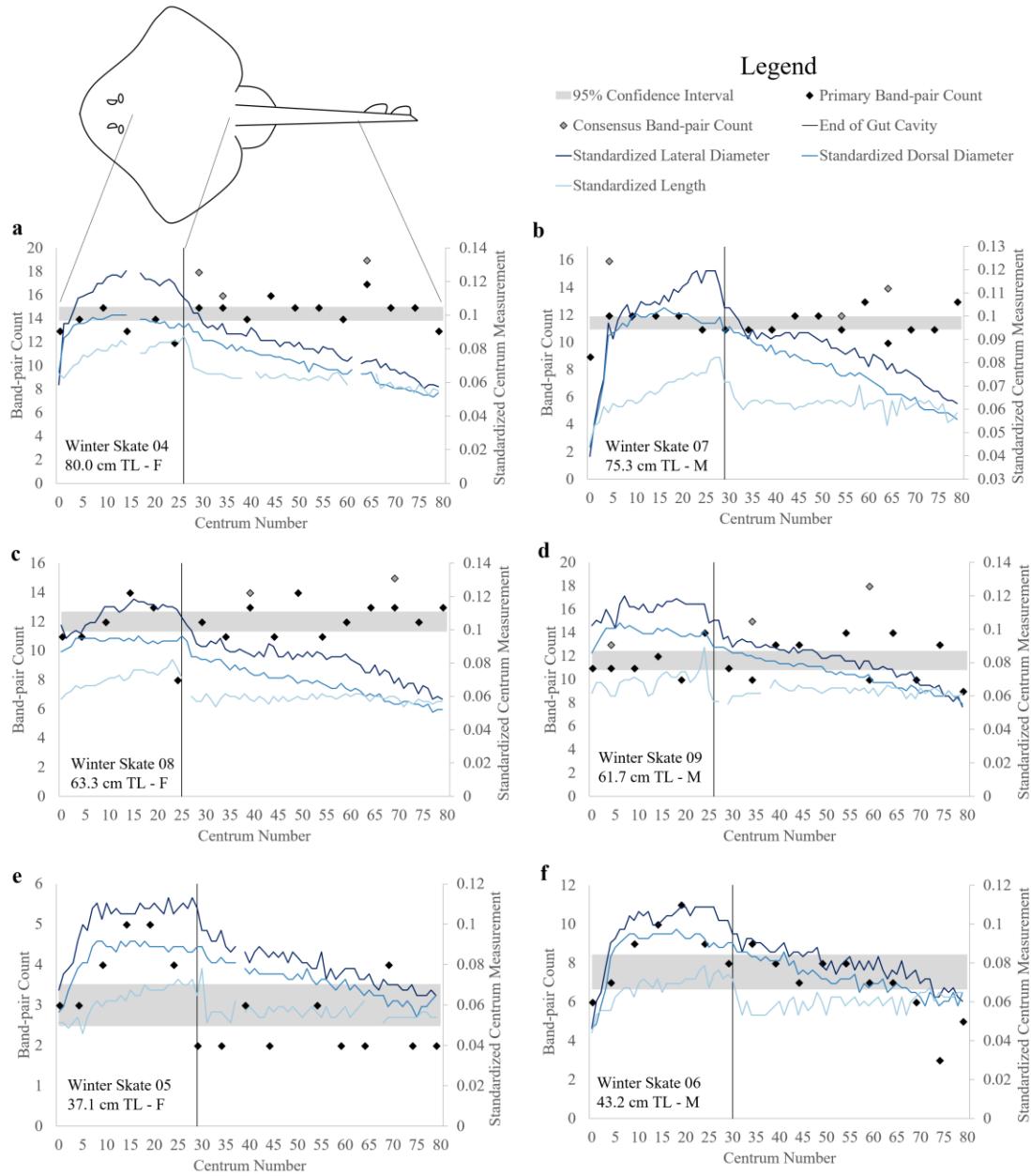


Figure 2. Band-pair count and standardized centrum measurements along the vertebral column for the six winter skates analyzed for band pairs.

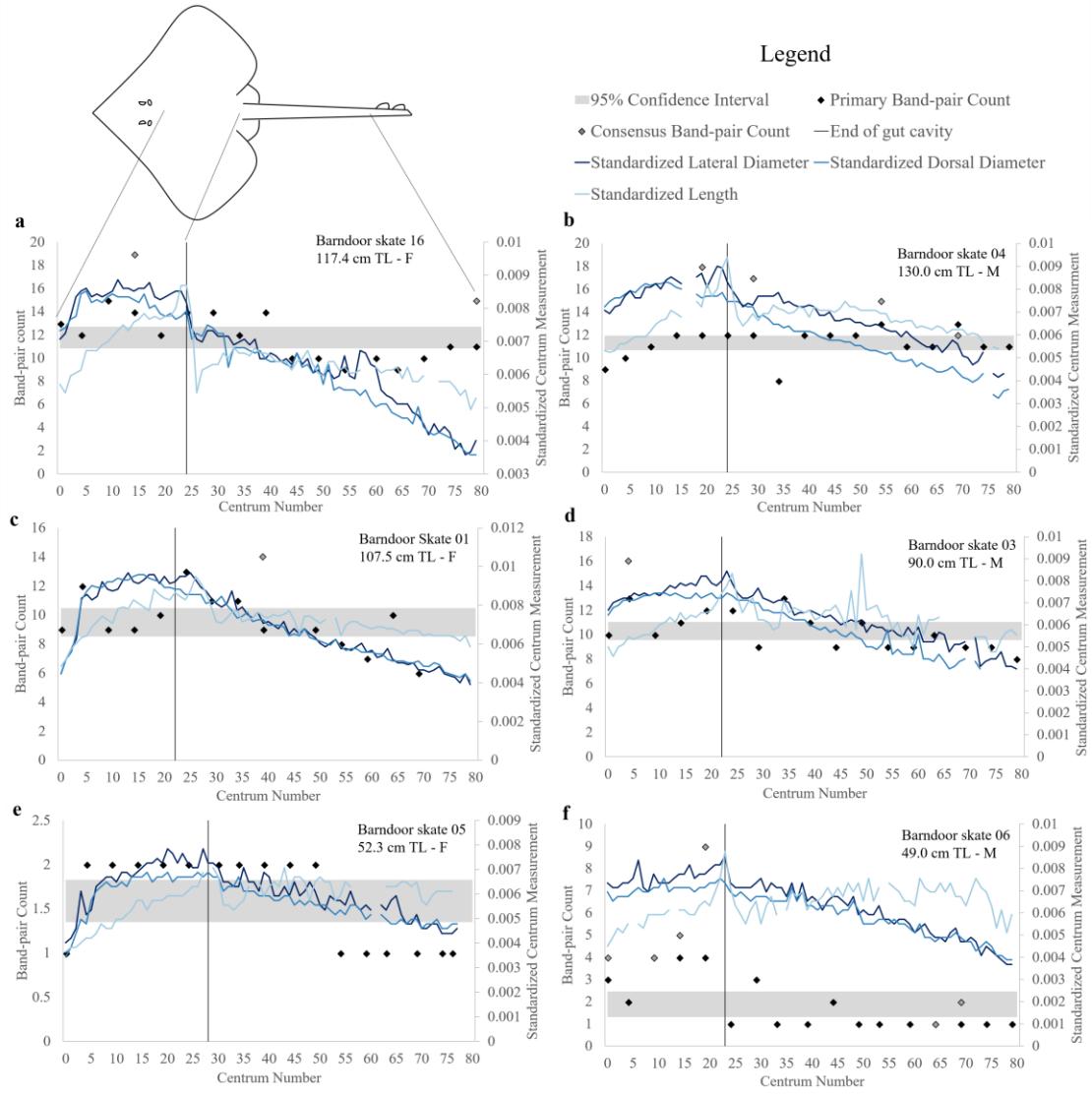


Figure 3. Band-pair count and standardized centrum measurements along the vertebral column for six barndoor skates analyzed for band pairs.

abdominal cavity, and both measurements were smaller in the caudal centra (Figures 4 & 5). Atlantic stingray and round ray centra were slightly ovoid (greater LD) along the abdominal cavity. LEN increased from the head until the transition from abdominal to caudal vertebrae after which LEN quickly decreased, but the decrease was less dramatic than that seen in the skate species. In both ray species LEN was constant along the tail.

For each species, the centrum morphology along the vertebral column was best described by individual variation (Figure 6). The best-fit GAMs modeled each individual with its own intercept and smoothing function for all species and measurements rather than by sex or species. The only exception was the LEN measurements in Atlantic stingrays, which was best modeled by each individual with its own intercept, but the same smoothing function for all individuals (Table 2).

#### *Band-pair Counts*

Counting every 5<sup>th</sup> vertebral centrum from one to 80 should result in 17 vertebral centra analyzed for band pairs from each individual. The number of centra counted was sometimes less than 17 (range 11-17) due to damage that made some centra unusable (Table 1). The within-reader CV was 6.86 - 14.88% for primary reader and 6.37 - 12.54% for the secondary reader (Table 3), while the between-reader CV ranged from 10.08 - 21.35% (Table 3). The number of centra per species with counts that differed by three or more band pairs, and thus were examined by both readers for a consensus, ranged from 9 – 16 (Table 3). Between-reader CV was calculated before centra were re-examined by both readers for a consensus;

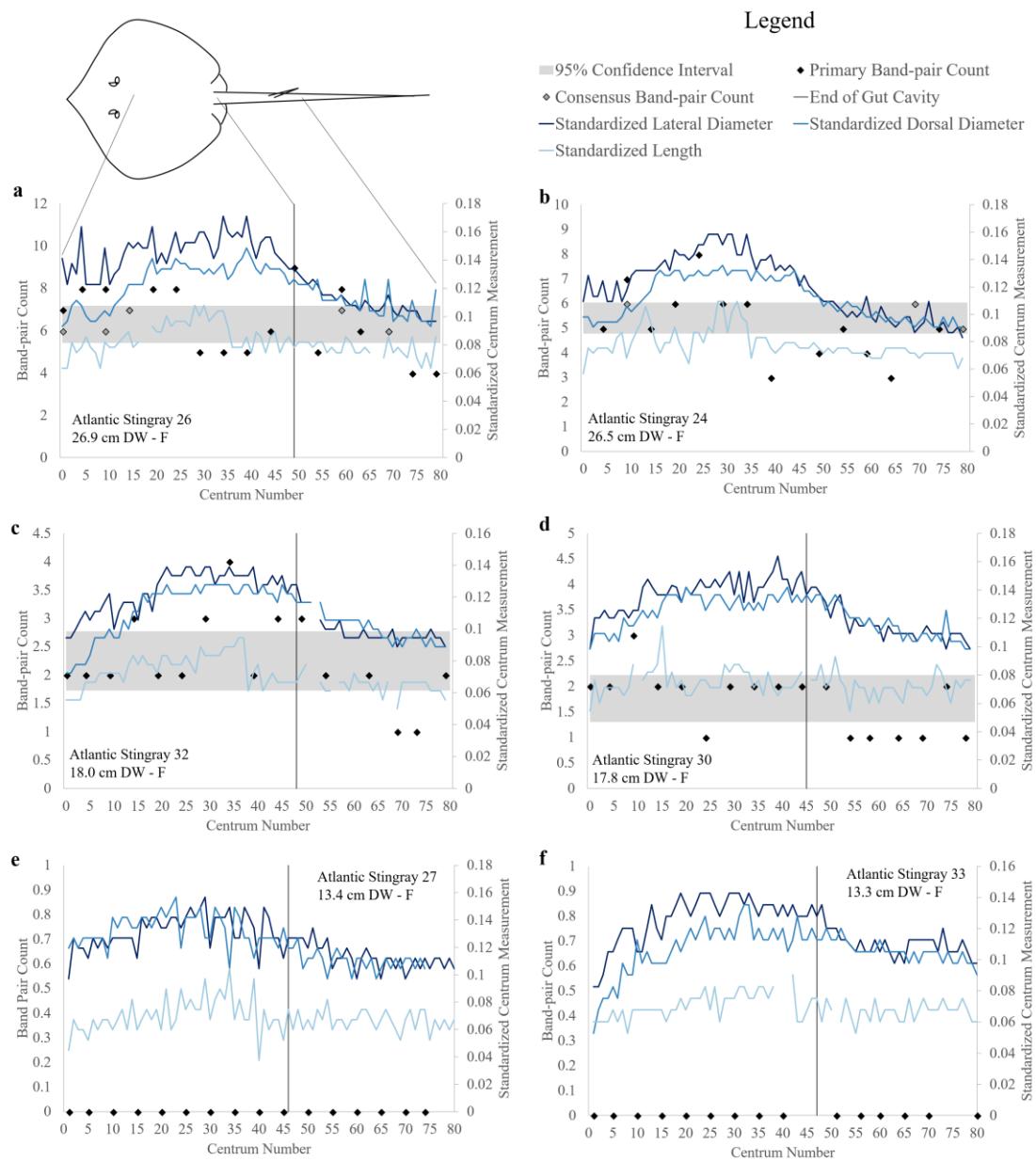


Figure 4. Band-pair count and standardized centrum measurements along the vertebral column for six Atlantic stingrays analyzed for band pairs.

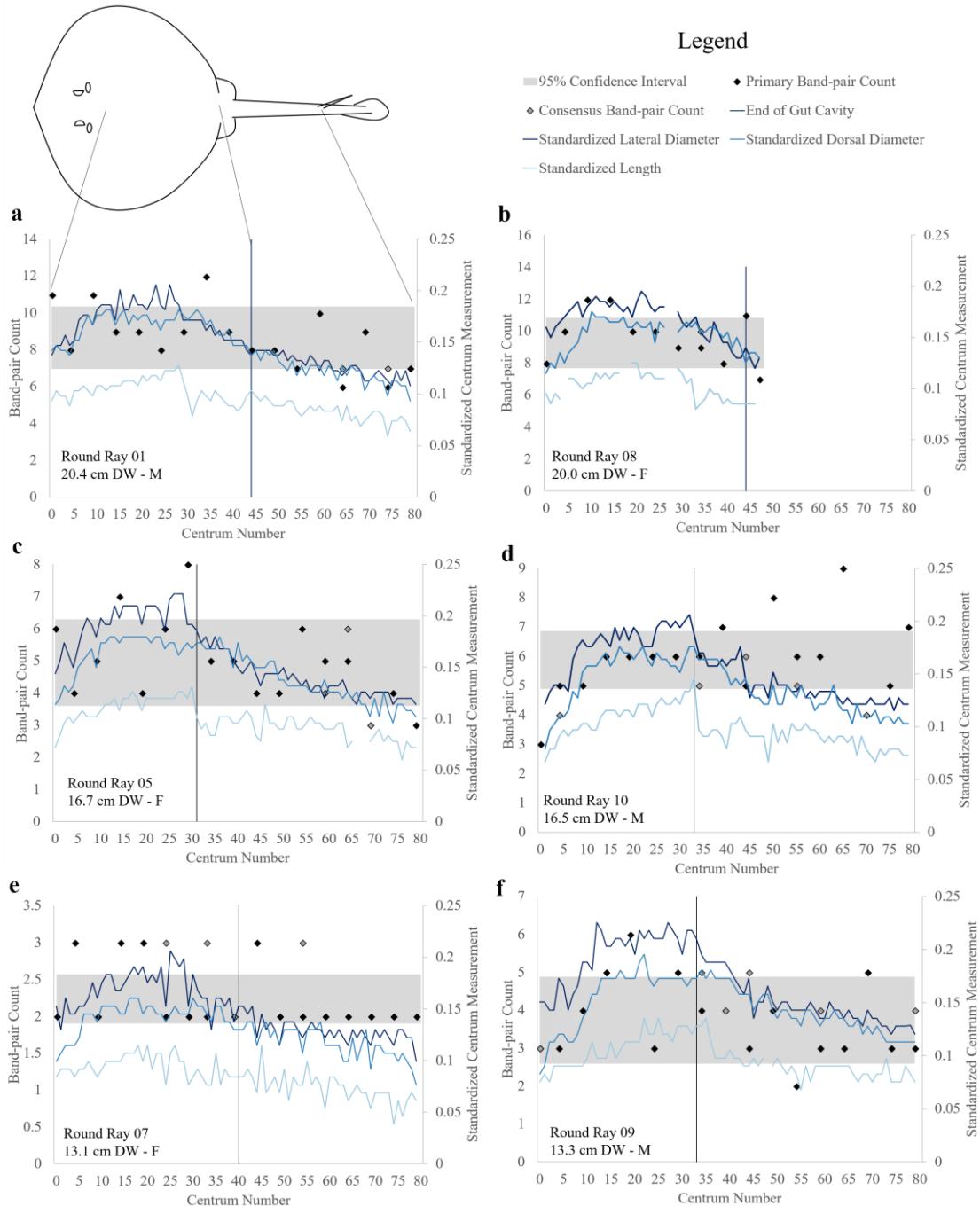


Figure 5. Band-pair count and standardized centrum measurements along the vertebral column for six round rays analyzed for band pairs.

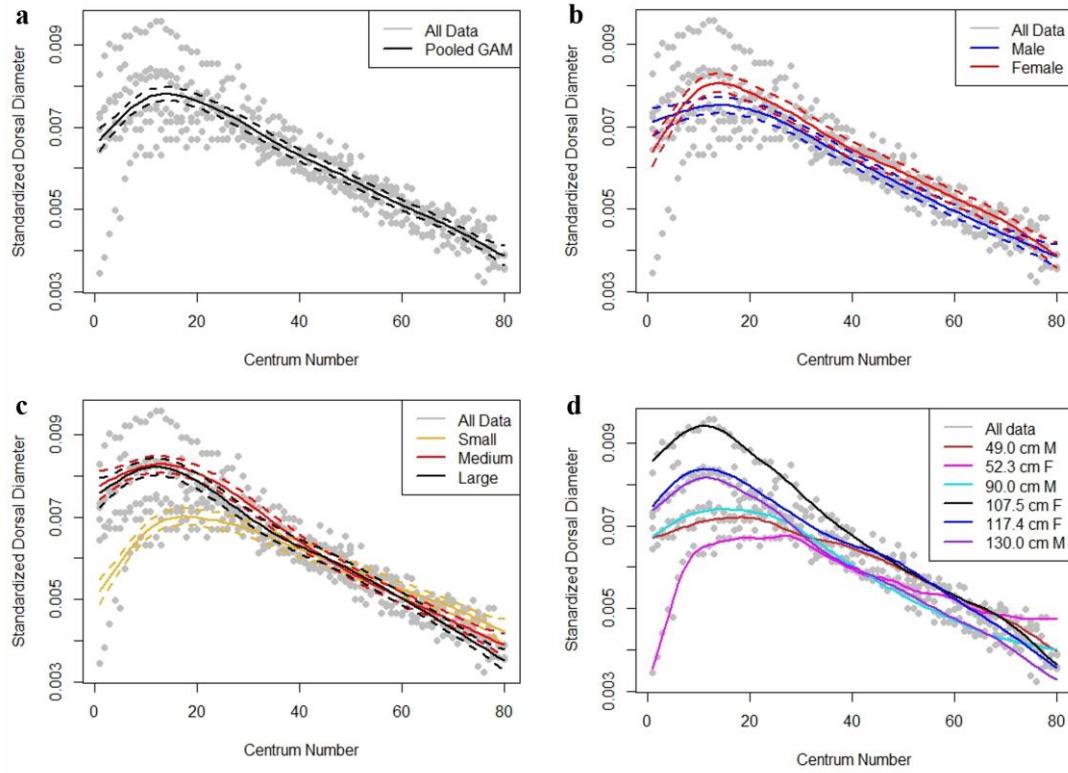


Figure 6. Example of generalized additive model variations with (a) all data pooled, data pooled (b) by sex, (c) by size class, and (d) by individual fit to barndoor skate data.

Table 2. Generalized additive model results for 10 different models to best describe centrum morphology along the vertebral column for five batoid species. k is the number of knots used in each model.

Species	Model	Dorsal Diameter			Lateral Diameter			Length			AIC				
		Deviance	k	Adjusted r <sup>2</sup>	AIC	Deviance	k	Adjusted r <sup>2</sup>	AIC	Deviance	k	Adjusted r <sup>2</sup>	AIC	AIC	
Little Skate	Pooled	3277.71	15	0.84	-25275.12	4419.48	3273.56	18	0.88	-23443.01	4433.93	3088.55	15	0.45	
	By Sex	Different Smoother	3265.75	18	0.84	-25263.06	4461.54	3261.56	18	0.88	-23453.14	4423.8	3072.59	18	0.47
	Different Intercept	3275.28	18	0.84	-25276.17	4448.43	3272.56	18	0.88	-23443.65	4433.29	3085.32	18	0.46	
	Different Smoother and Intercept	3264.76	18	0.84	-25261.29	4442.31	3260.57	18	0.88	-23454.93	4422.01	3071.55	18	0.48	
	By Size	Different Smoother	3256.68	18	0.85	-25230.62	4393.98	3250.89	18	0.89	-23733.30	4143.64	3060.25	18	0.52
	Different Intercept	3274.29	18	0.84	-25278.37	4446.23	3271.16	18	0.88	-23440.82	4436.12	3084.36	18	0.47	
	Different Smoother and Intercept	3254.69	18	0.85	-25229.92	4394.68	3248.95	18	0.89	-23734.11	4122.83	3058.12	18	0.53	
	By Individual Different Smoother	3034.36	18	0.9	-26446.93	5277.67	2963.43	18	0.93	-24876.66	5000.28	2756.94	18	0.65	
	Different Intercept	3334.89	20	0.9	-26696.52	3228.08	3232.78	18	0.92	-24667.08	5209.86	3045.69	18	0.58	
	Different Smoother and Intercept	2901.33	18	0.96	-29724.60	0	2813.94	18	0.97	-27876.94	0	2644.62	18	0.78	
Winter Skate	Pooled	462.05	15	0.86	-3614.77	926.251	4620.03	16	0.86	-3451.75	691.533	454.63	13	0.52	
	By Sex	Different Smoother	452.01	18	0.87	-3650.95	890.077	451.25	18	0.88	-3489.35	653.331	419.49	10	0.54
	Different Intercept	459.59	20	0.87	-3663.15	877.869	460.36	18	0.86	-3449.98	693.303	450.41	18	0.86	
	Different Smoother and Intercept	450.31	18	0.89	-3708.53	832.493	450.26	18	0.88	-3487.91	655.373	440.36	18	0.88	
	Different Smoother	443.47	15	0.93	-3896.50	644.521	439.29	17	0.92	-3677.35	465.93	417.25	10	0.58	
	Different Intercept	459.49	16	0.87	-3638.81	902.213	459.56	17	0.88	-3481.86	661.422	451.96	14	0.6	
	Different Smoother and Intercept	442.44	14	0.93	-3925.36	615.662	434.22	20	0.93	-3724.76	418.519	439.14	10	0.66	
	By Individual Different Smoother	412.76	17	0.95	-4084.52	456.506	397.4	20	0.96	-3999.54	143.739	426.11	12	0.65	
	Different Intercept	456.29	17	0.89	-3701.02	840.003	456.59	17	0.88	-3479.35	663.928	451.37	10	0.64	
	Different Smoother and Intercept	378.1	23	0.98	-4541.02	0	385	20	0.97	-4143.38	0	394.28	17	0.83	
Barndoor Skate	Pooled	459.00	10	0.79	-5574.04	1085.87	462.9	15	0.83	-5725.53	670.572	442.45	10	0.32	
	By Sex	Different Smoother	453.69	18	0.79	-5574.10	1085.806	458.24	10	0.83	-5731.21	665.29	437.25	10	0.33
	Different Intercept	457.93	10	0.8	-5594.87	1065.042	462.8	10	0.83	-5723.23	673.277	441.47	10	0.32	
	Different Smoother and Intercept	453.24	10	0.8	-5596.25	1063.661	457.26	10	0.83	-5730.57	665.939	436.28	10	0.33	
	Different Smoother	448.64	10	0.87	-5799.38	860.539	452.42	10	0.87	-5840.35	556.152	432.06	10	0.47	
	Different Intercept	457.05	10	0.82	-5632.87	107.036	461.75	15	0.86	-5808.05	587.557	440.27	10	0.4	
	Different Smoother and Intercept	446.04	10	0.89	-5852.63	807.275	449.73	10	0.89	-5922.22	474.187	429.11	10	0.55	
	By Individual Different Smoother	433.82	10	0.92	-5955.65	704.26	434.07	10	0.93	-6090.14	306.37	418.69	10	0.52	
	Different Intercept	453.72	10	0.89	-5807.76	852.147	457.46	10	0.89	-5875.56	520.941	437	10	0.57	
	Different Smoother and Intercept	406.41	18	0.98	-6659.91	0	616.74	15	0.96	-6396.50	0	388.06	18	0.82	
Atlantic Stringray	Pooled	695.09	10	0.59	-4384.39	950.027	694.26	15	0.69	-4296.73	606.958	676.88	10	0.29	
	By Sex	Different Smoother	691.18	15	0.61	-4420.81	913.605	690.54	10	0.69	-4295.07	608.621	673.52	10	0.69
	Different Intercept	693.28	15	0.59	-4382.33	952.085	694.03	10	0.69	-4307.25	596.443	677.05	10	0.69	
	Different Smoother and Intercept	690.92	15	0.61	-4418.96	915.457	689.51	10	0.69	-4307.12	596.567	672.5	10	0.69	
	By Size	Different Smoother	681.98	15	0.64	-4465.12	869.296	683.35	10	0.72	-4375.49	528.202	666.12	15	0.3
	Different Intercept	691.94	15	0.68	-4538.10	796.308	692.29	10	0.69	-4304.36	599.326	670.6	18	0.47	
	Different Smoother and Intercept	684.48	15	0.72	-4634.37	700.044	681.32	10	0.73	-4620.01	524.681	660.07	15	0.46	
	Different Smoother	685.12	10	0.66	-4487.95	846.458	654.97	10	0.74	-4386.82	516.868	648.52	10	0.28	
	Different Intercept	686.68	10	0.81	-4891.77	442.645	686.89	15	0.82	-4661.20	242.487	665.7	15	0.52	
	Different Smoother and Intercept	622.79	15	0.91	-5334.41	0	631	15	0.88	-4903.69	0	629.71	15	0.52	
	Round Ray	750.84	16	0.81	-4794.71	538.898	751.51	15	0.84	-4662.83	468.253	743.36	15	0.66	
	By Sex	Different Smoother	745.95	10	0.81	-4806.81	526.799	742.96	15	0.85	-4710.33	420.762	736.5	15	0.67
	Different Intercept	752.88	10	0.82	-4831.30	502.313	749.58	18	0.85	-4680.77	450.319	742.35	15	0.66	
	Different Smoother and Intercept	744.91	10	0.82	-4843.31	490.301	741.88	15	0.86	-4732.16	398.923	755.51	15	0.68	
	By Size	Different Smoother	749.22	15	0.81	-4808.90	524.709	749.47	15	0.85	-4685.51	445.518	730.4	15	0.66
	Different Intercept	744.75	14	0.82	-4841.70	491.916	738.3	10	0.86	-4720.63	410.46	728.5	15	0.67	
	Different Smoother and Intercept	685.33	17	0.86	-4924.13	409.486	698.01	11	0.87	-4763.74	367.347	695.76	15	0.7	
	By Individual Different Smoother	741.4	16	0.87	-5037.33	296.281	742.03	15	0.89	-4937.57	719.321	733.99	15	0.72	
	Different Intercept	656.47	23	0.92	-5333.61	0	680.26	12	0.92	-5131.09	0	681.04	15	0.77	
	Different Smoother and Intercept											-5097.25	0		

Table 3. Bias and precision of band-pair counts within and between readers. Asterisk indicates significant bias.

Species	Evans-Hoenig (1998) Bias test					
	n	df	$\chi^2$	p	CV	Consensus <sup>a</sup>
<b>Little Skate</b>	Primary reader	4	8.71	0.069	9.08	
	Second reader	3	2.38	0.497	11.89	
	Inter-reader	130	7	11.09	0.135	16.77
<b>Winter Skate</b>	Primary reader	6	11.30	0.800	6.86	
	Second reader	5	7.69	0.174	8.80	
	Inter-reader	99	6	7.22	0.301	10.08
<b>Barndoor Skate</b>	Primary reader	5	11.49	0.042	*	9.35
	Second reader	4	3.42	0.490		6.37
	Inter-reader	95	5	12.63	0.027	*
<b>Atlantic Stingray</b>	Primary reader	3	2.50	0.475		14.88
	Second reader	2	9.76	0.008	*	12.54
	Inter-reader	63	4	8.43	0.077	21.35
<b>Round Ray</b>	Primary reader	4	4.06	0.398		9.81
	Second reader	3	2.44	0.486		9.93
	Inter-reader	91	4	3.31	0.507	11.83

<sup>a</sup>Number of centra counted by both readers together for consensus.

post-consensus CV values would be lower. The Evans-Hoenig (1998) test of symmetry detected within-reader bias for primary reader only for barndoor skate data and within-reader bias for the secondary reader was detected only for Atlantic stingray data and between-reader bias was detected only for barndoor skate data (Table 3). Detailed examination of barndoor skate data revealed that the number of band pairs was undercounted on the second count of the primary reader relative to the first count for centra with >12 band pairs. The analysis of between-reader bias for the barndoor skate data showed that the secondary reader undercounted band pairs in the two smallest individuals compared to the primary reader. The secondary reader for Atlantic stingray overcounted on the second count relative to the first count for centra with three and four band pairs.

Significant differences in band-pair counts were found along the column of all individuals except in young-of-the-year (YOY; Figures 1-5). The band-pair counts for the three skate species were roughly correlated with the pattern of the DVD and LD measurements (Figures 1-3). Band-pair counts for the Atlantic stingray and the round ray did not exhibit a trend along the vertebral column, but still showed significant differences among different centra within an individual (Figures 4 & 5). Variation in band-pair counts along the vertebral column of individuals was a maximum of seven band pairs for little skate, eight band pairs for winter skate, 11 band pairs in barndoor skate, five band pairs in Atlantic stingray, and six band pairs for round ray. In general, abdominal centra had higher band-pair counts than caudal centra. The two smallest Atlantic stingray specimens examined were YOYs and did not have any band pairs in

any centra along the vertebral column, this explains why the band-pair counts were not significantly different among centra for these two individuals (Figure 4).

Dorso-ventral diameter, medio-lateral diameter, and length had significant correlations with the band-pair counts of little skate, barndoor skate, and round ray (Table 4). Atlantic stingray had significant correlations with DVD and LD, but not with LEN. Winter skate did not have any significant correlations with any centrum measurements.

## Discussion

The rationale for the use of skeletal hard parts like vertebral centra to estimate age is that band pairs are deposited as the hard part and the individual increase in body size relative to a consistent time period (Haskell 1948; Cailliet et al. 2006). Vertebral centra, which vary in morphology within an individual, vary in the number of band pairs within an individual. Variable band-pair counts among centra along the vertebral column has now been observed in 15 species representing 9 elasmobranch families (Natanson and Cailliet 1990; Natanson et al. 2008; Huvaneers et al. 2013; Natanson et al. 2018; current study). The presence of variation in band-pair counts among vertebral centra within an individual suggests that the mechanism that regulates the formation of band pairs does not result in annual band-pair formation for all centra in any given individual. An increase in centrum size (and subsequent increase in the number of band pairs) with somatic growth is required if centra are to be used to estimate age. However, in the case of centra, this makes them unreliable as a tool for ageing fish. In

Table 4. Linear mixed-effects model comparing band-pair counts with the three centrum measurements with individual included in the model as a random effect. Asterisk indicates significant correlation.

<b>Species</b>	<b>Measurement</b>	<b>Estimate</b>	<b>S.E.</b>	<b>df</b>	<b>t-value</b>	<b>p-value</b>
Little Skate	Dorsal Diameter	0.591	0.218	129	2.716	0.0075 *
	Lateral Diameter	0.435	0.140	129	3.101	0.0024 *
	Length	0.624	0.300	127	2.078	0.0398 *
Winter Skate	Dorsal Diameter	0.123	0.184	93	0.667	0.5066
	Lateral Diameter	0.092	0.146	93	0.628	0.5316
	Length	-0.325	0.364	92	-0.894	0.3737
Barndoor Skate	Dorsal Diameter	0.572	0.148	91	3.864	0.0002 *
	Lateral Diameter	0.646	0.154	91	4.205	0.0001 *
	Length	0.666	0.244	90	2.732	0.0076 *
Atlantic Stingray	Dorsal Diameter	0.865	0.326	88	2.649	0.0096 *
	Lateral Diameter	0.961	0.210	88	4.574	0.0000 *
	Length	0.915	0.499	87	1.833	0.0703
Round Ray	Dorsal Diameter	0.807	0.278	88	2.909	0.0046 *
	Lateral Diameter	0.625	0.264	88	2.371	0.0199 *
	Length	0.900	0.431	86	2.085	0.0400 *

addition, band-pair counts that vary among centra along the vertebral column of an individual cannot accurately reflect a single age estimate (Natanson et al. 2018).

The prevailing hypothesis is that band pairs are deposited to provide structural support within the vertebral column. This hypothesis was suggested as a result of the observation that band-pair deposition patterns were obviously not annual in the Pacific angel shark (Natanson and Cailliet 1990), which has now been observed in a variety of species over the years (Tanaka 1990; Chidlow et al. 2007; Natanson et al. 2008; Huveneers et al. 2013). Recently, this hypothesis has gained further support; band-pair deposition has been found to be more closely linked with somatic growth and the structural needs of the elasmobranch skeleton than with age (Natanson et al. 2018).

A complimentary assumption to annual band-pair deposition was the assumption that every centrum has the same number of band pairs, so any centrum could be used to estimate age. This assumption is only occasionally addressed in age and growth studies in which vertebrae from different regions of the vertebral column are compared (Natanson and Cailliet 1990; Officer et al. 1996; Piercy et al. 2006; Natanson et al. 2008). When differences in band-pair counts between more anterior and more posterior centra were detected, it was suggested that band pairs in smaller, caudal centra were more difficult to interpret (Brown and Gruber 1988; Officer et al. 1996; Natanson et al. 2006; Piercy et al. 2006). This study did not find it difficult to interpret band pairs in smaller centra, instead the observed variation in band-pair counts along the vertebral column in five species disproves the assumption that every centrum has the same number of band pairs.

To establish if band-pair counts accurately estimate age, counts must be validated. While many validation methods have been developed, none of these methods have successfully validated the band-pair counts throughout the entire lifespan of the fish. Several species of shark have had the band-pair counts validated as annual, but only up to approximately the age at sexual maturity (Casey and Natanson 1992; Andrews et al. 2011; Hamady et al. 2014; Natanson et al. 2014; Passerotti et al. 2014; Natanson et al. 2015; Natanson al. 2018). Despite over 35 years of scientists attempting to validate annual band-pair deposition of elasmobranch vertebral centra, the model does not fit. Support continually grows for a model of band-pair deposition in response to somatic growth rather than an annual cycle.

The precedent for acceptable CV values for band-pair counts of elasmobranchs is <10%, however this threshold is arbitrary; CV values for fish otoliths are frequently 5%, while shark vertebrae studies often report values over 10% (Campana 2001). Precision is influenced by species and structure used, not only the reader (Campana 2001). Atlantic stingray had particularly high CV values (Table 3) driven by low band-pair counts, particular the two medium specimens that had band-pair counts between one and four. The CV is much larger when comparing one and two band pairs (47.14%) versus 10 and 11 band pairs (6.73%). In theory the precision should be higher for fewer band pairs, but the band pairs of some centra are more difficult to interpret than others regardless of position along the vertebral column. Whether the difficulty in interpretation stemmed from the centrum itself or processing error, no centra were removed from the study based on readability. Instead, if band-pair counts differed by three or more band pairs, the centrum was examined by both readers

together and a consensus band-pair count reached. Using all centra regardless of readability highlights the variation among centra.

Centrum morphologies in these five batoid species were roughly similar to the centrum morphology of sharks, which also exhibited species-specific patterns (Natanson et al. 2018). Centrum morphology increased in size from behind the cranium to a peak or plateau in the abdominal cavity and in most cases decreased after the end of the abdominal cavity (Natanson et al. 2018). The Atlantic angel shark had the most similar centrum morphology to the batoids with the largest centrum in the middle of the abdominal cavity (approximately in line with the tips of the pectoral fins), while the largest centrum for the other shark species was at the end of the abdominal cavity (Natanson et al. 2018). In batoids, abdominal centra ranged from circular to ovoid, while caudal centra were circular. In sharks, centra were circular, except for the strongly ovoid centra of the Atlantic angel shark and the abdominal centra of very large lamnids (i.e. shortfin mako; Natanson et al. 2018).

While rough similarities exist across and within species, individual variation was the best descriptor of centrum morphology for batoids. Centrum morphology was not statistically similar by size class (small, medium, or large), sex, or within a species. This suggests that the conditions that an individual experiences influences the growth of the vertebral centra. For instance, in Atlantic salmon, *Salmo salar* Linnaeus 1758, different vertebral centra grew at different rates when exposed to different photoperiod regimes (Fjelldal et al. 2005). Besides Fjelldall et al. (2005), there is a dearth of research exploring variable centrum growth. Therefore, we rely on research on the plasticity of fish growth and suggest that factors affecting individual body

growth (food availability, temperature, population density, and genetics [McDowall 1994]) may also affect growth of vertebral centra.

Natanson et al. (2018) suggested that for sharks, “centra are functionally linked to body shape”. This finding is based on the positive correlation of body girth measurements to centrum size and is supported by Thomson and Simanek’s (1977) five categories of body and tail type, in which species of similar body shapes and swimming styles also had similar centrum morphology. While batoids possess vastly different swimming styles than many sharks, the Atlantic angel shark uses a swimming mode that is an intermediate between caudal fin propulsion and paired fin propulsion (Wilga and Lauder 2004). These dorso-ventrally flattened sharks also demonstrate a relationship between body shape and centrum morphology (Natanson et al. 2018), so it is reasonable to assume that this relationship extends to batoids. The body girth measurements used by Natanson et al. (2018) did not translate to a batoid body plan (James, unpub. data) so a different approach will have to be used to investigate the relationship between body shape and centrum morphology.

The paradigm of annual band-pair deposition within vertebral centra of elasmobranchs has been repeatedly called into question over the years (Natanson and Cailliet 1990; Francis et al. 2007; Harry 2017; Natanson et al 2018). Here we demonstrate that centrum morphology and band-pair count vary along the vertebral column of an individual, supporting the idea that band pair number is related to somatic growth and/or the structural needs of the individual (Natanson and Cailliet 1990; Tanaka 1990; Natanson et al. 2008; Natanson et al. 2018). Thus, we are unable to accurately determine the age of elasmobranchs based on band-pair counts and

caution should be applied when using band-pair counts as a proxy of age without validation (Beamish and MacFarlane 1983). Unfortunately, validation throughout the entire lifespan has not been achieved for any elasmobranch species. Therefore, two tasks now must be accomplished: investigating the impact of inaccurate ages on stock-assessment model results and determining an alternate method to age elasmobranchs.

### **Acknowledgements**

We thank M. Winton for providing R code. We thank R. Skyes, the crew of the F/V *Virginia Marise*, the crew of the R/V *Cap'n Bert*, and the Cape Cod Fishermen's Alliance for collection assistance. We thank K. Viducic, K. Wooley, and A. Tuminelli for data collection assistance. Collection occurred under a Rhode Island Collector's Permit #2015-06 and #2016-51. Research was supported by the Marine Anglers for Research and Conservation Memorial, and an American Elasmobranch Society Student Research Award, and the University of Rhode Island.

## **References**

- Andrews AH, Natanson LJ, Kerr LA, Burgess GH, Cailliet GM (2011) Bomb radiocarbon and tag-recapture dating of sandbar shark (*Carcharhinus plumbeus*) Fish Bull 109: 454-465
- Beamish RJ, McFarlane GA (1983) The forgotten requirement for age validation in fisheries biology. Trans Am Fish Soc 112 735-743
- Brown CA, Gruber SH (1988) Age assessment of the Lemon shark, *Negaprion brevirostris*, using tetracycline validated vertebral centra. Copeia 3(1998): 747-753
- Cailliet GM, and Goldman KJ (2004) Ch 14 Age determination and validation in Chondrichthyan fishes. Biology of sharks and their relatives 2004 CRC Press LLC
- Cailliet GM, Smith WD, Mollet HF, Goldman KJ (2006) Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. Environ Biol Fish 77: 211-228

Campana, SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J Fish Biol* 59: 197-242

Casey JG, Natanson LJ (1992) Revised estimates of age and growth of the Sandbar Shark (*Carcharhinus plumbeus*) from the western North Atlantic. *Can J Fish Aquat Sci* 49: 1474-1477

Chang WYB (1982) A statistical method for evaluating the reproducibility of age determination. *Can J Fish Aquat Sci* 39: 1208-1210

Chidlow JA, Simpfendorfer CA, Russ GR (2007) Variable growth band deposition leads to age and growth uncertainty in the Western Wobbegong Shark, *Orectolobus hutchinsi*. *Mar Freshw Res* 58: 856-865

Dean MN, Summers AP (2006) Mineralized cartilage in the skeleton of chondrichthyan fishes. *Zool* 109: 164-168

Ebert DA (2005) Reproductive biology of skates, *Bathyraja* (Ishiyama), along the eastern Bering Sea continental slope. *J Fish Biol* 66: 618-649

Evans GT, Hoenig JM (1998) Testing and viewing symmetry in contingency tables, with applications to readers of fish ages. *Biometrics* 54: 620-629

Fjelldal PG, Nordgarde U, Berg A, Grotmol S, Totland GK, Wargelius A, Hansen T (2005) Vertebrae of the trunk and tail display different growth rates in response to photoperiod in Atlantic salmon, *Salmo salar* L., post-smolts. Aquaculture 250: 516-524

Francis MP, Campana SE, Jones CM (2007) Age under-estimation in New Zealand Porbeagle Sharks (*Lamna nasus*): is there an upper limit to ages that can be determined from shark vertebrae? Mar Freshw Res 58: 10-23.

Haddon M (2001) Modeling and quantitative measures in fisheries. Chapman & Hall/CRC Press, Boca Raton FL

Hamady LL, Natanson LJ, Skomal GB, Thorrold SR (2014) Vertebral bomb radiocarbon suggests extreme longevity in white sharks. PLoS ONE 9(1): e84006

Harry, A.V. (2017). Evidence of systemic age underestimation in shark and ray ageing studies. Fish Fish 2017: 1-16

Haskell WL (1948) An investigation of the possibility of determining the age of sharks through annuli as shown in cross-sections of vertebrae. Annual Report of the

Marine Laboratory of the Texas Game, and Fish Commission FY 1948-49:  
212-217

Huvaneers C, Stead J, Bennett MB, Lee KA, Harcourt RG (2013) Age and growth determination of three sympatric wobbegong sharks: How reliable is growth band periodicity in Orectolobidae? *Fish Res* 147: 413-425

Ishiyama R (1951) Studies on the rays and skates belonging to the family Rajidae, found in Japan and adjacent regions. 2. On the age-determination of Japanese Black-Skate *Raja fusca*. *Bull Jap Soc Sci Fish* 16: 112-118

Jones BC, Geen GH (1977) Age determination of an elasmobranch (*Squalus acanthias*) by x-ray spectrometry. *J Fish Res Board Can* 34: 44-48

Kalish JM, Johnston J (2001) Determination of school shark age based on analysis of radiocarbon in vertebral collagen In Use of bomb radiocarbon chronometer to validate fish age, (Kalish JM, ed) Final Report, FDRC Project 93/109 Fisheries Research and Development Corporation, Canberra, Australia, pp 116-122

McDowall RM (1994) On size and growth in freshwater fish. *Ecol Freshw Fish* 3: 67-

Natanson L, Kohler N, Ardizzone D, Cailliet G, Wintner S, Mollet S (2006) Validated age and growth estimates for the shortfin mako, *Isurus oxyrinchus*, in the North Atlantic Ocean. Environ Biol Fish 77: 367-383

Natanson LJ, Cailliet GM (1990) Vertebral growth zone deposition in Pacific Angel Sharks. Copeia 1990: 1133-1145

Natanson LJ, Gervelis BJ, Winton MV, Hamady LL, Gulak SJB, Carlson JK (2014) Validated age and growth estimates for *Carcharhinus obscurus* in the northwestern Atlantic Ocean, with pre- and post-management growth comparisons. Environ Biol Fish 97: 881-896

Natanson LJ, Skomal GB, Hoffmann S, Porter M, Goldman KJ, Serra D (2018) Age and growth of elasmobranchs: do band pairs on vertebral centra record age? Mar Freshw Res <https://doi.org/10.1071/MF17279>

Natanson LJ, Sulikowski JA, Kneebone JR, Tsang PC (2007) Age and growth estimates for the Smooth Skate, *Malacoraja senta*, in the Gulf of Maine. Environ Biol Fish 80: 293-308.

Natanson LJ, Wintner S, Johansson F, Piercy AN, Campbell P, De Maddalena A, Gulak SJ, Human B, Fulgosi FC, Ebert DA, Hemida F, Mollen FH, Vanni S, Burgess GH, Compagno LJV, Wedderburn-Maxwell A (2008) Ontogenetic

vertebral growth patterns in the Basking Shark, *Cetorhinus maximus*. Mar Ecol Prog Ser 361, 267-278

Natanson LJ, Hamady LL, Gervelis BJ (2015) Analysis of bomb radiocarbon data for common thresher sharks, *Alopias vulpinus*, in the northwestern Atlantic Ocean with revised growth curves. Environ Biol Fish 99: 39-47

Officer RA, Gason AS, Walker TI, Clement JG (1996) Sources of variation in counts of growth increments in vertebrae from gummy shark, *Mustelus antarcticus*, and school shark, *Galeorhinus galeus*: implications for age determination. Can J Fish Aquat Sci 53: 1765-1777

Passerotti MS, Andrews AH, Carlson JK, Wintner SP, Goldman KJ, Natanson LJ (2014) Maximum age and missing time in the vertebrae of sand tiger shark (*Carcharias taurus*): validated lifespan from bomb radiocarbon dating in the western North Atlantic and southwestern Indian Oceans. Mar Freshw Res 65: 674-687

Piercy AN, Ford TS, Levy LM, Snelson FF (2006) Analysis of variability in vertebral morphology and growth ring counts in two Carcharhinid sharks. Environ Biol Fish 77: 401-406

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria URL <https://www.R-project.org/>

Ridewood, WG (1921) On the calcification of the vertebral centra in sharks and rays. Phil Trans Royal Soc B 210: 311-407.

Tanaka S (1990) Age and growth studies on the calcified structures of newborn sharks in laboratory aquaria using tetracycline. In Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of Fisheries NOAA Technical Report 90: 189-202

Thomson KS, Simanek, DE (1977) Body form and locomotion in sharks. Amer Zool 17:343-354.

Wilga CAD, Lauder GV (2004) Biomechanics of locomotion in sharks, rays, and chimeras. Carrier, JC, Musick, JA, and Heithaus, MR (eds.) Biology of Sharks and Their Relatives 2004 CRC Press LLC

Wood SN (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J Royal Stat Soc B 73(1): 3-36

## **CHAPTER 2**

### **The Battle of the Bands: Chemical Composition of Vertebral Opaque vs. Translucent Regions in 12 Elasmobranch Species**

Kelsey C James<sup>1</sup>

<sup>1</sup>Department of Fisheries, Animal and Veterinary Sciences. University of Rhode Island, Kingston, Rhode Island 02881, USA

*in preparation for submission to Journal of Morphology*

## **Abstract**

Elasmobranch vertebrae exhibit alternating opaque and translucent bands that have been used to estimate age for over 60 years. However, counts of these bands are being shown to underestimate age in a growing number of species. This evidence of opaque and translucent bands not reflecting age reinforces the need to understand the difference between these two band types. The bulk chemical composition of opaque and translucent bands of 12 elasmobranch species was examined using energy-dispersive X-ray spectrometry focusing on 11 elements. There is no difference in chemical composition between opaque and translucent bands in the little skate, *Leucoraja erinacea*, ( $p = 0.954$ ) or across the 12 species examined ( $p = 0.532$ ). Regardless of band type, vertebrae are composed mostly of oxygen, calcium, and phosphorus. The significance of optical differences between opaque and translucent band types requires further research.

**Keywords:** batoid, shark, mineralization, opaque, translucent

## **Introduction**

A defining characteristic of skates and rays is their cartilaginous skeleton. The cartilage skeleton is reinforced with apatite mineral deposited in distinct patterns (Ridewood 1921; Applegate 1967; Clement 1992). The main chemical components of apatite is calcium (Ca), phosphorus (P) and oxygen (O). Most of the elasmobranch skeleton is reinforced by tessellated cartilage – a mosaic of mineralized tiles called tesserae that encrust the unmineralized cartilage core (Kemp and Westrin 1979; Clement 1992; Dean and Summers 2006). In contrast, the vertebral centra are composed of areolar cartilage – a web-like scaffold of mineralized bands and struts interspersed with unmineralized cartilage, forming the hourglass-shaped vertebral body (Clement 1992; Dean and Summers 2006). The mechanism of formation of tessellated cartilage has been well-studied (Kemp and Westrin 1979; Clement 1992; Dean et al. 2009; Dean et al. 2015; Seidel et al. 2016), but the formation of areolar cartilage and the associated unmineralized cartilage in vertebrae has not received the same attention.

The alternating pattern of areolar cartilage and unmineralized cartilage in the vertebral centra has been the subject of extensive research. Early investigators attempted, with marginal success, to use the species-specific mineralization patterns to establish taxonomic relationships (Hasse 1879; Ridewood 1921). Haskell (1949) and Ishiyama (1951) noted that the mineralization pattern manifested as opaque and translucent bands in horizontal section and suggested that the bands might be related to time. This launched the field of elasmobranch age and growth; vertebral centra are still the preferred method to estimate age for elasmobranchs.

The mineralization pattern in vertebral centra has also been attributed to species-specific mechanical and structural needs (Kemp and Westrin 1979; Dingerkus et al. 1991; Clement 1992; Egerbacher et al. 2006; Porter et al. 2006; Porter et al. 2007; Natanson et al. 2018). Furthermore, the alternation of opaque and translucent bands has been proposed to behave as a viscoelastic composite that can withstand the stresses experienced during swimming (Porter et al. 2006; Huber et al. 2013). Several authors have suggested that the formation of the mineralization pattern is directly related to somatic growth rather than to age (Natanson and Cailliet 1990; Tanaka 1990; Natanson et al. 2008; Natanson et al. 2018).

Despite the varied areas of research into the vertebral centra mineralization pattern, the basic chemical composition of the opaque and translucent bands has not been conclusively determined. The goal of most examinations of the chemical composition of band pairs has been to verify age estimates from counting translucent and opaque bands. Jones and Geen (1977) were the first to use energy-dispersive X-ray spectrometry (EDS) on elasmobranch vertebrae and that study related Ca and P peaks to length-frequency age estimates in the spiny dogfish (*Squalus acanathias*). Cailliet and Radtke (1987) used the same method and related peaks in Ca concentrations along the centra with the number of opaque bands counted in the gray reef shark (*Carcharhinus amblyrhynchos*) and the common thresher shark (*Alopias vulpinus*). More recently, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used to attempt to verify band-pair counts in elasmobranchs (Hale et al. 2006; Christensen 2011; Scharer et al. 2012). This method has had limited success; the band counts in the round stingray (*Urotrygon halleri*) were only verified for

part of its lifespan (Hale et al. 2006), the opaque bands of the smalltooth sawfish (*Pristis pectinata*) did not have a relationship to Ca or P peaks (Scharer et al. 2012) and each opaque band of the white shark (*Carcharodon carcharias*) had three Ca peaks (Christensen 2011). The results of these studies indicated that Ca variation is not a reliable indicator for opaque and translucent band alternation. While our basic knowledge of elasmobranch cartilage composition suggests Ca and P variation between opaque and translucent bands, research to date does not solidly support this. The current study characterizes the bulk chemical composition of opaque and translucent bands of elasmobranch vertebrae in an effort to explain the optical differences between the two band types. Chemical composition of opaque and translucent bands was examined in 12 elasmobranch species using EDS focusing on 11 elements, Ca, P, O, sulfur (S), strontium (Sr), sodium (Na), chlorine (Cl), potassium (K), aluminum (Al), silicon (Si), and magnesium (Mg). These elements were chosen based on expected composition (Jones and Geen 1977; Cailliet and Radtke 1987; Porter et al. 2006) and preliminary testing.

## Methods

One abdominal vertebra from each of two individuals from each of eleven elasmobranch species (basking shark, *Cetorhinus maximus*, Atlantic angel shark, *Squatina dumeril*, white shark, *Carcharodon carcharias*, mako shark, *Isurus oxyrinchus*, porbeagle shark, *Lamna nasus*, common thresher shark, *Alopias vulpinus*, blue shark, *Prionace glauca*, dusky shark, *Carcharhinus obscurus*, Atlantic stingray, *Dasyatis sabina*, barndoor skate, *Dipturus laevis*, and winter skate, *Leucoraja*

*ocellata*) and one vertebra from each of 10 individuals of little skate, *L. erinacea*, were used in this study. These taxa demonstrate a wide variation in geographic range, life history characters, economic importance, and availability.

Centra were stored frozen before being sectioned horizontally through the focus to a thickness of 0.2-0.6 mm using a Ray Tech Gem Saw or an Isomet® low speed saw (Buehler, Lake Bluff, IL, USA). Next, a pin was used to indent an opaque and a translucent band for each section. Indentations were in the intermedialia for sharks and in the corpus calcareum for batoids (Figure 1). Photographs of each section were taken on a dissecting microscope (Nikon model SMZ1500®, Melville, NY, USA), a digital camera (Nikon model DSR12, Tokyo, Japan), and image processing software (NIS Elements, version 4.40, Nikon Corporation, Tokyo, Japan). The sections were dried for 24 hrs in a 60°C oven, attached to SEM stubs with PELCO tabs™, covered with a coating of Graphite Aerosol™ (Ted Pella, Redding, CA, USA), and observed with an SEM (Hitachi SU-1500 or Zeiss Supra40VP) equipped with an energy dispersive X-ray (EDX) spectrometer (IXRF systems, Austin, TX, USA or Oxford Instruments, Abingdon, United Kingdom ) at University of New Haven, New Haven, CT, USA, and the Marine Biological Laboratory, Woods Hole, MA, USA. The photograph of the indentations guided sampling by the scanning electron microscope (SEM).

Three measurements were made of each band type for each section focusing on detection of Ca, P, S, O, Sr, Na, Cl, K, Al, Si, and Mg. The concentration (% weight of elements sampled) of each element was recorded and average element concentrations

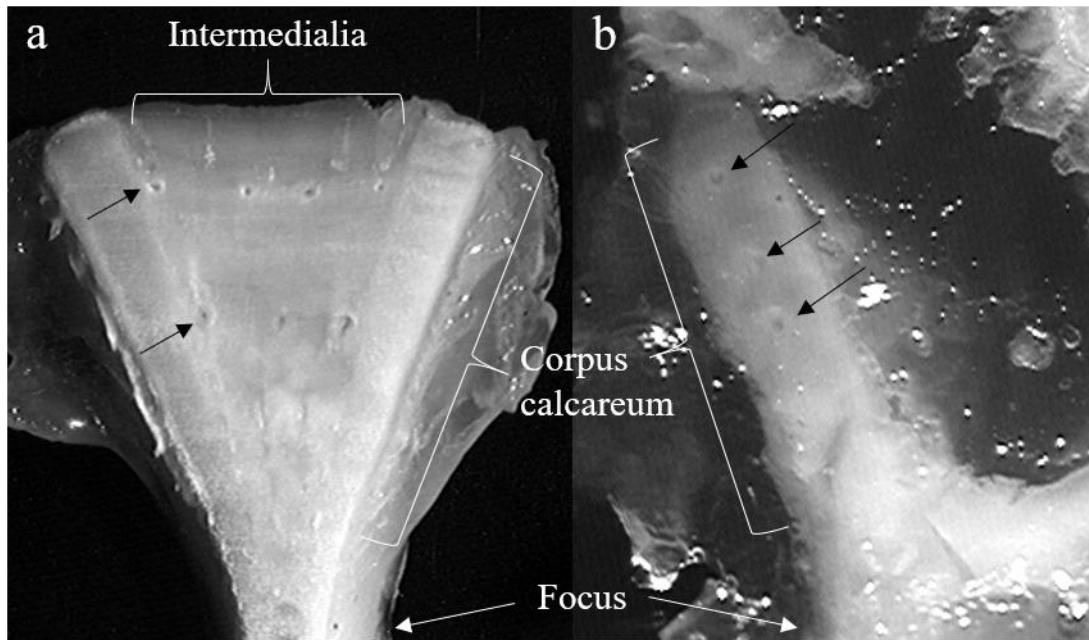


Figure 1. Pin indentations identified with black arrows in (a) the intermedialia of *Alopis vulpinus* marking an opaque band at the top of the photo and a translucent band in the middle of the photo and (b) the corpus calcareum of *Leucoraja ocellata* marking opaque bands.

were calculated from the three measurements of each band type. Correlations between elements were assessed using correlation coefficients ( $r$ ).

Differences in element concentrations were analyzed in the following groupings: band type (opaque vs translucent), species, species by band type, body plan (batoid or shark), and body plan by band type. Homogeneity of multivariate dispersion was tested with betadisper and differences among groups were tested with PERMANOVAs using adonis from the R package vegan (Oksanen et al. 2016; R Core Team 2014). PERMANOVAs were appropriate when the homogeneity of multivariate dispersion was not different, the groups had balanced designs (same sample size), or for unbalanced designs when the group with the larger sample size had the greater dispersion (Anderson and Walsh 2013).

## Results

Of the eleven elements analyzed, Ca, P, and O were found in the highest concentrations in the majority of sampled material (Figure 2; Table 1). Five elements (Al, K, Mg, Si, and Sr) combined comprised less than 6% in any sample. Sulfur (Figure 2d), Na, and Cl each comprised more than 10% in a few samples, but never more than 21%.

Elements were highly correlated. Six element pairs had  $r$  values greater than 0.8 (Table 2). There were negative correlations between O and P, O and Ca, S and P, and S and Ca and positive relationships between P and Ca and between Na and Cl. Multivariate dispersions were unequal for all comparisons ( $p < 0.001$ ) except between band types ( $p = 0.344$ ). Nevertheless, testing differences among groups was

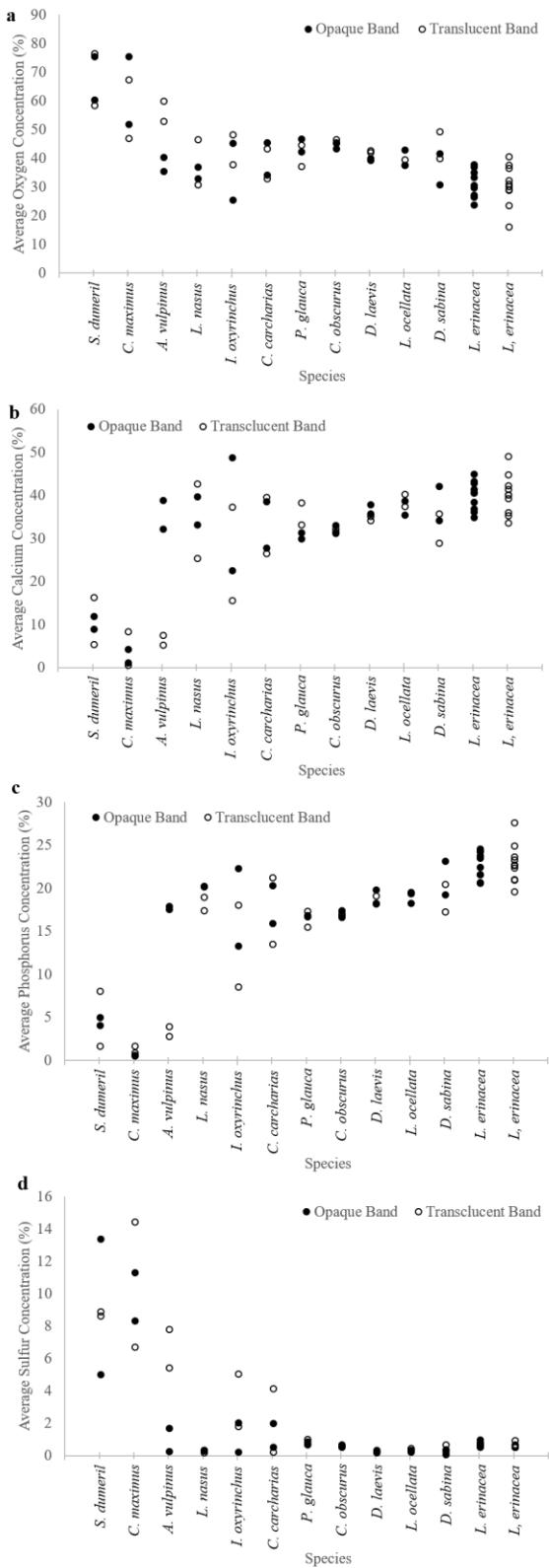


Figure 2. Observed percentage of (a) oxygen, (b) calcium, (c) phosphorus, and (d) sulfur for 12 elasmobranch species separated by opaque and translucent bands.

Table 1. Percentage composition for opaque and translucent bands of each of eleven elements for 32 opaque bands and 32 translucent bands.

	<b>Opaque band</b> <b>Mean ± S.E.</b>	<b>Translucent band</b> <b>Mean ± S.E.</b>
<b>Oxygen</b>	$40.455 \pm 2.181$	$42.199 \pm 2.189$
<b>Calcium</b>	$33.330 \pm 2.040$	$30.878 \pm 2.321$
<b>Phosphorus</b>	$17.960 \pm 1.150$	$16.575 \pm 1.308$
<b>Sodium</b>	$2.1712 \pm 0.437$	$2.833 \pm 0.690$
<b>Sulfur</b>	$1.825 \pm 0.569$	$2.400 \pm 0.615$
<b>Chlorine</b>	$1.577 \pm 0.581$	$2.357 \pm 0.851$
<b>Potassium</b>	$0.895 \pm 0.174$	$1.034 \pm 0.215$
<b>Magnesium</b>	$0.576 \pm 0.052$	$0.558 \pm 0.045$
<b>Aluminum</b>	$0.148 \pm 0.020$	$0.156 \pm 0.032$
<b>Silicon</b>	$0.171 \pm 0.028$	$0.174 \pm 0.037$
<b>Strontium</b>	$0.893 \pm 0.114$	$0.836 \pm 0.124$

Table 2. Pairwise correlation coefficients ( $r$ ) for eleven elements.

	Calcium	Phosphorus	Sodium	Sulfur	Chlorine	Potassium	Magnesium	Aluminum	Silicon	Strontium
	$r$	$r$	$r$	$r$	$r$	$r$	$r$	$r$	$r$	$r$
Oxygen	0.8879	-0.8937	0.3464	0.7591	0.2282	0.4234	0.5925	0.5538	-0.0149	-0.6747
Calcium	0.9725	-0.7089	-0.8425	-0.6104	-0.6470	-0.5141	-0.5766	-0.0498	0.6172	
Phosphorus		-0.6681	-0.8712	-0.5774	-0.6585	-0.4973	-0.6147	-0.0119	0.6978	
Sodium			0.4847	0.9273	0.5898	0.1191	0.2978	-0.0917	-0.4155	
Sulfur				0.3572	0.6155	0.5960	0.7322	0.3153	-0.4321	
Chlorine					0.5969	-0.0911	0.1872	-0.0780	-0.3604	
Potassium						0.3998	0.2466	-0.1358	-0.4611	
Magnesium							0.2830	-0.0520	-0.3627	
Aluminum								0.4301	-0.2877	
Silicon										0.5423

appropriate for all comparisons. Band type, body plan, and body plan by band type had balanced designs (same sample number per group). Species and species by band type had uneven sample sizes, but the group with the largest sample size did not have the smallest dispersion. The larger sample size of *L. erinacea* allowed for testing between band types within a species; there was no statistically significant difference ( $p=0.954$ ). For all 12 species combined there also was no statistically significant difference between band types ( $p = 0.532$ ). Convex hulls of opaque and translucent band types overlapped extensively in the first two principle coordinates (Figure 3).

Statistically significant differences in chemical composition were detected among species ( $p = 0.001$ ). Convex hulls of *S. dumeril* and *P. glauca* did not overlap with the other ten species for the first two principle coordinates (Figure 4). *Squatina dumeril* had higher percentages of O and S, and lower percentages of Ca and P when compared with the other eleven species. *Prionace glauca* was similar in concentrations of O, Ca, P, and S as a majority of the other species, but the overall composition was different.

The elemental composition was also significantly different among species by band type ( $p = 0.001$ ). The translucent band of *C. maximus* was different from the opaque band of *C. maximus* and more similar to *S. dumeril* and *P. glauca* (Figure 5).

The elemental composition was significantly different by body plan (shark vs batoid;  $p = 0.001$ ; Figure 6) and body plan by band type ( $p = 0.001$ ; Figure 7). The shark body plan had a larger convex hull than the batoid body plan. The opaque and translucent convex hulls for detecting a difference in body plan by band type completely overlapped for both sharks and batoids.

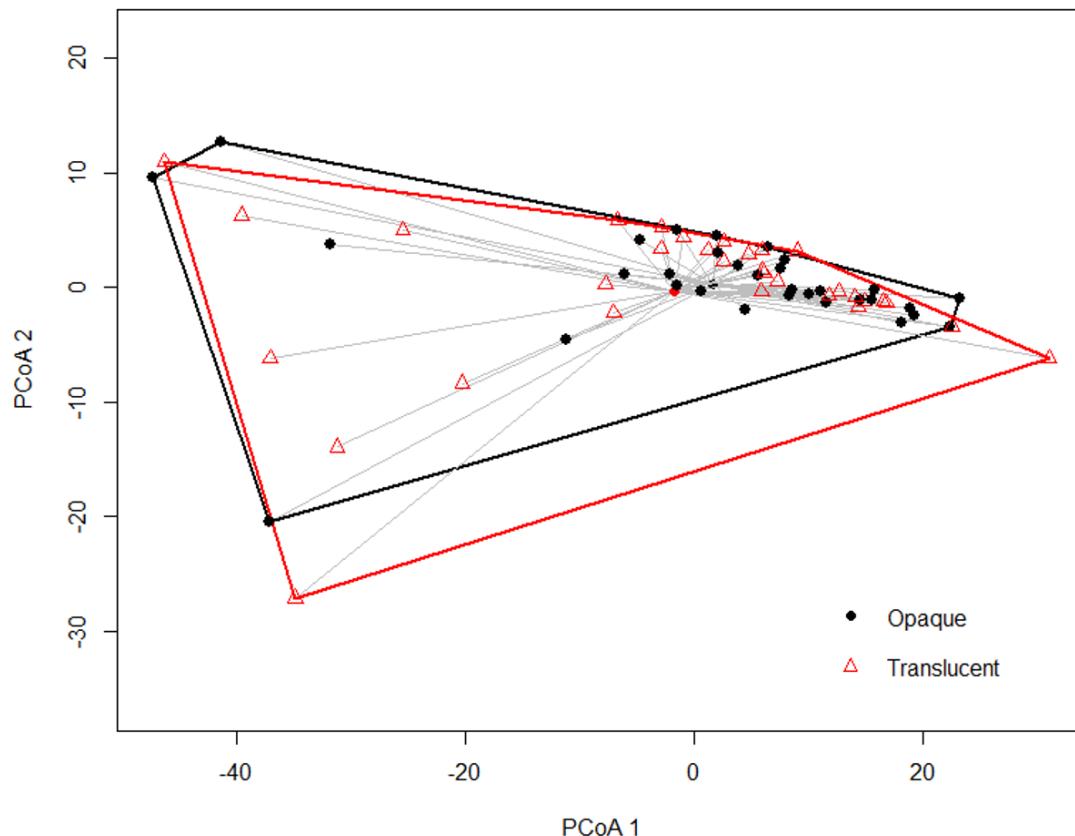


Figure 3. First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae between opaque and translucent bands using eleven elements. Each polygon represents a convex hull. Grey lines represent the distance to the centroid.

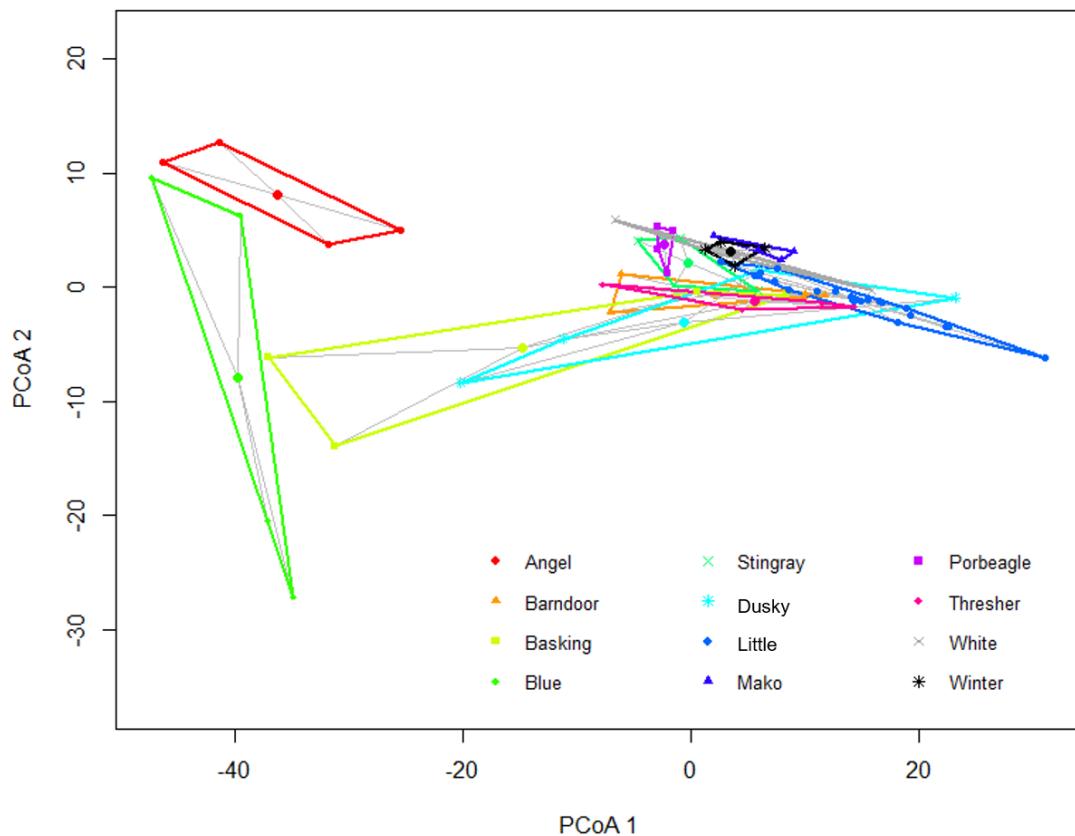


Figure 4. First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae among 12 species using eleven elements. Each polygon represents a convex hull. Grey lines represent the distance to the centroid.

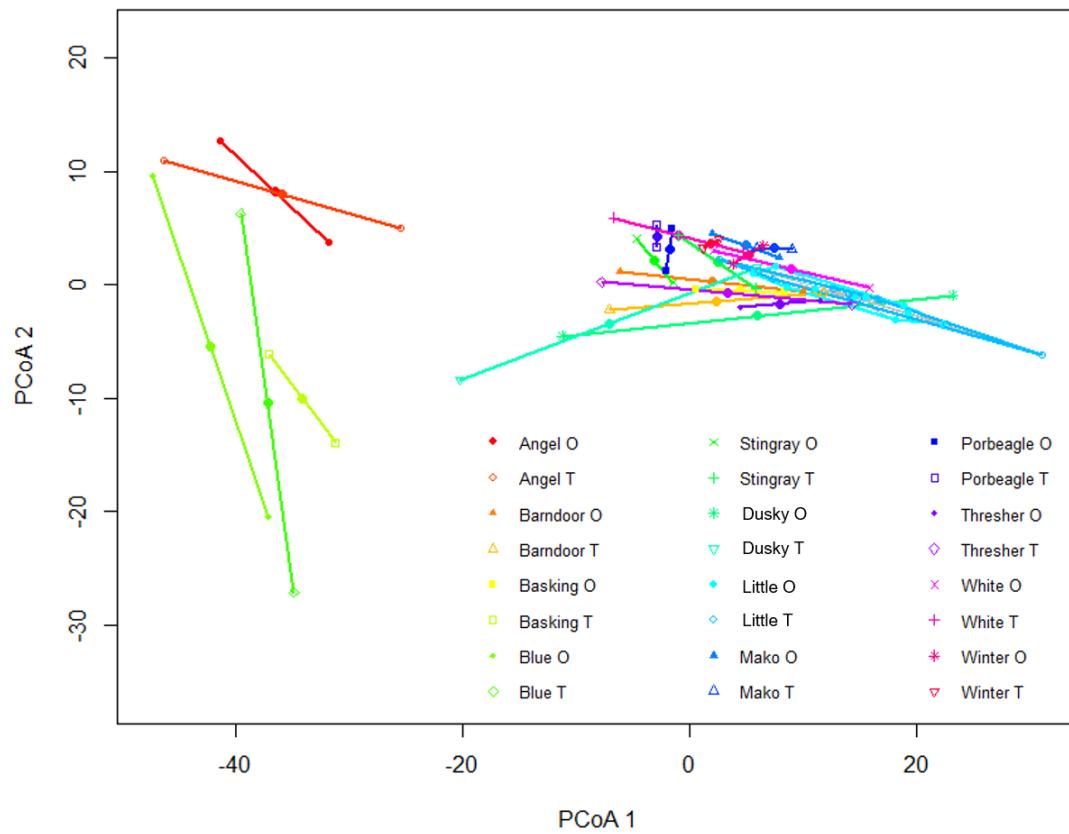


Figure 5. First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae among 12 species and band type (O = opaque; T = translucent) using eleven elements. Each line connects each data point with its centroid. Each polygon represents a convex hull. Grey lines represent the distance to the centroid.

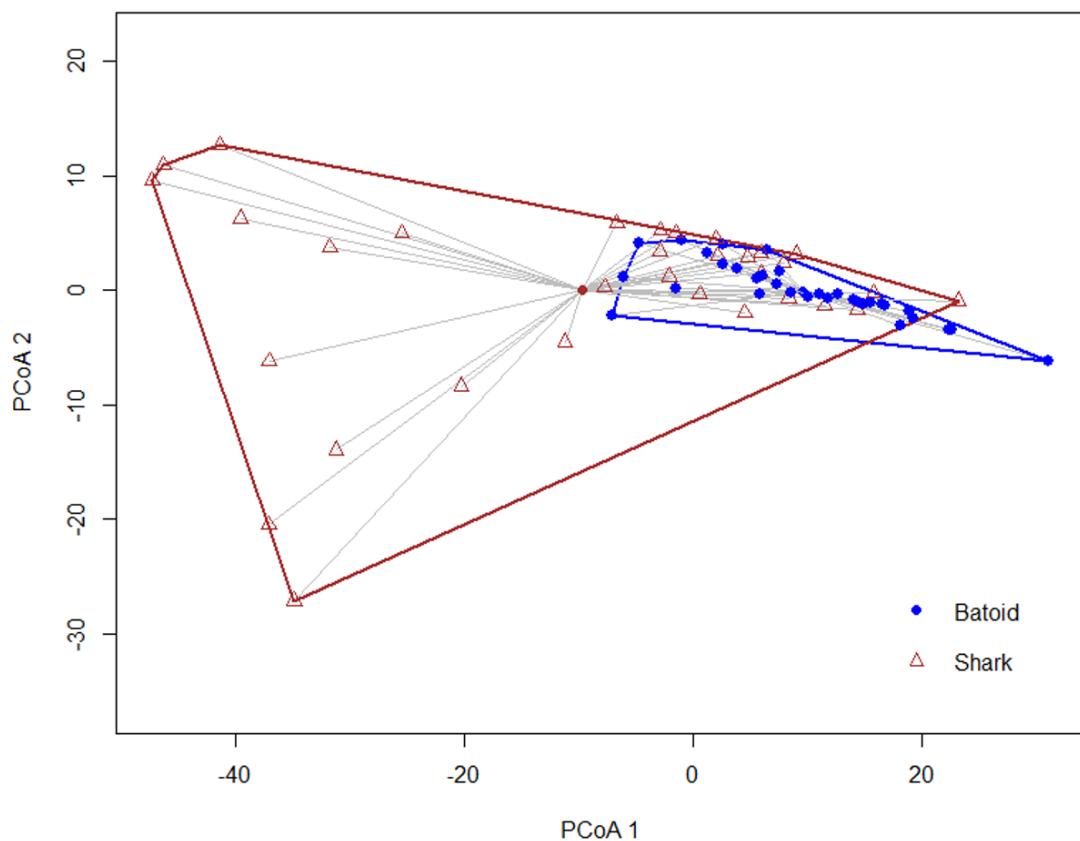


Figure 6. First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae among shark and batoid body plan using eleven elements. Each polygon represents a convex hull. Grey lines represent the distance to the centroid.

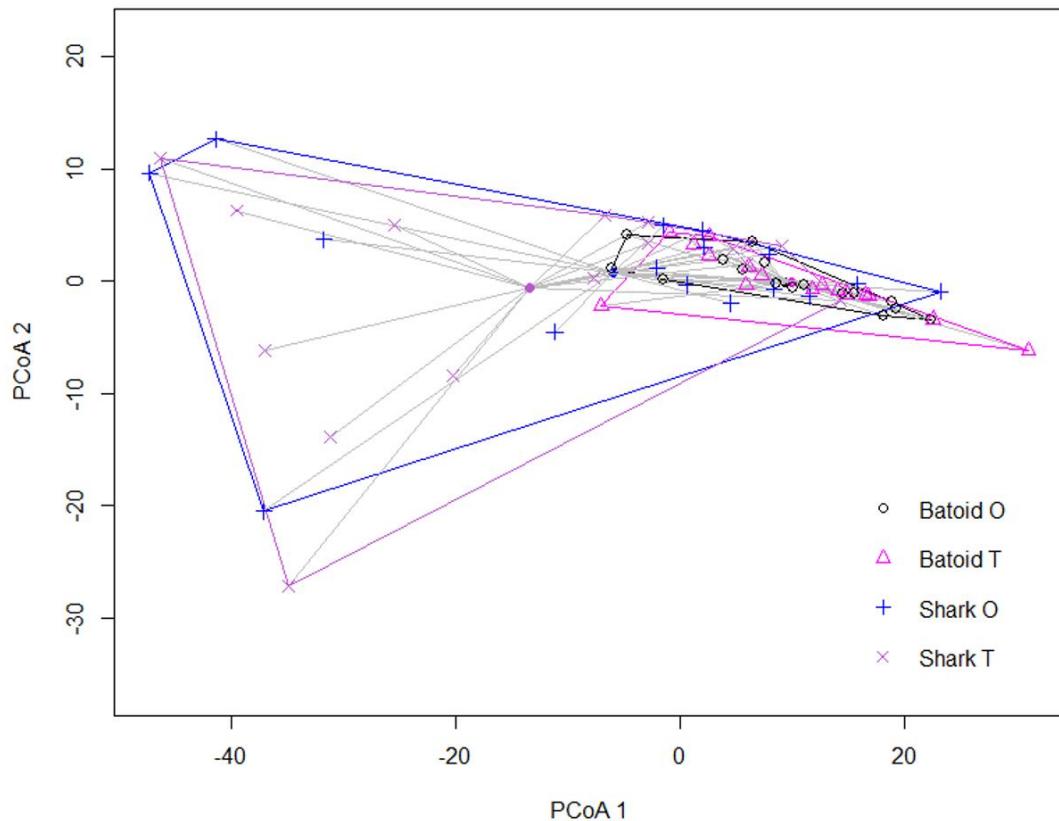


Figure 7. First two principal coordinates (PCoA1 and PCoA2) describing the chemical composition of elasmobranch vertebrae among shark and batoid body plan and band type using eleven elements. Each polygon represents a convex hull. Grey lines represent the distance to the centroid.

## **Discussion**

There was no chemical difference between band types (opaque and translucent) within *L. erinacea* and across species. This is at odds with previous research that detected peaks and troughs of Ca and P that corresponded with opaque and translucent bands (Jones and Geen 1977; Cailliet and Radtke 1987; Hale et al. 2006; Christensen 2011). The discrepancy may be due in part to the different method and slightly different goal of this study. Here, opaque and translucent bands were pinpointed and directly sampled, whereas previous studies have taken samples in a transect from focus to centrum edge. Additionally, the goal here was to identify distinct differences in chemical composition between band types to better characterize them, while previous studies (Cailliet and Radtke 1987; Hale et al. 2006; Christiansen 2011) matched Ca and P peaks and troughs to opaque and translucent bands in an effort to estimate age. While matching peaks and troughs to the band types was occasionally successful, the direct comparison of the chemical composition of the two band types across 12 species shows that there is no difference in chemical composition between opaque and translucent bands.

However, in many cases the element concentrations were highly variable within each band type and among individuals both within and between species (Figure 2). Variability of elements is evident in other chemical analyses of elasmobranch vertebrae, but not always discussed. For instance, Jones and Geen (1977) tracked Ca and P from focus to centrum edge and some troughs had similar values as peaks for both elements. Hale et al. (2006) found Ca peaks that varied 3.5x (~400,000 to ~1,400,000 Ca counts) within an individual and varied by 46.7x (~30,000 to

~1,400,000 Ca counts) among individuals. Christiansen (2011) also detected highly variable Ca peaks in *C. carcharias* centra using LA-ICP-MS; 1.6x (~1,250,000 to ~2,000,000 normalized Ca concentrations) within individuals and 2.6x (~700,000 to ~2,000,000 normalized Ca concentrations) among individuals. Additionally, Christiansen (2011) did not detect differences in amount of Ca between opaque and translucent bands. Cailliet and Radtke (1987) showed consistent relative magnitudes of peaks and troughs but had two instances of two Ca and P peaks for one opaque band. The variation of each element among bands of the same type along a centrum (opaque vs. opaque) may preclude detecting differences among bands of different types (opaque vs. translucent).

Chemical differences (regardless of band type) were detected among species particularly among the most common elements: Ca, P, O, and S. *Squatina dumeril*, and the translucent band of *C. maximus* were different from other species with lower Ca and P and higher O and S concentrations. *Prionace glauca* was not different in the concentrations in most common elements, but the overall composition was different from a majority of other species. Porter et al. (2006) showed that for several species of sharks, mineral content was positively correlated with vertebral stiffness and stiffer skeletons indicated increased swimming abilities; therefore, lower concentrations of Ca and P may reflect the reduced swimming stresses experienced by *S. dumeril*, a benthic sit-and-wait predator.

Different areas of vertebral centra are used for ageing by fishery biologists studying sharks and batoids. Both have opaque and translucent bands, but batoids often do not have an intermedia so ageing is exclusively done from the corpus

calcareum, whereas for sharks, the intermedialia can be heavily relied upon for ageing. For this study, the lack of an intermedialia in batoids led to a difference in sampling technique between sharks and batoids (Figure 1). The opaque and translucent bands are more easily distinguished in the intermedialia for sharks; therefore, this is where pin indentations and sampling occurred. Batoid vertebrae are smaller and the different band types were more difficult to pin-point on the corpus calcareum, which was the only option in the absence of an intermedialia. Despite this, the chemical composition of opaque and translucent bands in the batoids was less variable than that of sharks (Figure 5). Most of the sharks had similar chemical compositions to the batoids, so it is unlikely there is a large difference between sampling intermedialia and corpus calcareum.

The suggested difference between opaque and translucent bands within elasmobranch vertebrae is different amounts of mineralization between the two band types: opaque and translucent but results on which band type is more mineralized directly conflict. Some studies document higher amounts of Ca and P in the opaque band (Cailliet and Radtke 1987; Hale et al. 2006) while other studies claim the translucent bands are hypermineralized (Richards et al. 1963; Johnson 1979; Officer et al. 1997). The direct sampling of opaque and translucent bands separately in this study shows that Ca and P are present throughout both band types. Therefore, the optical differences between the band types are not based on a difference in basic chemical composition. Perhaps the difference between band types is structural rather than chemical. Opaque and translucent refer to whether light passes through a substance or is reflected; therefore, the crystalline structure may differ between opaque and

translucent bands. This warrants further research as opaque and translucent bands are used to estimate age, but the fundamental difference between the two is still unknown.

### **Acknowledgements**

I thank L. Natanson and D. Bengtson for their support and guidance. I thank C. O'Brien for helping this get started and use of the SEM at University of New Haven. I thank L. Kerr for training and use of the SEM at the Marine Biological Laboratory. I thank B. Flammang, J. Collie, and J. Webb for manuscript edits.

## References

- Anderson, M.J., and Walsh, D.C.I. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogenous dispersions: What null hypothesis are you testing? *Ecological Monographs*. **83**: 557-574.
- Applegate, S.P. 1967. A survey of shark hard parts. In. P.W. Gilbert, R.F. Matthewson, and D.P. Rall (eds.). Sharks, Skates, and Rays. The John Hopkins Press, Baltimore, Maryland. 37-67 pp.
- Cailliet, G.M. and R.I. Radtke. 1987. A progress report on the electron microprobe analysis technique for age determination and verification in elasmobranchs. In. The Age and Growth of Fish. (eds.) R.C. Summerfelt and G.E. Hall. Iowa State University Press, Iowa. 359-369 pp.
- Christensen, H.M. 2011. Developing and applying elemental composition of shark vertebrae as a tool for quantifying life history characteristics over ontogeny. University of Windsor, Ontario, Canada. M.S. Thesis.
- Clement, J.G. 1992. Re-examination of the fine structure of endoskeletal mineralization in Chondrichthyans: Implications for growth, ageing and calcium homeostasis. *Australian Journal of Marine and Freshwater Research*. **43**: 157-181.

Dean, M.N. and A.P. Summers. 2006. Mineralized cartilage in the skeleton of Chondrichthyan fishes. *Zoology*. **109**: 164-168.

Dean, M.N., C.G., Mull, S.N. Gorb, and A.P. Summers. 2009. Ontogeny of the tessellated skeleton: insight from the skeletal growth of the round stingray *Urobatis halleri*. *Journal of Anatomy*. **215**: 227-239.

Dean, M.N., Ekstrom, L., Monsonego-Ornan, E., Ballantyne, J., Witten, P.E., Riley, C., Habraken, W., and Omelon, S. 2015. Mineral homeostasis and regulation of mineralization processes in the skeletons of sharks, rays, and relatives (Elasmobranchii). *Seminars in Cell & Developmental Biology*. **46**: 51-67.

Dingerkus, G., B. Seret, and E. Guilbert. 1991. Multiple prismatic calcium phosphate layers in the jaws of present-day sharks (Chondrichthyes; Selachii). *Experientia*. **47**: 38-40.

Egerbacher, M., M. Helmreich, E. Mayrhofer, and P. Bock. 2006. Mineralization of hyaline cartilage in the small-spotted dogfish *Scyliorhinus canicula* L. *Scripta Medica (BRNO)*. **79(4)**: 199-212.

Hale, L.F., J.V. Dudgeon, A.Z. Mason, and C.G. Lowe. 2006. Elemental signatures in the vertebral cartilage of the round stingray, *Urobatis halleri*, from Seal Beach, California. *Environmental Biology of Fishes*. **77**: 317-325.

Haskell, W.L. 1949. An investigation of the possibility of determining the age of sharks through annuli as shown in cross-sections of vertebrae. Annual Report of the Marine Laboratory of the Texas Game, and Fish Commission FY 1948-49: 212-217

Hasse, C. 1879. Das natürliche System der Elasmobranchier: eine morphologische und paläontologische Studie. (Gustav Fischer: Jena.)

Huber, D.R., D.E. Neveu, C.M. Stinson, P.A. Anderson, and I.K. Berzins. 2013. Mechanical properties of sand tiger shark (*Carcharias taurus*) vertebrae in relation to spinal deformity. *The Journal of Experimental Biology.* 216: 4256-4263.

Ishiyama, R. 1951. Studies on the rays and skates belonging to the family Rajidae, found in Japan and adjacent regions. 2. On the age-determination of Japanese Black-Skate *Raja fusca*. *Bull Jap Soc Sci Fish* 16: 112-118

Johnson, G.F. 1979. The biology of the Little Skate, *Raja erinacea*, in Block Island Sound, Rhode Island. Master's Thesis. University of Rhode Island, Kingston, RI, USA.

Jones, B.C. and G.H. Geen. 1977. Age determination of an elasmobranch (*Squalus acanthias*) by x-ray spectrometry. *Journal of the Fisheries Research Board of Canada*. **34**: 44-48.

Kemp, N.E. and S.K. Westrin. 1979. Ultrastructure of calcified cartilage in the endoskeletal tesserae of sharks. *Journal of Morphology*. **160**: 75-102.

Natanson, L.J., and G.M. Cailliet. 1990. Vertebral growth zone deposition in Pacific angelsharks. *Copeia*. **1990(4)**: 1133-1145.

Natanson, L.J., S. Wintner, F. Johansson, A.N. Piercy, P. Campbell, A. De Maddalena, S.J. Gulak, B. Human, F.C. Fulgosi, D.A. Ebert, F. Hemida, F.H. Mollen, S. Vanni, G.H. Burgess, L.J.V. Compagno, and A. Wedderburn-Maxwell. 2008. Ontogenetic vertebral growth patterns in the basking shark, *Cetorhinus maximus*. *Marine Ecology Progress Series*. **361**: 267-278.

Natanson L.J., Skomal, G.B., Hoffmann, S., Porter, M., Goldman, K.J., and Serra, D. (2018). Age and growth of elasmobranchs: do band pairs on vertebral centra record age? *Marine and Freshwater Research*  
<https://doi.org/10.1071/MF17279>

Officer, R.A., R.W. Day, J.G. Clement, and L.P. Brown. 1997. Captive gummy sharks, *Mustelus antarcticus*, form hypermineralized bands in their vertebrae

during winter. *Canadian Journal of Fisheries and Aquatic Sciences*. **54**: 2677-2683.

Oksanen, J. F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, and H. Wagner. 2016. Vegan: Community Ecology Package. R package version 2.3-5. <http://CRAN.R-project.org/package=vegan>.

Porter, M.E., J.L. Beltran, T.J. Koob, and A.P. Summers. 2006. Material properties and biochemical composition of mineralized vertebral cartilage in seven elasmobranch species (Chondrichthyes). *The Journal of Experimental Biology*. **209**: 2920-2928.

Porter, M.E., T.J. Koob, and A.P. Summers. 2007. The contribution of mineral to the material properties of vertebral cartilage from the smooth-hound shark *Mustelus californicus*. *The Journal of Experimental Biology*. **210**: 3319-3327.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.

Richards, S.W., Merriman, D., and Calhoun, L.H. 1963. Studies on the marine resources of southern New England, IX. The biology of the little skate, *Raja*

*erinacea*, Mithcill. Bulletin of the Bingham Oceanographic Collection Yale University **18**: 5-67.

Ridewood, W.G. 1921. On the calcification of the vertebral centra in sharks and rays. *Philosophical Transactions of the Royal Society of London Series B*. **210**: 311-407.

Scharer, R.M., W.F. Patterson III, J.K. Carlson, and G.R. Poulakis. 2012. Age and growth of endangered smalltooth sawfish (*Pristis pectinata*) verified with LA-ICP-MS analysis of vertebrae. *PLoS One*. 7(10): e47850

Seidel, R., Lyons, K., Blumer, M., Zaslansky, P., Fratzl, P., Weaver, J.C., and Dean, M.N. 2016. Ultrastructural and developmental features of the tessellated endoskeleton of elasmobranchs (sharks and rays). *Journal of Anatomy*. **229**: 681-702.

Tanaka, S. 1990. Age and growth studies on the calcified structures of newborn sharks in laboratory aquaria using tetracycline. In Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of Fisheries NOAA Technical Report 90: 189-202

## **CHAPTER 3**

### **Vertebral Growth and Band-Pair Deposition in Sexually Mature Little Skates**

#### ***Leucoraja erinacea: Is Adult Band-Pair Deposition Annual?***

Kelsey C James<sup>1</sup>

<sup>1</sup>Department of Fisheries, Animal and Veterinary Sciences. University of Rhode Island, Kingston, Rhode Island 02881, USA

*in review in Journal of Fish Biology*

## ABSTRACT

The number of band pairs in the vertebral centra of elasmobranchs is used as a proxy of age for the construction of stock-assessment models. Band pairs are generally assumed to be annual, but evidence to the contrary and the difficulty of confirming their annual periodicity has warranted further study into the processes underlying band-pair deposition. Mature male and female little skates *Leucoraja erinacea* were injected with oxytetracycline and maintained in captivity for 13 months to document centrum growth and the frequency of band-pair deposition. Of the 41 individuals analyzed, 63% did not deposit a full band pair over a 13-month period. Thus, a majority of individuals did not exhibit annual band-pair deposition. All females were reproductively active and ate approximately 1.5 times more food than did males, but monthly growth rate, total centrum growth, and the numbers of band pairs deposited were not significantly different between sexes. Age underestimation of larger/older elasmobranchs is being identified in an increasing number of elasmobranch species. The effect of inaccurate age estimates from band-pair counts on stock assessment results needs to be addressed.

**Keywords:** age; batoids; decreased frequency; growth; oxytetracycline; vertebral centra

## INTRODUCTION

In fishes, accurate age-based characteristics, such as age at sexual maturity and longevity, are crucial for the construction of stock assessment models in order to

correctly estimate population productivity. Age is generally determined by counting growth zones in a hard structure that grows in proportion to body size or weight. Size-at-age data compiled from individuals across the entire size range of a species are used to estimate these age-based characteristics.

Vertebral centra are the most commonly used structure for age determination in elasmobranch fishes. The centra have characteristic band pairs, each of which is composed of one opaque and one translucent band (Figure 1; Lagler, 1952; Cailliet *et al.*, 2006). The assumption has been that one band pair is deposited per year (Ishiyama, 1951), but Natanson & Cailliet (1990) and Natanson *et al.*, (2008) showed that band-pair deposition is correlated with somatic growth, but not age, in two species, the Pacific angel shark *Squatina californica* Ayres 1859 and the basking shark *Cetorhinus maximus* (Gunnerus 1765). Furthermore, no predictable temporal pattern of band-pair deposition (annual or otherwise) could be identified in several other elasmobranch species (Tanaka, 1990; Chidlow *et al.*, 2007; Huvaneers *et al.*, 2013). Age validation studies (confirming the accuracy of age estimates with a determinate method; Cailliet, 1990) support the assumption of annual band-pair deposition for a part of the lifespans of only some species, and other studies have shown that band-pair deposition slows and/or stops at older ages (Kalish & Johnston, 2001; Francis *et al.*, 2007; Harry, 2017).

While most of the research illustrating a decreased frequency (less than one band pair per year) or cessation of band-pair deposition has been in large-bodied sharks, decreased frequency of band pair-deposition has also been documented in larger individuals in several batoid species: Little Skate *Leucoraja erinacea* (Mitchill

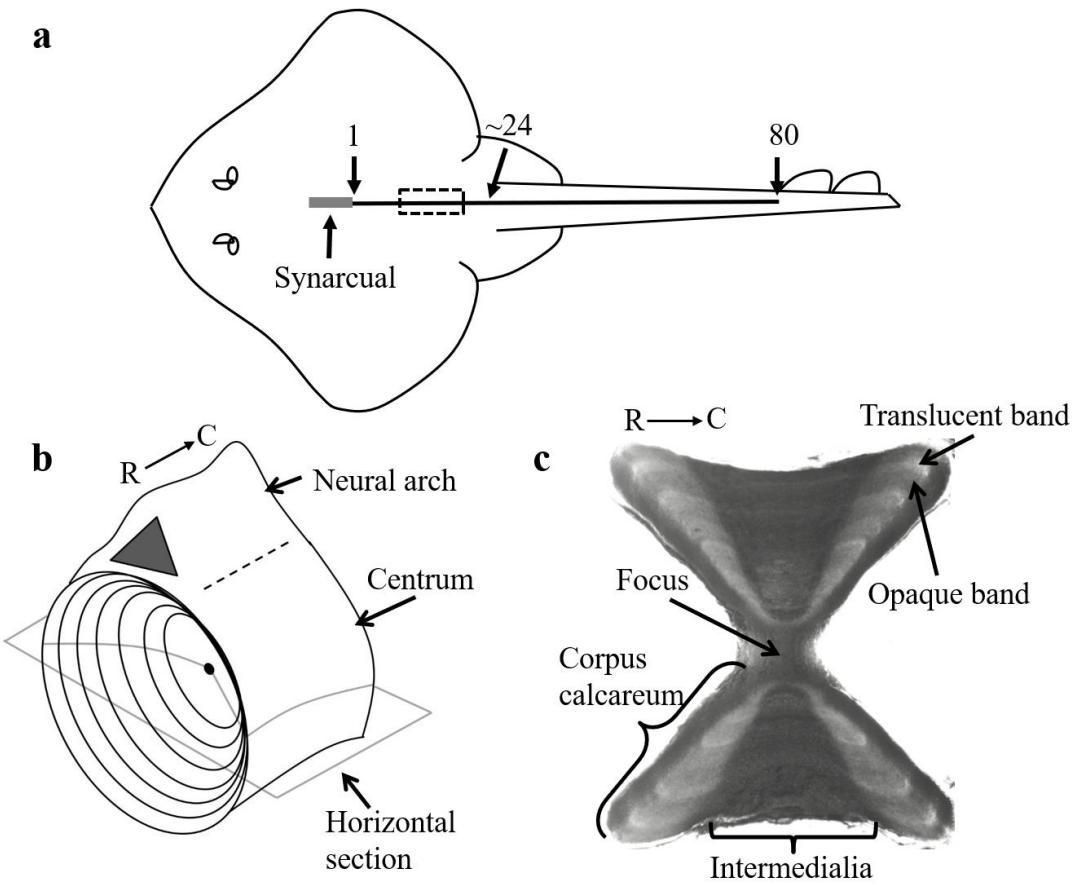


Figure 1. (a) Diagram of a typical *Leucoraja erinacea* indicating the position of different vertebrae (1, ~24, and 80). The transition between abdominal and caudal vertebrae varies among individuals but occurs at vertebra 24 on average. The dotted box indicates where vertebrae were sampled. (b) Parts of a vertebra and (c) resulting horizontal section used for ageing. The rectangle in (b) is represented in (c). (c) is a section from a Pacific starry skate, *Raja stellulata*.

1825) (Natanson, 1993), Winter Skate *L. ocellata* (Mitchill 1815) (McPhie & Campana, 2009), and Blue-Spotted Maskray *Neotrygon kuhlii* (Müller & Henle 1841) (Pierce & Bennett, 2009). *Leucoraja erinacea* is an interesting example because three independent experimental studies have documented annual band-pair deposition in juveniles and in some adults (Natanson, 1993; Ciccia *et al.*, 2009; Sagarese & Frisk, 2010). However, Natanson (1993) noted two mature, ovipositing females that only deposited a partial opaque band in one year of captive growth. That study concluded that “annual banding may not occur when the females are reproductively active” (Natanson, 1993). This result, in combination with the documented decreased frequency of band-pair deposition in larger/older elasmobranch species, suggested that reproductive state may affect band-pair deposition.

Instead of age, a proportional relationship between somatic growth and frequency of band-pair deposition has been suggested in several shark species (Natanson & Cailliet, 1990; Tanaka, 1990; Natanson *et al.*, 2008). The relationship between somatic growth and total centrum growth over a period of time has long been established (Haskell, 1948) and is the basis for using hard parts, like vertebral centra, as indicators of age (Cailliet *et al.*, 2006). Natanson & Cailliet (1990) suggested that centra grow (and therefore band pairs are deposited) in proportion to increases in body mass as dictated by structural needs. As an individual approaches maximum total length, body growth and centrum growth decrease; therefore, the frequency of band-pair deposition may also decrease (Francis *et al.*, 2007; Natanson *et al.*, 2014; Natanson *et al.*, 2018).

The goal of the present study was to investigate the frequency of band-pair deposition and centrum growth in sexually mature individuals. *Leucoraja erinacea* was used as a model organism based on its use in previous research and its resilience in captivity. Annual band-pair deposition was confirmed in juvenile *L. erinacea* (Natanson, 1993; Sagarese & Frisk, 2010), but not in reproductive, mature females. Here, monthly growth rate, total centrum growth, and frequency of band-pair deposition over a period of 13 months were compared between sexually mature (ovipositing) females and sexually mature males using chemical markers. The characterization of centrum growth in adults is critical to assess the validity of using band-pair counts for accurate age estimates and their subsequent use for stock assessments and fisheries management.

## METHODS

Forty-four sexually mature *L. erinacea* (22 male and 22 female  $> 40$  cm  $L_T$  (Total Length); Sosebee, 2005) were collected from Narragansett Bay, Rhode Island (RI), USA in February 2015 and May – November 2015 (water temperature  $< 19^\circ\text{C}$ ) using an otter trawl with a tow duration of 30 min. Healthy individuals, indicated by an active defense response to handling, were transported in seawater-filled coolers to the aquarium facility at the Narragansett Bay Campus, University of Rhode Island. Total length ( $L_T$ ) was measured as the straight-line from snout tip to tail tip to the nearest 0.1 cm. Fish were weighed (Total Weight [ $W_T$ ]) to the nearest 0.5 g upon capture, and monthly thereafter. Pectoral fin clips, notching the fin in strategic locations, were used to identify individuals. Water temperature in holding tanks was

maintained at ambient Narragansett Bay temperatures, but was not allowed to go below 5°C or above 20°C to acknowledge the normal thermal range for the species (1–21°C; Bigelow & Schroeder, 1953). Temperature, dissolved oxygen, and pH were monitored daily.

Skates were acclimated for at least two weeks. On 4 December 2015, each skate was injected intramuscularly in the thickest part of the pectoral fin muscle with 25 mg kg<sup>-1</sup> body weight of oxytetracycline (OTC). Skates were fed every other day by offering 2-g pieces of herring or squid to each individual until food was refused. Food consumption was recorded at each feeding for each individual and egg deposition was recorded daily. At the end of 13 months (4 January 2017) skates were euthanized via overdose with tricaine methanesulfonate (400 mg L<sup>-1</sup> water) and measured (L<sub>T</sub>) and weighed (W<sub>T</sub>). Monthly growth rate (g month<sup>-1</sup>) was calculated as  $\frac{(W_{Tfinal} - W_{Tinitial})}{13}$ . Sexual maturity status was determined by visually inspecting the condition of the gonads (Ebert 2005).

Two adjacent abdominal vertebral centra (Figure 1) were extracted from each skate. One centrum used for oxytetracycline analysis (hereafter referred to as the OTC section) was embedded in TAP® Clear-Lite casting resin (TAP® Plastics, Dublin, CA, USA), and sectioned horizontally through the focus (Figure 1) with a low-speed saw (Buehler Isomet®, Lake Bluff, IL, USA) with paired diamond-edged blades separated by 0.2-mm or 0.4-mm spacer. The section was visualized with a dissecting microscope (Nikon model SMZ1500®, Melville, NY, USA) using UV light (366 nm). Images were captured with a digital camera (Nikon model DSR12, Tokyo, Japan) and image processing software (NIS Elements, version 4.40, Nikon Corporation, Tokyo,

Japan). Total centrum growth was defined as the distance from the beginning of the OTC mark to the centrum edge (to the nearest 0.01 mm) using Adobe® Photoshop® (Adobe Systems Incorporated, San Jose, CA, USA). The other centrum extracted from each individual (hereafter referred to as the histology section) was processed histologically to enhance band pairs (Natanson *et al.*, 2007) and photographed with reflected light using the camera system described above. The total centrum growth measured from the image of the OTC section was divided by the length of the corpus calcareum (CC) then superimposed on the image of the histology section to determine into which band type the OTC was incorporated, and the number of band pairs located distal to the OTC mark (new cartilage deposition; Figure 2). The number of band pairs distal to the OTC mark was compared between sexes and with respect to each of seven growth variables (food consumption, monthly growth rate, centrum growth, change in  $L_T$ , final  $L_T$ , final  $W_T$ , and total band-pair count, detailed below) using logistic regressions (R Core Team 2017).

Band pairs were counted in captured digital images of the histology sections. The birth band was identified as the first fully-formed band beyond the focus that was associated with an angle change in the CC. Two readers counted the band pairs in each centrum twice, without knowledge of fish size or sex. Precision, to assess repeatability of counts, was determined using the coefficient of variation (CV; Chang, 1982). Coefficients of variation <10% were interpreted as reflecting acceptable within and between reader precision. Bias, as a result of either systematic or random error, was assessed with the Evans-Hoenig (1998) test of symmetry. Final band-pair counts

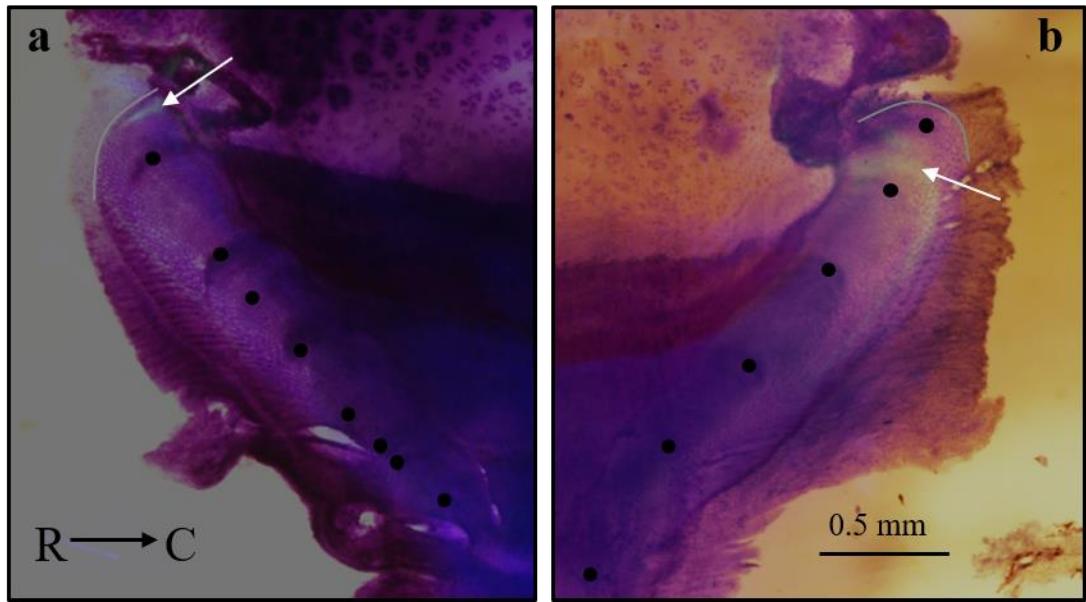


Figure 2. Sample histological sections with oxytetracycline (indicated by white arrow) superimposed. (a) Oxytetracycline in the ultimate band. (b) Oxytetracycline with a band pair formed distally. Oxytetracycline was deposited in a translucent band in both images. Light grey line indicates the edge of the corpus calcareum. Orientation is from left to right: rostral (R) to caudal (C).

were assigned from the primary reader's second count (KCJ) or from consensus between readers if the band-pair counts differed by more than two band pairs.

Food consumption per individual was summed for the entire experimental period (13 months). Ten growth variables: food consumption, monthly growth rate, total centrum growth, change in  $L_T$ , initial  $L_T$ , final  $L_T$ , initial  $W_T$ , final  $W_T$ , total band-pair count, and band pairs distal to OTC were compared between sexes using t-tests (R Core Team 2017). Food consumption and monthly growth rate and food consumption and total centrum growth were compared in males and females using analyses of covariances (ANCOVAs; R Core Team 2017).

## RESULTS

Forty-one skates (21 females and 20 males) were used for analysis; three fish were eliminated due to procedural error and mortality. During the experimental period water temperature ranged from 5 to 21 °C, pH ranged from 7.8 to 8.2, and dissolved oxygen ranged from 2.4 to 11.9 mg L<sup>-1</sup>. Total fish length did not differ significantly between the sexes at the beginning or end of the 13-month experimental period, but females had a significantly higher mean mass than males both at the beginning and the end of the experimental period (Table I).

All but one female was observed laying at least one egg during the experimental period. A total of 1395 eggs were laid during the experimental period representing a mean of 65.2 eggs per reproductively active female per year. Peak egg deposition occurred between June and October 2016. The total number of eggs laid per month increased with increasing temperature over the experimental period (Figure 3). Upon

Table I. Differences in ten growth variables in males versus females at the end of the 13-month experimental period for *Leucoraja erinacea* analyzed using t-tests. L<sub>T</sub> is total length and W<sub>T</sub> is total weight. Asterisk indicates significant difference.

Variable	Females Mean ± S.E.	Males Mean ± S.E.	t statistic	df	p-value
<b>Food Consumption (g)</b>	2640 ± 52	1723 ± 58	11.866	39	< 0.001 *
<b>Monthly Growth (g mo.<sup>-1</sup>)</b>	4.8 ± 0.8	2.6 ± 1.8	1.101	26	0.281
<b>Centrum Growth (mm)</b>	0.05 ± 0.01	0.04 ± 0.01	1.085	32	0.286
<b>Change in L<sub>T</sub> (cm)</b>	-1.1 ± 0.4	-1.1 ± 0.4	0.060	39	0.952
<b>Initial L<sub>T</sub> (cm)</b>	48.1 ± 0.4	48.0 ± 0.6	0.185	31	0.855
<b>Final L<sub>T</sub> (cm)</b>	47.0 ± 0.3	46.9 ± 0.5	0.272	39	0.787
<b>Initial W<sub>T</sub> (cm)</b>	651.0 ± 10.3	599.9 ± 18.1	2.457	30	0.020 *
<b>Final W<sub>T</sub> (cm)</b>	713.0 ± 12.3	633.7 ± 20.0	3.381	32	0.002 *
<b>Total Band Pair Count</b>	9 ± 0.5	9 ± 0.5	1.416	32	0.166
<b>Band Pairs Distal to OTC</b>	0.7 ± 0.1	0.6 ± 0.1	0.079	39	0.937

OTC, oxytetracycline

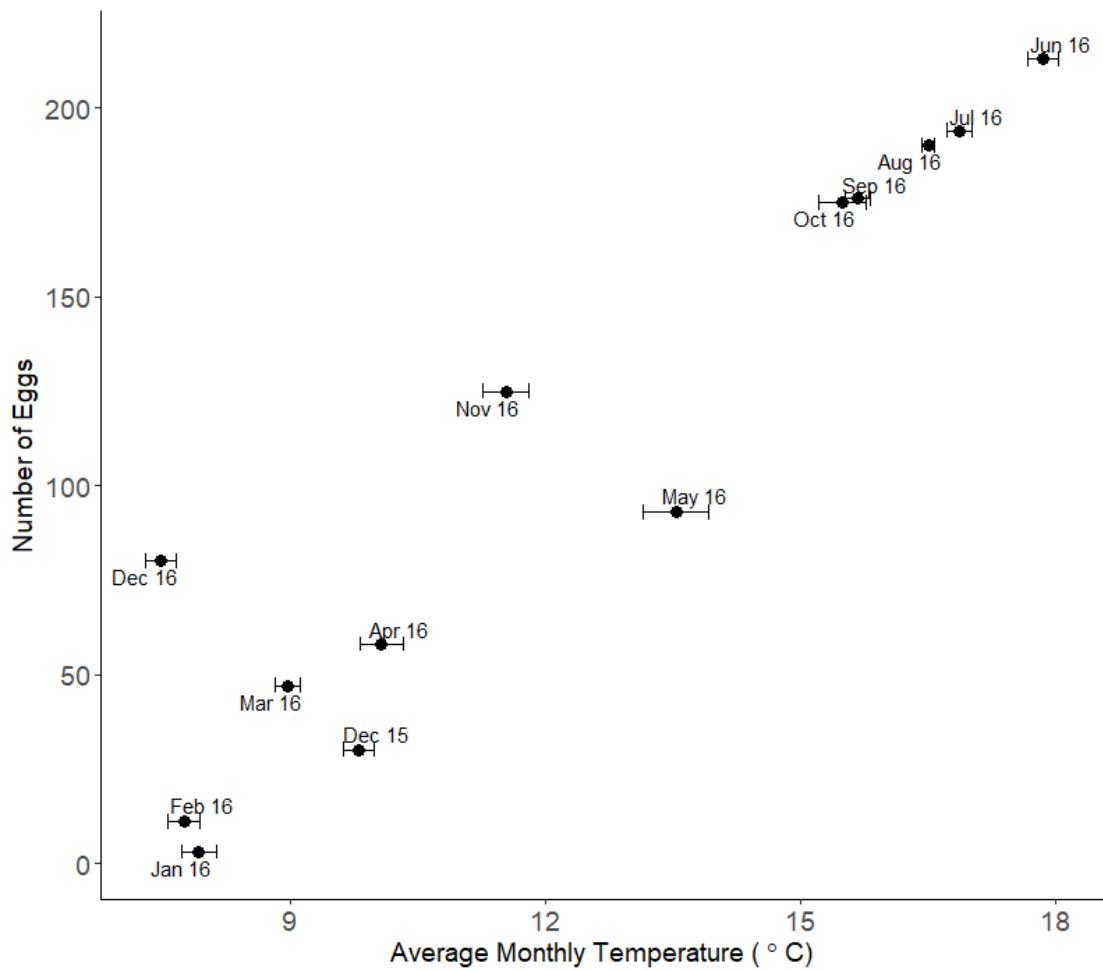


Figure 3. Monthly total number of eggs laid by 20 *Leucoraja erinacea* by mean monthly water temperature (°C) in 2015 and 2016. Error bars represent standard error.

dissection, the one female that had not been observed to lay eggs was determined to be sexually mature based on the presence of well-developed shell glands, ovaries, and uteri, but no eggs were present in either ovary. In the remaining females, each ovary had 1- 6 eggs,  $\geq$  10 mm in diameter.

A mean of 2192 g of food ( $\pm$  SE = 82 g) was consumed per individual during the experimental period. Females consumed significantly more food than males (Table I; Figure 4a), but the mean monthly growth rate was not significantly different between sexes (Table I; Figure 4b).

Total band-pair counts ranged from 4 to 15 for all 41 individuals, but the number of band pairs was not significantly different between males and females (Table I). The within-reader precision of the primary reader was good (CV = 8.57%), while the within-reader precision of the secondary reader was greater than 10% (CV = 12.03%). Comparing the second counts between readers, between-reader CV was 21.08% with 22 centra differing by more than two band pairs. These 22 centra were re-examined by both readers to determine a consensus. The between-reader CV value without these 22 centra was acceptable at 9.05%. The Evans-Hoenig test of symmetry detected within-reader bias for the primary reader ( $\chi^2 = 13.45$ , d.f. = 4,  $p < 0.01$ ), and between-reader bias ( $\chi^2 = 30.33$ , d.f. = 8,  $p < 0.001$ ). Within-reader bias was not detected for the secondary reader ( $\chi^2 = 3.12$ , d.f. = 3,  $p > 0.05$ ).

All 41 individuals had a fluorescent OTC mark. Six individuals (15%; 1 female, 5 males) did not have an OTC mark across the corpus calcareum (CC; where band pairs are visible), therefore the number of band pairs distal to the OTC mark and

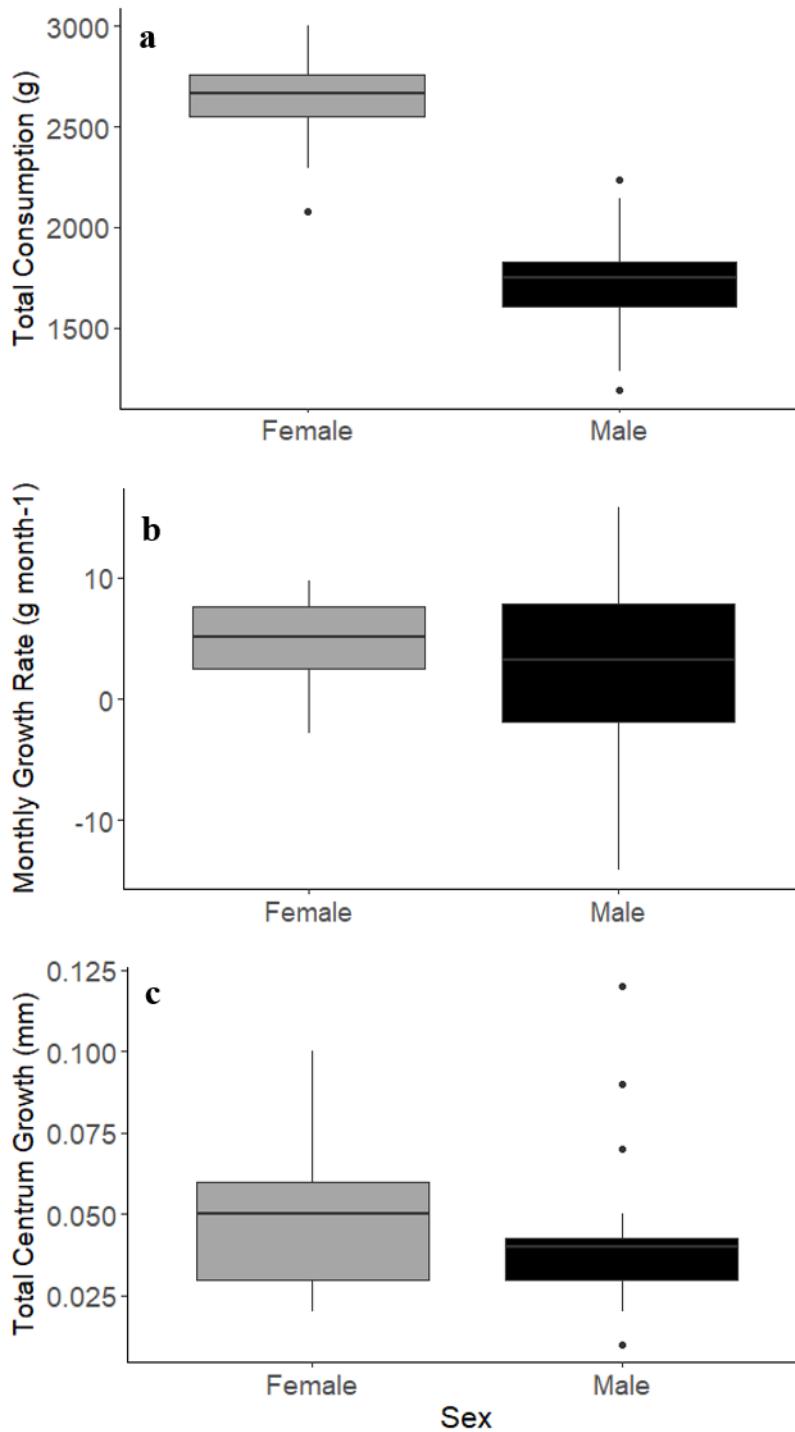


Figure 4. (a) Total consumption over 13 months by sex (Females = 21; Males = 20). (b) Monthly growth rate over 13 months by sex (Females = 21; Males = 20). (c) Total centrum growth over 13 months by sex (Females = 20; Males = 15). Horizontal line is median, box is inter-quartile range, vertical line is 95% confidence intervals and points fall outside the 95% confidence intervals.

total centrum growth could only be measured in 35 individuals. Oxytetracycline staining was observed on the outer edge of CC in 78% of individuals, diffuse throughout the CC in 34% of individuals, in the arch tissue surrounding the centrum in 58.5% of individuals, at the focus in 19.5% of individuals, and diffuse in the intermedia in 12% of individuals, in addition to across the CC in most individuals (Figure 5). Mean total centrum growth measured from the beginning of the OTC mark to the edge of the centrum was 0.05 mm (SE = 0.004) did not significantly differ between males and females (Table I; Figure 4c).

Oxytetracycline was incorporated into the translucent band in 74% of individuals (15 females and 11 males) and into the opaque band in 26% of individuals (5 females and 4 males; Table II). Sixty-three percent of individuals had OTC in the ultimate band, having formed only 0.5 band pairs (11 females and 11 males), and 34% had formed one band pair (9 females and 3 males). One male (#10) had formed 1.5 band pairs during the 13-month experimental period (Figure 2; Table II) and was excluded from subsequent statistical analyses, because its category (1.5 band pairs) represented a sample size of one. Skates that deposited a full band pair were smaller in size (final  $L_T$ ) and their centra grew more during the experimental period (Table III). The number of band pairs deposited (0.5 or 1) was not significantly different between males and females (Table I), or with reference to any of the following growth variables: food consumption, monthly growth rate, change in  $L_T$ , final  $W_T$ , or total band-pair count (Table III).

The relationship between total food consumption and monthly growth rate was significantly different between sexes ( $F = 12.015$ , d.f. = 1,  $p < 0.01$ ). Monthly growth

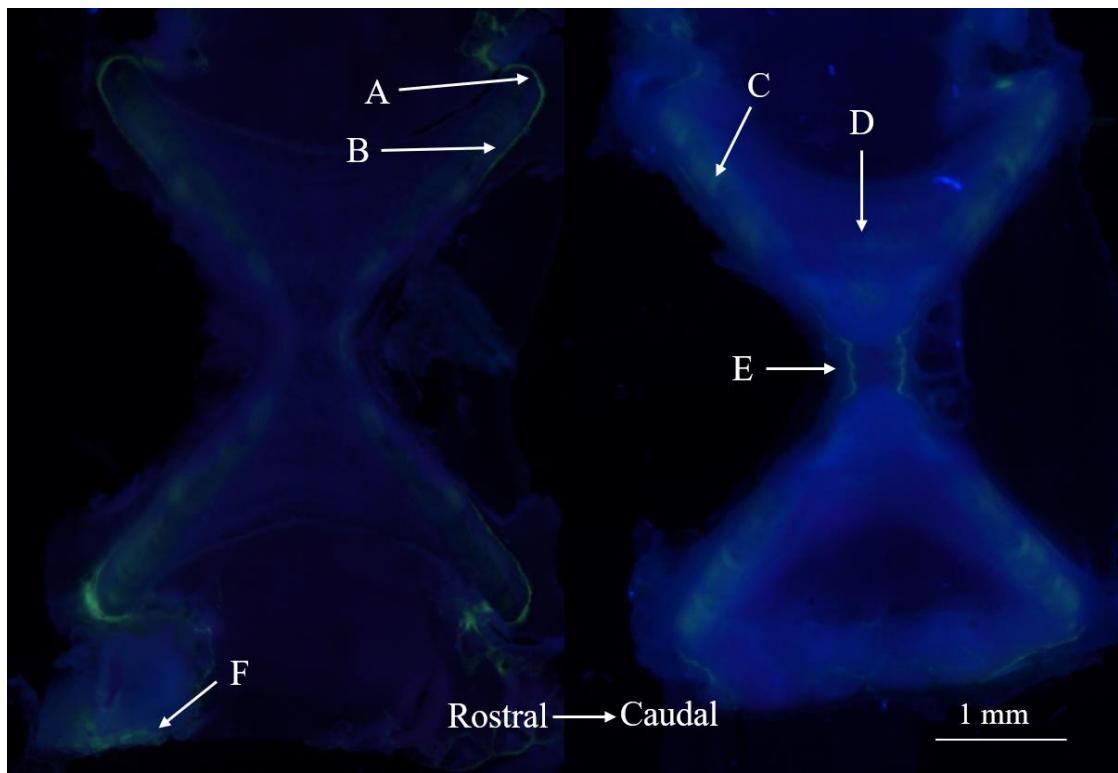


Figure 5. Locations of oxytetracycline in *Leucoraja erinacea* centra. Oxytetracycline was expected to be across the corpus calcareum (A), but also occurred on the outer edge of the corpus calcareum (B), diffuse in the corpus calcareum (C), diffuse in the intermedia (D), at the focus (E), and in the arch tissue surrounding the centrum (F). Centra belong to specimens #23 and #99.

Table II. Sex, location of oxytetracycline mark, size, and weight of captive *Leucoraja erinacea* used in this study.

<b>Individual<sup>†</sup></b>	<b>Sex</b>	<b>OTC<sup>‡</sup></b>	<b>Initial LT (cm)</b>	<b>Final LT (cm)</b>	<b>Initial Wt (g)</b>	<b>Final Wt (g)</b>
26	M	O	46.7	44.3	523.5	592.0
11	F	O	47.0	46.6	683.0	743.0
88	M	O	46.8	46.8	463.5	615.0
69	F	O	48.1	49.9	673.0	766.5
23	M	T	45.2	44.5	682.0	498.0
99	M	T	43.9	44.6	639.5	490.5
33	F	T	46.1	45.6	564.0	590.5
52	F	T	48.1	46.4	631.0	730.0
7	F	T	50.4	47.0	722.5	788.5
18	F	T	47.9	47.0	558.0	685.0
6	M	T	49.6	47.1	569.0	618.0
66	M	T	48.1	47.7	581.0	674.5
PCP	F	T	49.8	47.9	631.5	698.0
15	F	T	50.4	48.0	612.0	715.0
50	F	T	49.6	48.2	632.0	646.5
60	F	T	47.7	48.5	687.0	667.0
4	M	T	48.1	48.5	590.0	682.5
3	M	T	52.2	48.9	691.0	713.5
70	M	T	48.9	49.1	559.0	704.0
82	F	T	49.6	49.5	662.0	777.5
120	M	T	50.0	50.1	550.0	676.5
39	M	T	52.2	51.5	660.0	865.5
14	F	O, T	44.5	46.2	608.0	653.0
36	F	O, T	48.0	46.5	643.5	729.5
40	F	O, T	46.6	46.6	610.0	702.5
100	M	O, T	48.2	47.5	481.0	650.5
RRRF	F	T, O	49.2	44.0	709.5	672.0
31	M	T, O	45.3	44.1	524.0	513.5
16	F	T, O	45.7	44.2	598.5	647.0
LEO	F	T, O	47.7	45.2	686.0	699.5
CRC	F	T, O	47.8	46.6	679.0	718.0
1	M	T, O	48.9	46.7	579.0	604.0
92	F	T, O	51.1	46.8	674.0	789.0
13	F	T, O	47.4	47.8	707.0	742.5
10	M	O, T, O	47.8	47.6	580.0	591.0

44	M	-	45.5	42.9	665.0	597.0
8	M	-	44.9	45.1	566.0	599.0
90	M	-	44.1	45.6	624.5	555.5
29	M	-	51.1	47.5	808.0	717.5
9	M	-	52.3	47.6	661.5	715.5
55	F	-	48.0	47.9	699.5	813.0

<sup>†</sup>Numeric or alphabetic code for individual skates; <sup>‡</sup>OTC, in which band the oxytetracycline mark was and subsequent band types present distal to this mark; O, Opaque band; T, Translucent band; -, no oxytetracycline detected in the corpus calcareum.

Table III. Differences between the number of band pairs present distal to the oxytetracycline mark (0.5, 1) and seven growth variables in males and females at the end of 13-month experimental period for *Leucoraja erinacea* analyzed with logistic regressions. Asterisk indicates significant difference.

<b>Variable</b>	<b>0.5 Band Pair</b>	<b>1 Band Pair</b>	<b>p-value</b>
	<b>Mean ± S.E.</b>	<b>Mean ± S.E.</b>	
<b>Food Consumption (g)</b>	2213 ± 1120	2371 ± 141	0.407
<b>Monthly Growth (g mo.<sup>-1</sup>)</b>	4.8 ± 1.5	4.0 ± 1.3	0.705
<b>Centrum growth (mm)</b>	0.04 ± 0.00	0.06 ± 0.01	0.035 *
<b>Change in Lt (cm)</b>	-0.9 ± 0.3	-1.5 ± 0.6	0.247
<b>Final Lt (cm)</b>	47.6 ± 0.4	46.0 ± 0.4	0.024 *
<b>Final Wt (g)</b>	679.0 ± 19.0	676.8 ± 20.7	0.939
<b>Total Band Pair Count</b>	9 ± 0.5	8 ± 0.6	0.143

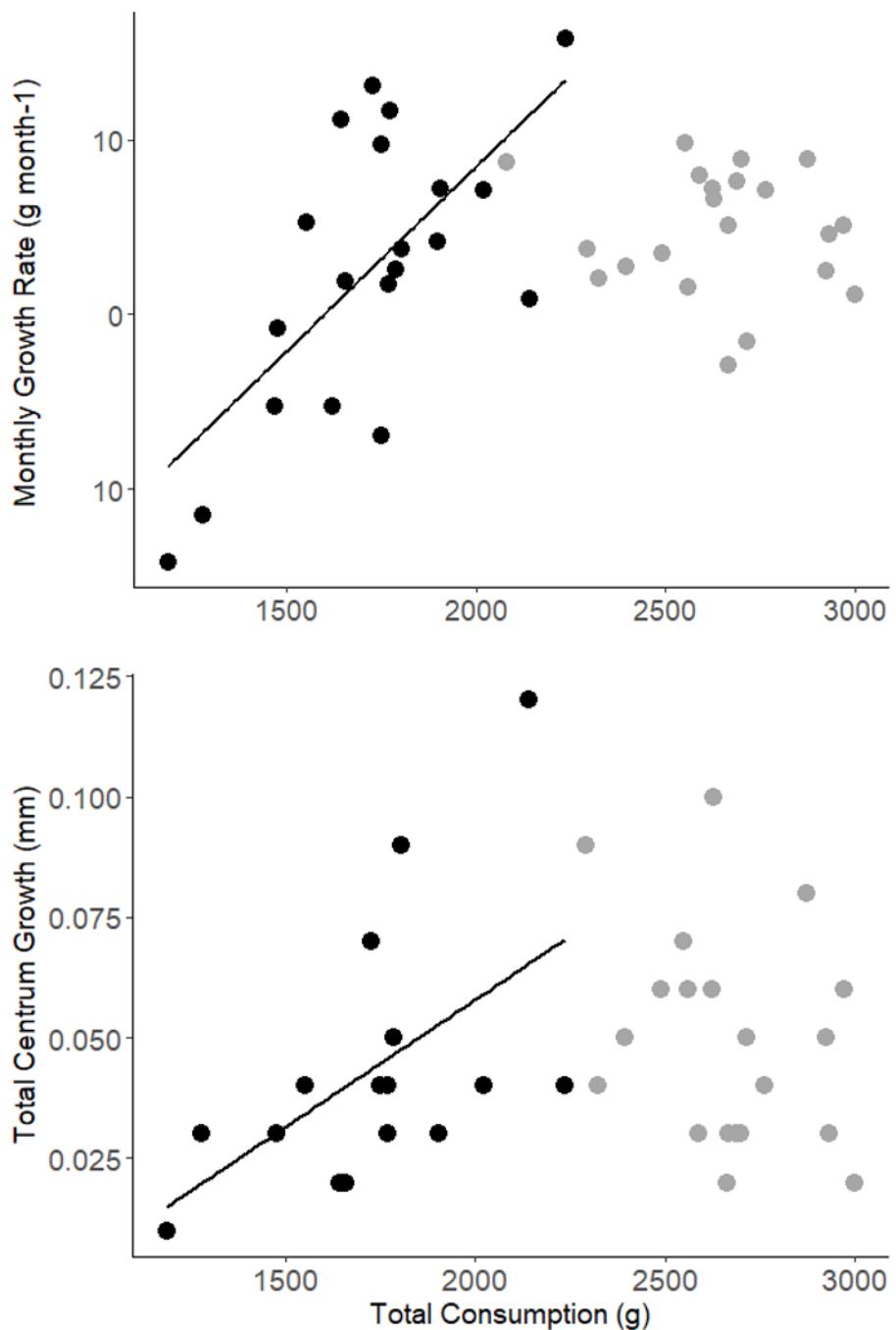


Figure 6. (a) Monthly growth rate for males (black circles, n=20) and females (grey circles, n=21). Male linear regression:  $y = 0.021x - 33.775$ ;  $p < 0.01$ ; adj  $r^2 = 0.42$ . Female linear regression:  $y = -0.0015x + 8.746$ ;  $p > 0.05$ ; adj  $r^2 = -0.04$ . (b) Total centrum growth by individuals in males (black circles, n=15) and females (grey circles, n=20). Male linear regression:  $y = 5.29 \times 10^{-5}x - 0.048$ ;  $p > 0.05$ ; adj  $r^2 = 0.22$ . Female linear regression:  $y = -3.23 \times 10^{-5}x + 0.136$ ;  $p > 0.05$ ; adj  $r^2 = 0.03$ .

rate showed a positive correlation with food consumption in males but was not related to food consumption in females (Figure 6a). The relationship between food consumption and total centrum growth was significantly different between sexes ( $F = 6.226$ , d.f. = 1,  $0.05 > p > 0.01$ ). Total centrum growth showed a positive correlation with food consumption in males, but was not related to food consumption in females (Figure 6b).

## DISCUSSION

The current study adds more evidence to the growing body of literature showing that elasmobranchs do not demonstrate annual band-pair deposition after sexual maturity (e.g. throughout their entire lifespan). A recent review of elasmobranch age validation studies concluded that age was likely underestimated in 30% of elasmobranch populations examined (Harry 2017). Age validation studies on several shark species show annual band-pair deposition up to the approximate age of sexual maturity, followed by band-pair deposition that is not annual (Casey & Natanson, 1992; Natanson *et al.*, 2014; Natanson *et al.*, 2018). The present study shows that adult *L. erinacea* of both sexes do not always have annual band-pair deposition. This corroborates the finding by Natanson (1993) of two adult female *L. erinacea* that did not deposit a full band pair in one year. In fact, many adult males do not deposit a full band in one year either suggesting a link between maturation and decreased frequency of band-pair deposition.

The frequency of band-pair deposition was variable among sexually mature individuals in this study, so it is not surprising that the band type (opaque or

translucent) in which OTC was deposited was not synchronous among individuals. Oxytetracycline appeared either in a translucent band (74% of individuals) or an opaque band (26% of individuals), despite the fact that all individuals were injected on the same day. This could be explained by the timing of injection (December), which seems to be a transition period between deposition of opaque and translucent bands in *L. erinacea* (Johnson, 1979; Waring, 1984). On the other hand, Natanson (1993) suggested that opaque bands are formed in fall/winter in *L. erinacea*. Nevertheless, synchronous seasonal switching of band types is not likely occurring in *L. erinacea* since OTC appeared in both band types among individuals and the rate of band-pair deposition was variable. The inconsistencies in previous studies (Johnson, 1979; Waring, 1984; Natanson, 1993) of the season that different band types are deposited in *L. erinacea* may be in part due to an adult pattern of decreased centrum growth and decreased frequency of band-pair deposition. Discrepancies in the timing of band-pair deposition observed in other elasmobranchs (e.g. Shortfin Mako *Isurus oxyrinchus* Rafinesque 1810; Wells *et al.*, 2013; Kinney *et al.*, 2016) is likely the result of band-pair deposition not being annual in older individuals (Harry, 2017; Natanson *et al.*, 2018).

The absence of an OTC mark in vertebral centra is well documented in captive and wild mark-recapture experiments in elasmobranchs (e.g. Smith, 1984; Sagarese & Frisk, 2010). In this study, OTC failed to mark the CC of six individuals (15%). In Natanson (1993), two out of 13 (15%) *L. erinacea* failed to incorporate OTC into their vertebral centra. Other studies of captive elasmobranchs had an OTC mark failure rate as high as 81.03% (Sagarese & Frisk, 2010). Mark-recapture studies of wild animals

had OTC mark failure rates from 6.38% (Walker *et al.*, 2001) to 66% (McFarlane & Beamish, 1987). Smith (1984) attributed the absence of OTC to insufficient mineralization of the vertebral centra directly after injection. Oxytetracycline deposited outside of the CC was common in the current study indicating active mineralization at these different sites (e.g. outer edge of CC) at the time of injection. Oxytetracycline was also present diffusely in both the intermedia and throughout the CC in several individuals. Holden & Vince (1973) observed faint and moderate fluorescence closer to the focus than the bright OTC mark in previously deposited opaque bands. Smith (1984) reported on one instance of faint fluorescence closer to the focus than the bright OTC mark and several instances of faint fluorescence of the entire centrum. The simplest explanation is that these areas are actively mineralizing at the time of OTC injection while the CC, where band pairs are counted for *L. erinacea*, is not actively mineralizing. Additionally, one individual in the current study which was not used for analysis was injected with OTC at two different times (approximately 8 months apart), but only one OTC mark was seen in the centrum. In other growth studies of captive elasmobranchs, multiple injections of OTC sometimes resulted in fewer than expected OTC marks (Tanaka, 1990; Huvaneers *et al.*, 2013).

The higher energetic cost of reproduction for females is well documented in many invertebrate and vertebrate taxa (Haywood & Gillooly, 2011). Female skates in this study consumed 1.5 times more food per individual than males (Figure 4a), which suggests a direct link between food consumption and reproductive output, given the energy needed for egg production. While females likely allocated food to egg production, there were no statistically significant differences between sexes in body or

total centrum growth, or number of band pairs. Therefore, the physiological mechanism regulating centrum growth or number of band pairs is likely not different between males and females.

The inability to accurately age elasmobranchs throughout their lifespan using current techniques raises concerns for current stock-assessment practices. The systematic under-ageing of larger, older individuals will lead to biased growth parameters with implications for stock assessments (Harry, 2017). Some stock-assessment methods used for teleosts do incorporate ageing bias and imprecision (Methot, 1990; Reeves, 2003; Punt *et al.*, 2008), and conclude that increases in the variability of assessment results that are particularly sensitive to the magnitude of the biases (Bradford, 1991; Reeves, 2003; Bertignac & de Pontual, 2007). The discussion of the implications of age underestimation in elasmobranchs has started. Harry (2017) addressed the potential effects of age underestimation on growth and mortality, highlighting complicated and conflicting consequences. As age underestimation is identified in more elasmobranch species, the effects of biased growth parameters on stock assessment results must be better understood and an alternate method to estimate elasmobranch age must be found.

I thank L. Natanson and D. Bengtson for their support and guidance. I thank R. Sykes, the crew of the F/V Virginia Marise, the crew of the R/V Cap'n Bert, M. Kane, and J. Langan for collection assistance. I also thank E. Baker, K. Viducic, K. Fagan, A. Frey, L. Byrnes, K. Law, R. Quinn, C. Ward, and R. LaGarenne for husbandry assistance. I thank J. Webb, B. Flammang, and J. Collie for manuscript edits. Specimen collection

was conducted with a Rhode Island Scientific Collector's Permit from the Rhode Island Department of Environmental Management Division of Fish and Wildlife: 2015-006. Research was conducted under an approved University of Rhode Island IACUC Protocol (AN1415-002). Research was supported by the Marine Anglers for Research and Conservation Memorial Fund and an American Elasmobranch Society Student Research Award.

## REFERENCES

- Bertignac, M. & de Pontual, H. (2007). Consequences of bias in age estimation on assessment of the northern stock of European Hake (*Merluccius merluccius*) and on management advice. *ICES Journal of Marine Science* **64**, 981-988.
- Bigelow, H.B. & Schroeder, W.C. (1953). Fishes of the Gulf of Maine. Fisheries Bulletin 53. 577 p.
- Bradford, M.J. (1991). Effects of ageing errors on recruitment time series estimated from sequential population analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 555-558.
- Cailliet, G.M. (1990). Elasmobranch age determination and verification: An updated review. In Elasmobranchs as living resources: Advances in the biology, ecology, systematics, and the status of the fisheries. *NOAA Technical Report NMFS* **90**, 528 p.

Cailliet, G.M., Smith, W.D., Mollet, H.F. & Goldman, K.J. (2006). Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. *Environmental Biology of Fishes* **77**, 211-228.

Casey, J.G. & Natanson, L.J. (1992). Revised estimates of age and growth of the Sandbar Shark (*Carcharhinus plumbeus*) from the western North Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* **49**, 1474-1477.

Chang, W.Y.B. (1982). A statistical method for evaluating the reproducibility of age determination. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 1208-1210.

Chidlow, J.A., Simpfendorfer, C.A. & Russ, G.R. (2007). Variable growth band deposition leads to age and growth uncertainty in the Western Wobbegong Shark, *Orectolobus hutchinsi*. *Marine and Freshwater Research* **58**, 856-865.

Cicia, A.M., Driggers, W.B., Ingram, G.W., Kneebone, J., Tsang, P.C.W., Koester, D.M., and Sulikowski, J.A. Size and age estimates at sexual maturity for the little skate *Leucoraja erinacea* from the western Gulf of Maine, U.S.A. *Journal of Fish Biology*. **75**: 1648-1666.

Ebert, D.A. (2005). Reproductive biology of skates, *Bathyraja* (Ishiyama), along the eastern Bering Sea continental slope. *Journal of Fish Biology* **66**, 618-649.

Evans, G.T. & Hoenig, J.M. (1998). Testing and viewing symmetry in contingency tables, with applications to readers of fish ages. *Biometrics* **54**, 620-629.

Francis, M.P., Campana, S.E., & Jones, C.M. (2007). Age under-estimation in New Zealand Porbeagle Sharks (*Lamna nasus*): is there an upper limit to ages that can be determined from shark vertebrae? *Marine and Freshwater Research* **58**, 10-23.

Harry, A.V. (2017). Evidence of systemic age underestimation in shark and ray ageing studies. *Fish and Fisheries* **2017**, 1-16.

Haskell, W.L. (1948). An investigation of the possibility of determining the age of sharks through annuli as shown in cross-sections of vertebrae. *Annual Report of the Marine Laboratory of the Texas Game, and Fish Commission. FY 1948-49*, 212-217.

Haywood, A. & Gillooly, J.F. (2011). The cost of sex: Quantifying energetic investment in gamete production by males and females. *PLoS ONE* **6**, e16577

Holden, M.J. & Vince, M.R. (1973). Age validation studies on the centra of *Raja clavata* using tetracycline. *Journal du Conseil International pour l'Exploration de la Mer* **35**: 13-17.

Huvaneers, C., Stead, J., Bennett, M.B., Lee, K.A., & Harcourt, R.G. (2013). Age and growth determination of three sympatric wobbegong sharks: How reliable is growth band periodicity in Orectolobidae? *Fisheries Research* **147**, 413-425.

Ishiyama, R. (1951). Studies on the rays and skates belonging to the family Rajidae, found in Japan and adjacent regions. 2. On the age-determination of Japanese Black-Skate *Raja fusca*. *Bulletin of the Japanese Society for the Science of Fish* **16**, 112-118.

Johnson, G.F. (1979). The biology of the Little Skate, *Raja erinacea*, in Block Island Sound, Rhode Island. Master's Thesis. University of Rhode Island, Kingston, RI, USA.

Kalish, J.M. & Johnston, J. (2001). Determination of school shark age based on analysis of radiocarbon in vertebral collagen. In. *Use of bomb radiocarbon chronometer to validate fish age*, (Kalish, J.M., ed). *Final Report, FDRC Project 93/109. Fisheries Research and Development Corporation*, Canberra, Australia, pp. 116-122.

Kinney, M.J., Wells, R.J.D. & Kohin, S. (2016). Oxytetracycline age validation of an adult Shortfin Mako Shark *Isurus oxyrinchus* after 6 years at liberty. *Journal of Fish Biology* **89**, 1828-1833.

Lagler, K.F. (1952). *Freshwater Fishery Biology*. Dubuque, IA: Brown.

McFarlane, G.A. & Beamish, R.J. (1987). Validation of the dorsal spine method of age determination for Spiny Dogfish. In *Age and Growth of Fish*, pp. 287-300. Ames, IA: Iowa State University Press.

McPhie, R.P. & Campana, S.E. (2009). Bomb dating and age determination of skates (family Rajidae) off the eastern coast of Canada. *ICES Journal of Marine Science* **66**, 546-560.

Methot, R.D. (1990). Synthesis model: an adaptive framework for analysis of diverse stock assessment data. *International North Pacific Fisheries Commission Bulletin* **50**, 259-277.

Natanson, L.J. (1993). Effect of temperature on band deposition in the Little Skate, *Raja erinacea*. *Copeia* **1993**, 199-206.

Natanson, L.J. & Cailliet, G.M. (1990). Vertebral growth zone deposition in Pacific Angel Sharks. *Copeia* **1990**, 1133-1145.

Natanson, L.J., Sulikowski, J.A., Kneebone, J.R., & Tsang, P.C. (2007). Age and growth estimates for the Smooth Skate, *Malacoraja senta*, in the Gulf of Maine. *Environmental Biology of Fishes* **80**, 293-308.

Natanson, L.J., Wintner, S., Johansson, F., Piercy, A.N., Campbell, P., De Maddalena, A., Gulak, S.J., Human, B., Fulgosi, F.C., Ebert, D.A., Hemida, F., Mollen, F.H., Vanni, S., Burgess, G.H., Compagno, L.J.V., & Wedderburn-Maxwell, A. (2008). Ontogenetic vertebral growth patterns in the Basking Shark, *Cetorhinus maximus*. *Marine Ecology Progress Series* **361**, 267-278.

Natanson, L.J., Gervelis, B.J., Winton, M.V, Hamady, L.L., Gulak, S.J.B., & Carlson, J.K. (2014). Validated age and growth estimates for *Carcharhinus obscurus* in the northwestern Atlantic Ocean, with pre- and post-management growth comparisons. *Environmental Biology of Fishes* **97**, 881-896.

Natanson, L.J., Skomal, G.B., Hoffmann, S., Porter, M., Goldman, K.J., & Serra, D. (2018). Age and growth of elasmobranchs: do band pairs on vertebral centra record age? *Marine and Freshwater Research* <https://doi.org/10.1071/MF17279>

Pierce, S.J. & Bennett, M.B. (2009). Validated annual band-pair periodicity and growth parameters of Blue-Spotted Maskray *Neotrygon kuhlii* from south-east Queensland, Australia. *Journal of Fish Biology* **75**, 2490-2508.

Punt, A.E., Smith, D.C., Krusic-Golub, K., & Robertson, S. (2008). Quantifying age-reading error for use in fisheries stock assessments, with application to species in Australia's southern and eastern scalefish and shark fishery. *Canadian Journal of Fisheries and Aquatic Sciences* **65**, 1991-2005.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Reeves, S.A. (2003). A simulation study of the implications of age-reading errors for stock assessment and management advice. *ICES Journal of Marine Science* **60**, 314-328.

Sagarese, S.R., & Frisk, M.G. (2010). An investigation on the effect of photoperiod and temperature on vertebral band deposition in Little Skate *Leucoraja erinacea*. *Journal of Fish Biology* **77**, 935-946.

Smith, S.E. (1984). Timing of vertebral band deposition in tetracycline-injected leopard sharks. *Transactions of the American Fisheries Society* **113**(3): 308-313.

Sosebee, K.A. (2005). Maturity of skates in northeast United States waters. *Journal of Northwest Atlantic Fisheries Science* **35**, 141-153.

Tanaka, S. (1990). Age and growth studies on the calcified structures of newborn sharks in laboratory aquaria using tetracycline. In Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of Fisheries. *NOAA Technical Report* **90**: 189-202.

Walker, T.I., Brown, L.P., & Clement, J.G. (2001). Age validation from tagged school and gummy sharks injected with oxytetracycline. *FRDC Project No. 97/110, Final Report to Fisheries Research and Development Corporation*. 30 pp.

Waring, G.T. (1984). Age, growth and mortality of the Little Skate off the northeast coast of the United States. *Transactions of the American Fisheries Society* **113**, 314-321.

Wells, R.J.D., Smith, S.E., Kohin, S., Freund, E., Spear, N., & Ramon, D.A. (2013). Age validation of juvenile Shortfin Mako (*Isurus oxyrinchus*) tagged and

marked with oxytetracycline off southern California. *Fishery Bulletin* **111**,  
147-160.

## **CHAPTER 4**

### **Age Misestimation in Elasmobranchs – Does it Affect our Ability to Accurately Assess Fishery Stocks?**

Kelsey C James<sup>1</sup>, Vanessa Trijoulet<sup>2</sup>, and Katherine Sosebee<sup>2</sup>

<sup>1</sup>Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, Kingston, Rhode Island, 02881, USA

<sup>2</sup>Population Dynamics Branch, Northeast Fisheries Science Center, NOAA Fisheries, Massachusetts, 02543, USA

*in preparation for submission to Fisheries Research*

## **Abstract**

Band pairs in vertebral centra have been used to age elasmobranchs for over 60 years. However, the instances where the number of band pairs underestimates the age of the individual are increasing, particularly in large adults of many species. These age estimates are still used in the construction of stock-assessment models that dictate how elasmobranch fisheries are managed. To reconcile the fact that the input data into stock assessment models is biased we examined the effect of intentionally biased age data on stock assessment model results. Length-at-age data for little skate, *Leucoraja erinacea*, and winter skate, *L. ocellata*, were biased  $\pm 10\%$  and  $\pm 25\%$  of the lifespan for (1) all ages and (2) mature ages only. For each species, these eight scenarios and an unbiased (normal) scenario were modeled with the von Bertalanffy growth model and applied to a statistical catch-at-age model. The effects of biased age data were subtle and had the largest effect on estimating spawning stock biomass. As age underestimation is identified in more elasmobranch species, research on the implications of biased age estimates that are incorporated into stock assessment results will be crucial until an alternate method to estimate elasmobranch age is found.

**Keywords:** ageing bias scenario, maximum sustainable yield, statistical catch-at-age model, stock assessment, skates

## **1. Introduction**

Elasmobranchs, the sharks, skates, and rays, have been a successful evolutionary lineage for 450 million years. Their biggest threat has only arrived relatively recently with the development of global fisheries targeting them (Catarci 2004; Vannuccini 1999). Elasmobranchs are characterized by slow growth, late age at maturity, small litters, and extended longevity; these traits make them particularly sensitive to over-exploitation (Cortes 2002; Holden 1973; Stevens et al. 2000).

Achievement of sustainable elasmobranch fisheries requires correct evaluation of the status of elasmobranch populations, which requires knowledge of their life history characteristics (Cortes 1998; Heppell et al. 1999). There is large variation among elasmobranch species in productivity and resilience, the ability to respond to perturbations. This ability ranges from populations that are relatively fishing-resilient to populations that are unable to recover after moderate exploitation (Cortes 2002; Smith et al. 1998). Such variation requires substantial knowledge of life-history characteristics in order to provide the data necessary to determine population status and potential for recovery after exploitation.

Accurate stock assessments require a knowledge of life history characteristics including age at first reproduction, years reproductively active, and growth rates, to determine a population's status. These characters are traditionally determined through estimates of length-at-age, which require the species to have a hard structure that records growth over time in a reliable and permanent way (Cailliet et al. 2006). Several hard structures exhibit growth patterns useful for ageing elasmobranchs, and the most effective for many species is an analysis of the vertebral centra (Cailliet et al.

2006). These structures grow via accretion, producing detectable band pairs composed of one opaque and one translucent band that may be counted and have been used as a proxy for age (Cailliet et al. 2006).

The validity of using band pair counts for age has been brought into question over the years starting with species for which band pair formation was related to somatic growth, growth of the body, rather than time or age (Natanson and Cailliet 1990; Tanaka 1990; Natanson et al. 2008). An increasing number of studies demonstrate underestimation of age for larger, older individuals (Andrews et al. 2011; Francis et al. 2007; Hamady et al. 2014; Harry 2017; Kalish and Johnston 2001; Natanson et al. 2014; Natanson et al. 2015; Natanson et al. 2018; Passerotti et al. 2014). Indeed, for many of these studies annual band pair deposition is validated up to the approximate age at maturity (Natanson 2014; Natanson et al. 2015; Passerotti et al. 2014). This systematic under-ageing of larger, older individuals needs to be accounted for in stock assessments. Alternatively, the overestimation of age may occur if band pair deposition occurs more frequently than annually. For instance, juvenile shortfin mako sharks (*Isurus oxyrinchus*) in the Pacific Ocean deposit two band pairs a year for approximately the first five years (Wells et al. 2013). Thus, the effects of ageing errors need to be addressed for elasmobranch stock assessments.

Age bias was intentionally introduced to age-at-length data of two skate species as a case study. *Leucoraja erinacea*, little skate, and *L. ocellata*, winter skate, are sympatric species that are targeted by trawl for the lobster bait fishery and wing fishery, respectively, in addition to being bycatch in other fisheries (NMFS 2007). They are managed as a part of the Northwest Atlantic skate complex which includes

seven skate species, but fisheries assessment does not currently incorporate age structure of the population. Nevertheless, little and winter skate provide an interesting case study particularly as a data-poor example. This study is the first to investigate the effect of ageing bias on the stock assessment outputs on elasmobranch species.

## 2. Methods

### *Simulated Bias*

Length-at-age data for *L. erinacea* and *L. ocellata* were obtained from Frisk and Miller (2006). Ageing bias was applied to the length-at-age data to simulate minor and major underestimation and overestimation of all ages and of mature individuals only. Minor bias is a deviation of 10% of the maximum age from true age while major bias is a deviation of 25% of the maximum age from true age. The maximum age of *L. erinacea* was 12 years (Frisk and Miller 2006); the 10% and 25% bias were one year and three years respectively. The maximum age of *L. ocellata* was 20 years (Frisk and Miller 2006); the 10% and 25% bias were two and five years respectively. These age biases were applied over all ages, and for mature ages only by adding or subtracting the bias (in years) from the length-at-age data. *Leucoraja erinacea* matures at eight years old and *L. ocellata* matures at 12 years old (Frisk and Miller 2006). This resulted in eight bias scenarios plus the unbiased scenario for each species (Table 1).

Biased simulated data and original data were modeled with the von Bertalanffy growth function

$$L_a = L_\infty(1 - e^{-k(a-t_0)})$$

Table 1. Bias scenarios and corresponding age at maturity, maximum age, and von Bertalanffy parameters used as input into the statistical catch-at-age model for little and winter skates.

Species	Bias	Ages	Age of Maturity	Maximum Age	Von Bertalanffy parameters		
	Scenario	Biased			$L_\infty$	$k$	$t_0$
<b>Little Skate</b>	None		8	12	55.45	0.197	-1.148
	-10%	All	7	10†	51.20	0.305	-1.148
	+10%	All	9	13	55.45	0.197	0.102
	-25%	All	5	9	48.48	0.597	-0.956
	+25%	All	11	15	55.45	0.197	1.977
	-10%	Mature only	7	11	60.92	0.165	-1.261
	+10%	Mature only	9	12†	52.45	0.225	-1.048
	-25%	Mature only	5	9	63.02	0.172	-1.113
	+25%	Mature only	11	12†	50.35	0.251	-0.956
<b>Winter Skate</b>	None		12	20	116.07	0.075	-1.694
	-10%	All	10	18	108.63	0.090	-3.030
	+10%	All	15	22	116.07	0.075	0.356
	-25%	All	7	15	93.15	0.168	-3.276
	+25%	All	18	25	116.07	0.075	3.431
	-10%	Mature only	10	18	164.40	0.045	-2.199
	+10%	Mature only	15	22	99.17	0.101	-1.289
	-25%	Mature only	7	15	192.38	0.040	-1.938
	+25%	Mature only	18	23†	89.88	0.125	-0.924

†Maximum age group was less than predicted by bias because the estimated von Bertalanffy parameters created ages with zero frequency in the length-at-age matrix

where  $L$  is the length at age  $a$ ,  $L_\infty$  is the asymptotic length,  $k$  is the growth coefficient and  $t_0$  is the theoretical age at length zero (Ricker 1979; von Bertalanffy 1938).

Maximum age, age at maturity, and von Bertalanffy parameters changed with each bias scenario (Figure 1; Table 1). In several cases the maximum age group was truncated to a younger age because the biased von Bertalanffy parameters created ages with zero frequency in the length-at-age matrix. For example, the negative 10% bias for little skate estimated that 49-cm TL individuals were 9 years old and 50 cm TL individuals were 11 years old; therefore, the maximum age group for negative 10% for the little skate was 10 rather than 11 years.

Indices of relative abundance and length-frequency data were used from the National Marine Fisheries Service (NMFS) groundfish spring (for *L. erinacea*) and fall (for *L. ocellata*) surveys from 1994 to 2014. Total catch (kg) and length-frequencies were used from fishery-dependent data provided by Dr. Sosebee. The simulated von Bertalanffy parameters (Table 1) were used to estimate ages for the total catch and survey length-frequency data. These ages were used in combination with the indices of relative abundance and total catch to test the sensitivity of a statistical catch-at-age model to various ageing bias scenarios.

#### *Statistical Catch-at-age Model*

The model developed for this study was an age-structured statistical catch-at age model. The predicted number of fish ( $\widehat{N}$ ) was modelled as a cohort where  $A$  is the age-plus group:

$$\widehat{N}_{t+1,a+1} = N_{t,a} e^{-Z_{t,a}}$$

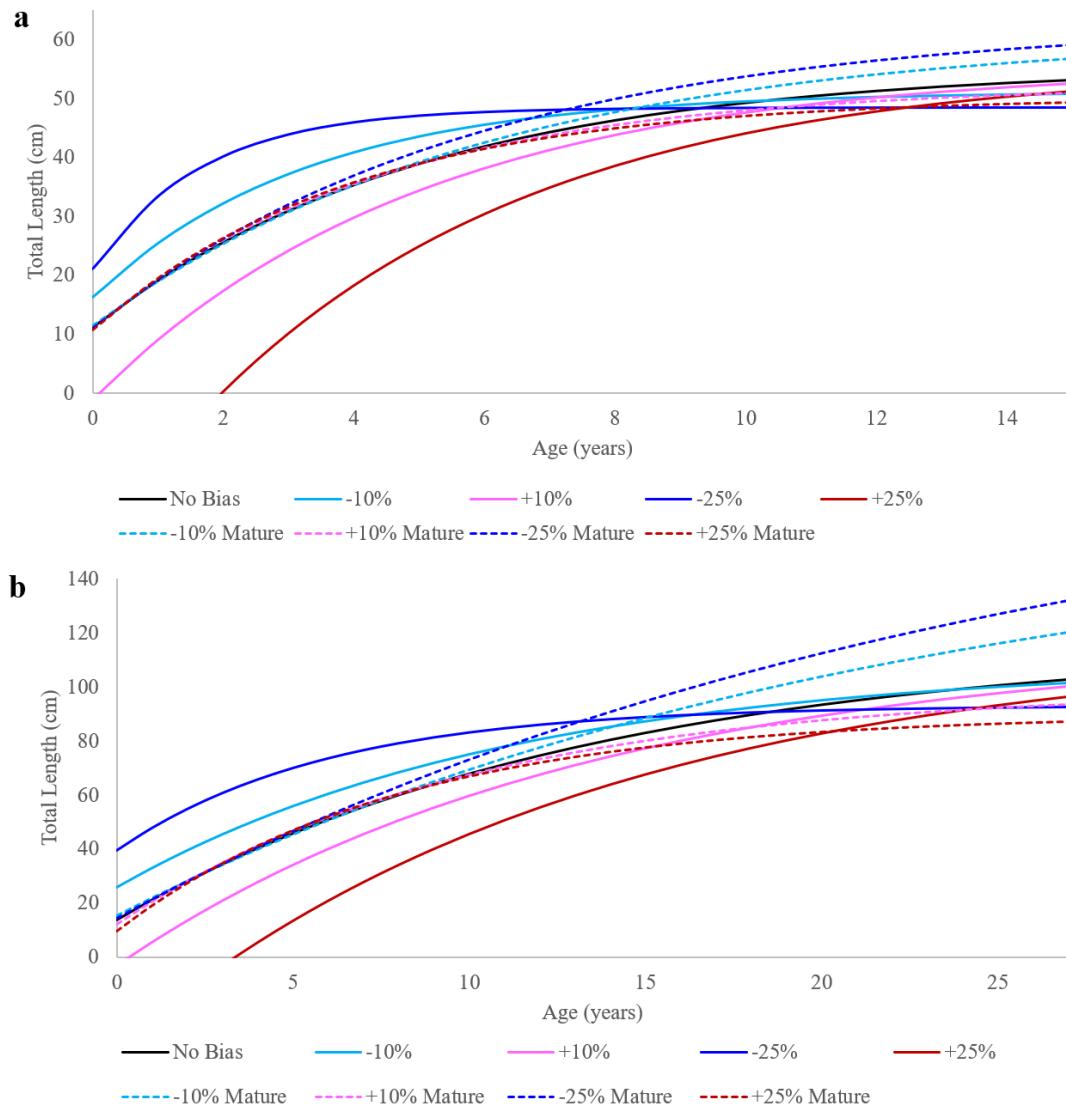


Figure 1. Von Bertalanffy parameter estimates for the no-bias and eight bias scenarios for (a) *L. erinacea* and (b) *L. ocellata*.

$$\widehat{N}_{t+1,A} = N_{t,A} e^{-Z_{t,A}} + N_{t+1,A} e^{-Z_{t+1,A}}$$

The model estimated the number of fish at age in the first year of simulation and fish recruitment at age zero. The total mortality on fish was given by

$$Z_{t,a} = F_{t,a} + M_{t,a}$$

where  $M$  was assumed known for each species and set equal to  $k$ . The fishing mortality  $F$  each year  $t$  is the product of estimated fully selected fishing mortality  $E$  and fishing selectivity:

$$F_{t,a} = f_t s_a^F$$

where  $f$  is the fully selected fishing mortality parameter. The fishing selectivity ( $s^F$ ) at age  $a$  was estimated following a logistic form where  $\gamma^F$  and  $A50^F$  were estimated parameters on a logit scale

$$s_a^F = \frac{1}{1 + e^{(-\frac{a - A50^F}{\gamma^F})}}$$

Selectivity was scaled so it is at its maximum at the maximum age class.

Predicted fishing catches in numbers ( $C$ ) were calculated following the Baranov equation:

$$C_{t,a} = \frac{F_{t,a}}{Z_{t,a}} N_{t,a} (1 - e^{-Z_{t,a}})$$

The predicted survey abundance ( $I$ ) was calculated as follows where  $\psi$  is the fraction of the year elapsed when the survey takes place:

$$I_{t,a} = q s_a^{surv} N_{t,a} e^{-\psi_t Z_{t,a}}$$

The catchability ( $q$ ) is estimated on a logit scale with  $logit\_q$  being estimated within the model:

$$q = \frac{1}{1 + e^{-\text{logit\_}q}}$$

The survey selectivity ( $s^{surv}$ ) was assumed to be follow a logistic curve:

$$s_a^{surv} = \frac{1}{1 + e^{\left(-\left(\frac{a - A_{50}^{surv}}{\gamma^{surv}}\right)\right)}}$$

The spawning stock biomass ( $SSB$ ) was calculated within the model as follows where  $w^{SSB}$  is the observed weight in the  $SSB$ ,  $mat$  is the proportion of mature fish at age and  $\phi$  is the fraction of year elapsed when the spawning takes place:

$$SSB_t = \sum_{a=1}^A (N_{t,a} w_{t,a}^{SSB} mat_{t,a} e^{-\phi Z_{t,a}})$$

The model is fitted to annual observations of total catch and survey abundance indices and corresponding age compositions for each species independently. We assumed log aggregated catch and log aggregated survey indices were normally distributed with given variance. Catch and survey index age compositions were assumed to be multinomially distributed for little skate and logistic-normally distributed for winter skate.

The model was developed in the R package (R Core Team 2017) Template Model Builder (TMB) (Kristensen et al. 2016). The estimated outputs from the various model scenarios were qualitatively compared.

The statistical catch-at-age successfully estimated parameters and standard error estimates for most bias scenarios. For little skate, the -25% and +25% bias for all ages did not successfully estimate parameters and standard errors. For winter skate, the +25% bias for all ages and +25% bias for mature ages only did not successfully estimate parameters and standard errors. These four models were left out of

subsequent analyses. The model had difficulty estimating some parameters for some scenarios, particularly recruitment at the end of the time series (little skate scenario +10%; winter skate scenario +10%) and age at 50% of selectivity for either the survey or fishing (little skate scenarios -10% mature only, -25% mature only; winter skate scenarios -25%, -10% mature). These models were included.

### 3. Results

The no-bias scenarios qualitatively fit the total catch data well for both species (Figure 2); however, the qualitative fit to the survey data was worse than for total catch data (Figure 3). The intentionally biased scenarios were similar to catch and survey data (Figures 2, 3). For winter skate, the last five years of the time series were not modeled well by any scenario; in general total catch data were overestimated and survey data were underestimated (Figures 2b, 3b). The direction of bias (positive or negative) did not result in a consistent over- or under-estimation of catch or survey data.

Estimates of SSB had high variability throughout the time series with most bias scenarios overestimating SSB for little skate and underestimating SSB for winter skate compared to the no-bias scenario (Figure 4). For little skate, the more extreme bias scenarios deviated most from the no-bias scenario (Figure 4a). For winter skate, the end of the time series had similar declining trends in SSB values for all the scenarios except -25% (Figure 4b).

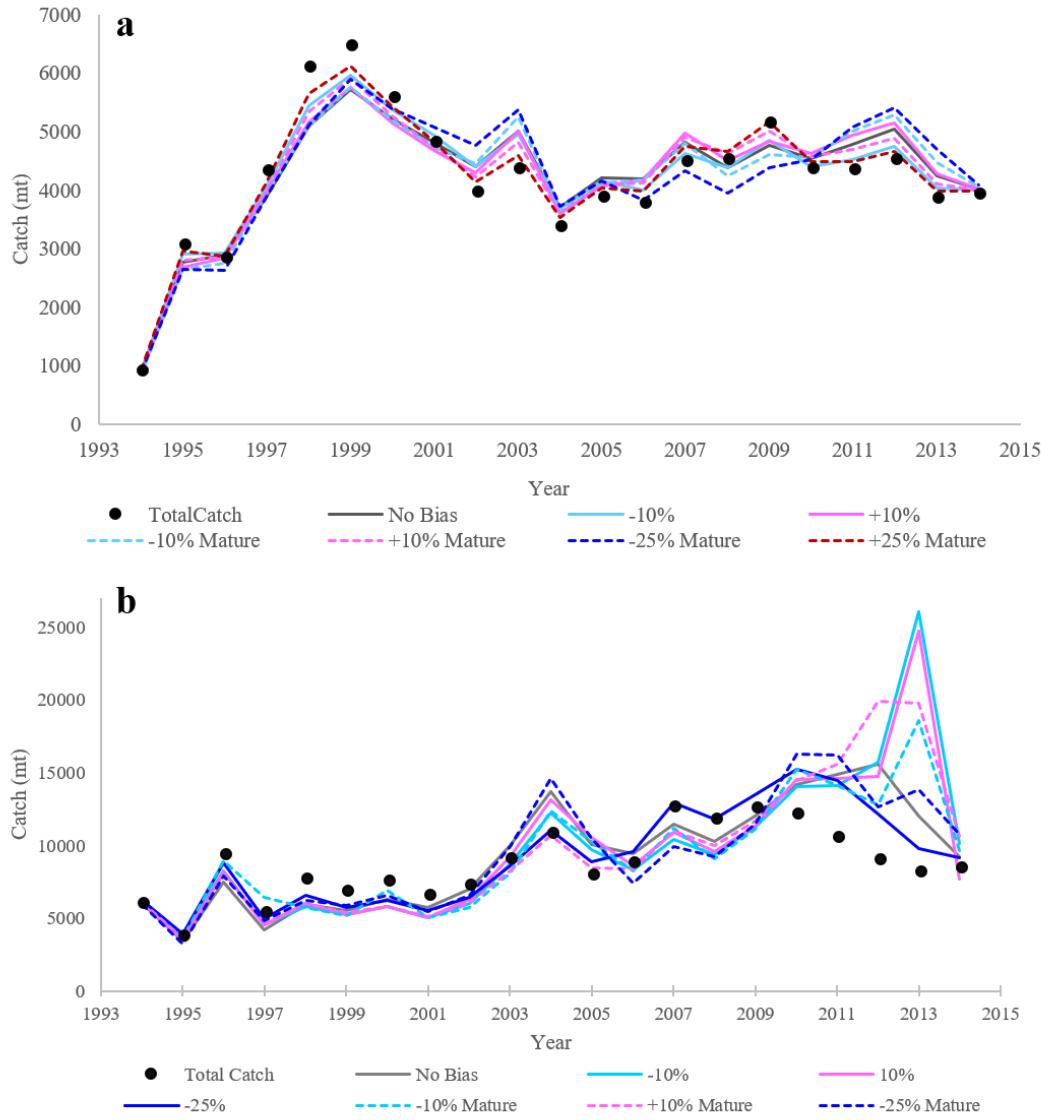


Figure 2. Observed and estimated total catch data for seven scenarios for (a) little skate and (b) winter skate.

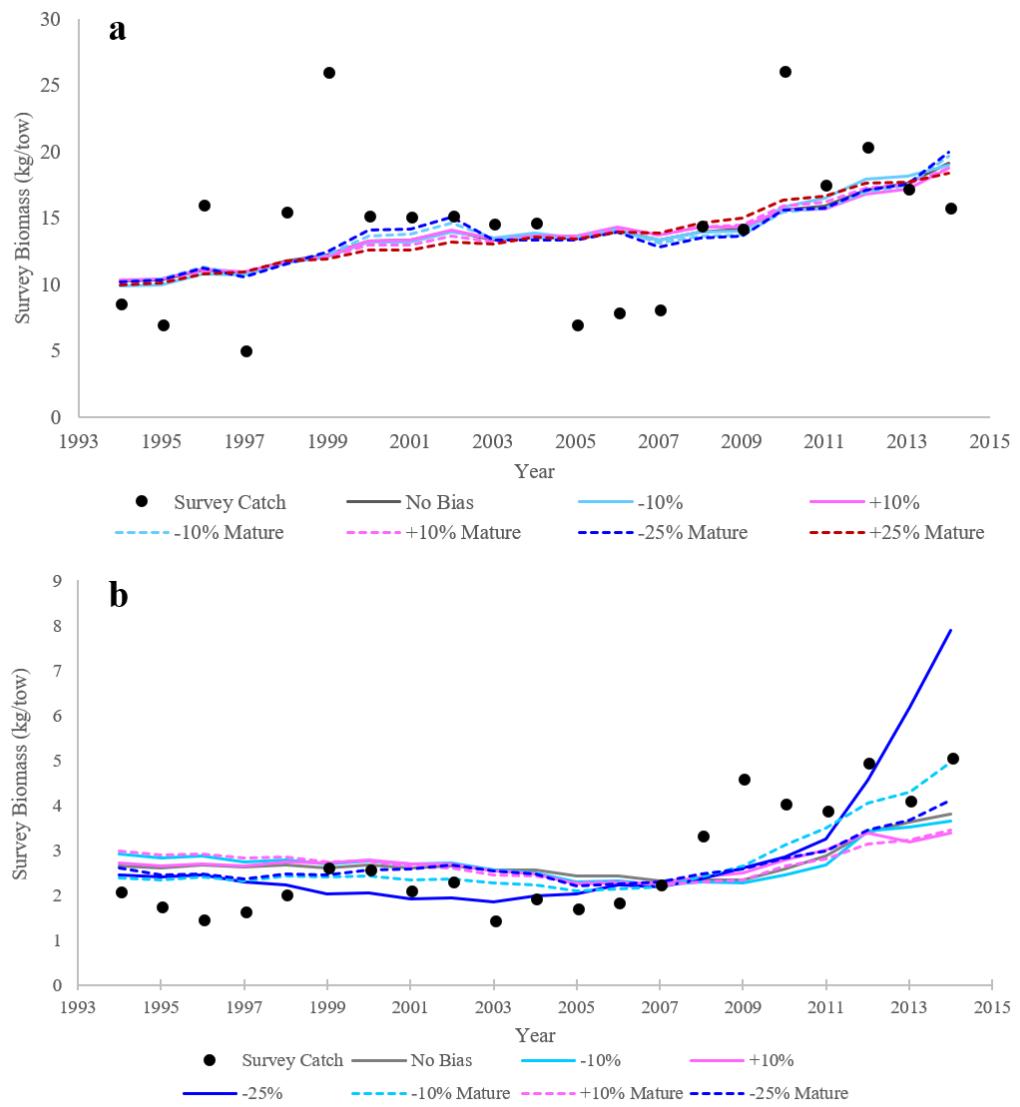


Figure 3. Observed and estimated survey catch data for seven scenarios for (a) little skate and (b) winter skate.

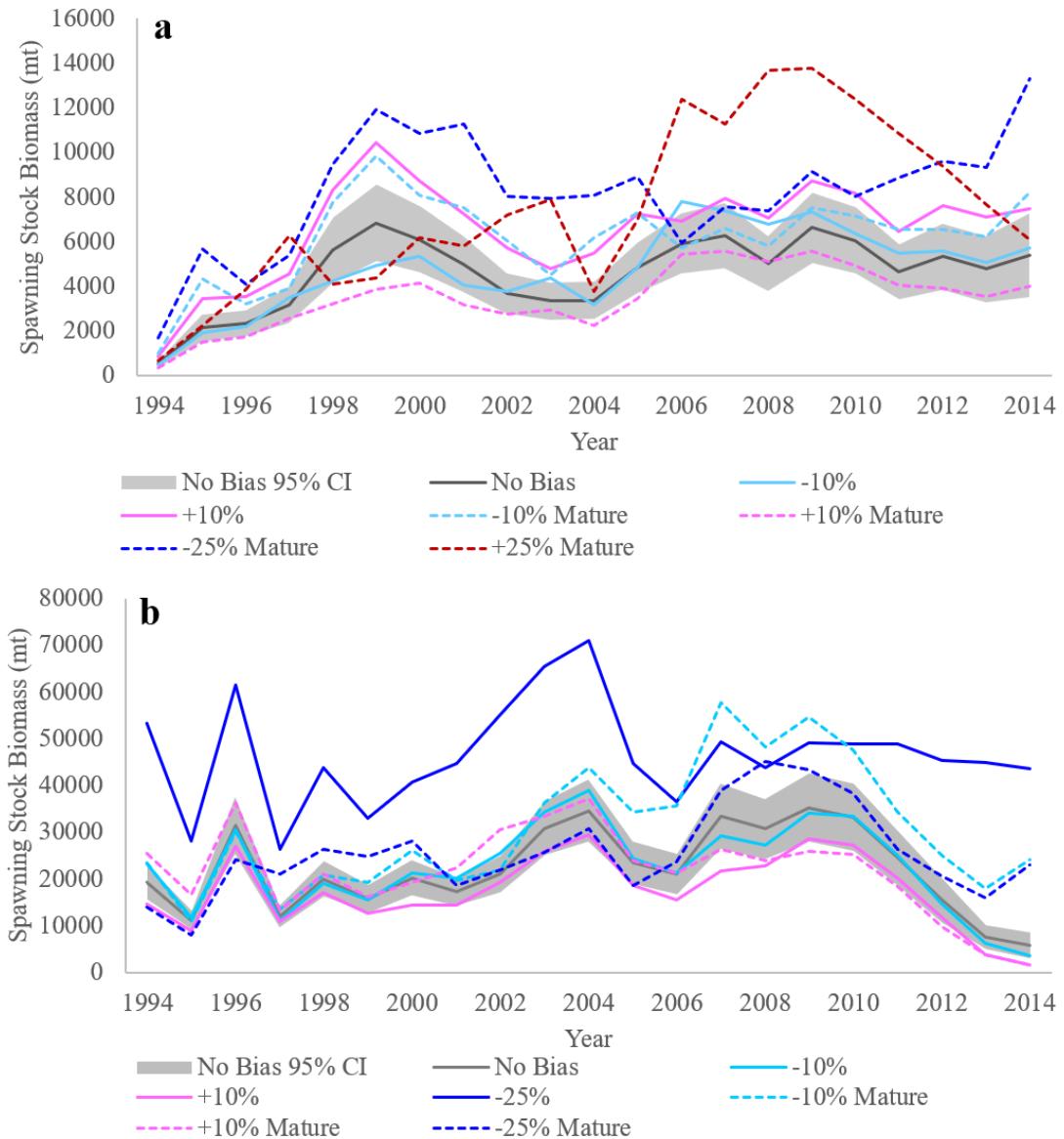


Figure 4. Estimated spawning stock biomass (SSB) for seven scenarios for (a) little skate and (b) winter skate. The 95% confidence interval around the no-bias scenario is shown in grey.

Estimates of F were predictable by bias scenario for little skate: when all ages were biased, a positive bias did not affect the estimated F (was similar to the no-bias scenario) while a negative bias decreased the estimated F, when only mature ages were biased, a positive bias decreased F while a negative bias increased F (Figure 5a). For the winter skate, estimates of F were overestimated by most of the bias scenarios, particularly in the last five years of the time series (Figure 5b).

#### **4. Discussion**

Intentionally biased age estimates introduced variability into stock assessment results, mostly into estimates of SSB. The variability was not predictable and does not align with similar research on teleosts (Bertignac and de Pontual 2007; Bradford 1991; Catalano and Bence 2012; Reeves 2003). Bradford (1991) simulated a fish population and applied two ageing error scenarios, relatively accurate and substantial underestimating of age, to determine the effect of ageing error on the estimation of a time series of recruitment via a sequential population analysis. Both scenarios reduced the estimated inter-annual variability in recruitment by up to 50% and 66%, respectively (Bradford 1991). Another simulation study generated three ageing error scenarios to examine the effect of ageing error on predicted stock trends. It found that, in general, ageing error resulted in prediction of similar stock trends no matter the bias, but spawning stock biomass estimates were more variable and fishing mortality was consistently underestimated (Reeves 2003). In contrast to Reeves (2003), the bias scenarios applied here did not have similar stock trends, particularly SSB, among scenarios.

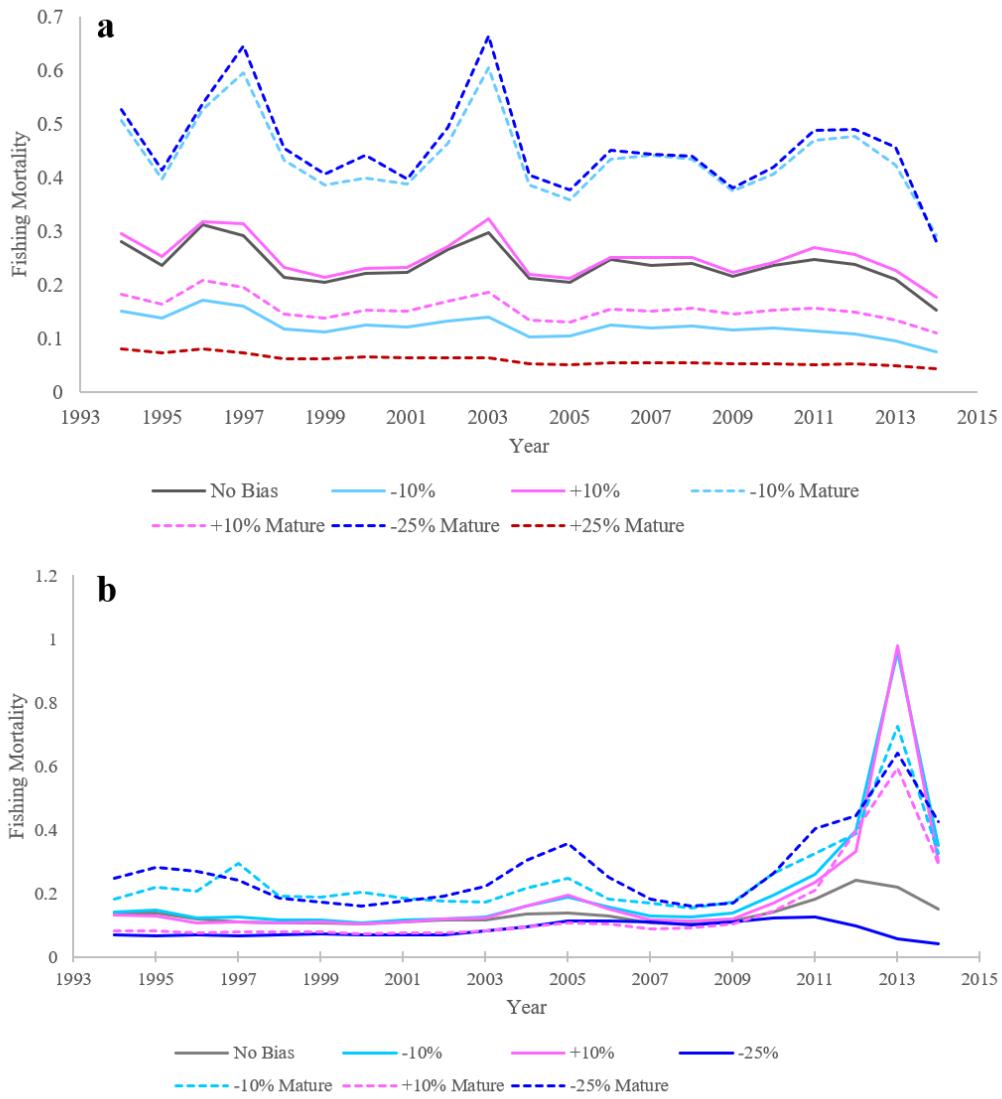


Figure 5. Estimated fishing mortality ( $F$ ) for seven scenarios for (a) little skate and (b) winter skate.

Furthermore, the direction of bias (negative or positive) did not have a predictable effect (i.e. consistent over- or under-estimation) on the estimated parameters. Reeves (2003) also noted this for estimates of SSB and attributed it to the complicated interactions among the factors used to estimate SSB. Some of the unpredictability seen here may be a result of fishing catch limits that were introduced for little and winter skate in 2010 (NEFMC 2009). This restricts the catch so the catch is relatively constant year-to-year and does not reflect the population size. The effect of catch limits is seen in the poor estimation of parameters for winter skate in the last five years of the time series. Many factors have the potential to affect our estimations of population size. The potential effects of age underestimation on growth and mortality for elasmobranchs are complicated with conflicting consequences (Harry 2017).

The Northeast Skate Complex in the Greater Atlantic Region that includes little and winter skates is managed using index-based reference points from the National Marine Fisheries Service Bottom Trawl Survey (Miller et al. 2009). Total allowable landings (TAL) are 4,218 mt for little skate and 8,372 mt for winter skate (Northeast Skate Complex 2018). Both species are not overfished and are not experiencing overfishing according to the index-based reference points (Northeast Skate Complex 2018). The stocks being healthy is supported by all the bias scenarios estimates of survey biomass over time (Figure 3). While the gold standard of stock assessment is to incorporate as much life history data as possible, for little and winter skate, the fishery-independent survey is providing sufficient information on the stocks to manage them appropriately.

With the increasing instances of age underestimation, uncertainty in ageing accuracy needs to be accounted for in elasmobranch stock assessments. The biased age scenarios affect stock assessment results. In this case study, the effects were subtle and not always predictable. In fisheries where age structure and accurate catch data are known, these simulated bias scenarios could be very informative to the range of resiliency of a population to mitigate the uncertainty around age estimates.

### **Acknowledgements**

We thank M. Frisk for contributing the unbiased length-at-age data. We thank M. Winton for providing R code for the von Bertalanffy model fitting.

## **References**

- Andrews, A.H., Natanson, L.J., Kerr, L.A., Burgess, G.H., and Cailliet, G.M. 2011. Bomb radiocarbon and tag-recapture dating of sandbar shark (*Carcharhinus plumbeus*). Fishery Bulletin. 109: 454-465.
- Bertignac, M., and de Pontual, H. 2007. Consequences of bias in age estimation on assessment of the northern stock of European hake (*Merluccius merluccius*) and on management advice. ICES Journal of Marine Science. 64: 981-988.
- Bradford, M.J. 1991. Effects of ageing errors on recruitment time series estimated from sequential population analysis. Canadian Journal of Fisheries and Aquatic Sciences. 48: 555-558.
- Cailliet, G.M., Smith, W.D., Mollet, H.F., and Goldman, K.J. 2006. Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. Environmental Biology of Fishes. 77: 211-228.
- Catalano, M.J., and Bence, J.R. 2012. The sensitivity to assumed ageing error of the stock assessment used to recommend lake whitefish yield for 2008 from management unit WFH01 of Lake Huron. QFC Technical Report 2011-01.

Catarci, C. 2004. World markets and industry of selected commercially-exploited aquatic species with an international conservation profile. FAO Fisheries Circular. No. 990. Rome, FAO. 186 p.

Cortes, E. 1998. Demographic analysis as an aid in shark stock assessment and management. *Fisheries Research*. 39: 199-208.

Cortes, E. 2002. Incorporating uncertainty into demographic modeling: application to shark populations and their conservation. *Conservation Biology*. 16(4): 1048-1062.

Francis, M.P., Campana, S.E., and Jones, C.M. 2007. Age under-estimation in New Zealand porbeagle sharks (*Lamna nasus*): is there an upper limit to ages that can be determined from shark vertebrae? *Marine and Freshwater Research*. 58: 10-23.

Frisk, MG., and Miller, T.J. 2006. Age, growth, and latitudinal patterns of two Rajidae species in the northwestern Atlantic: little skate (*Leucoraja erinacea*) and winter skate (*Leucoraja ocellata*). *Canadian Journal of Fisheries and Aquatic Sciences*. 63: 1078-1091.

Hamady, L.L., Natanson, L.J., Skomal, G.B., and Thorrold, S.R. 2014. Vertebral bomb radiocarbon suggests extreme longevity in white sharks. PLoS ONE 9(1): e84006.

Harry, A.V. 2017. Evidence for systemic age underestimation in shark and ray ageing studies. Fish and Fisheries. 2017: 1-16.

Heppell, S.S., Crowder, L.B., and Menzel, T.R. 1999. Life table analysis of long-lived marine species, with implications for conservation and management. In: J.A Musick (ed.) Life in the slow lane: ecology and conservation of long-lived marine animals. Symposium 23. American Fisheries Society, Bethesda, Maryland. 137-148.

Holden, M.J. 1973. Are long-term sustainable fisheries for elasmobranchs possible? Rapports et Procès Verbaux des Rèunions du Conseil International pour l'Exploration de la Mer. 164: 360-367.

Kalish, J. and Johnston, J. 2001, Determination of school shark age based on analysis of radiocarbon in vertebral collagen. In: Use of the bomb radiocarbon chronometer to validate fish age. Final Report FRDC Project 93/109. Pp. 116-129.

Kristensen, K., A. Nielsen, C. W. Berg, H. Skaug, and B. M. Bell. 2016. TMB: Automatic Differentiation and Laplace Approximation. Journal of Statistical software 70:1-21.

Miller, T., Muller, R., O'Boyle, B., and Rosenberg, A. 2009. Report by the Peer Review Panel for the Northeast Data Poor Stocks Working Group. Northeast Fisheries Science Center. 38 p.

Natanson, L.J., Wintner, S., Johansson, F., Piercy, A.N., Campbell, P., De Maddalena, A., Gulak, S.J., Human, B., Fulgosi, F.C., Ebert, D.A., Hemida, F., Mollen, F.H., Vanni, S., Burgess, G.H., Compagno, L.J.V., and Wedderburn-Maxwell, A. 2008. Ontogenetic vertebral growth patterns in the basking shark, *Cetorhinus maximus*. Marine Ecology Progress Series. 361: 267-278.

Natanson, L.J., Gervelis, B., Winton, M.V., Hamady, L.L., Gulak, S.J.B., and Carlson, J.K. 2014. Validated age and growth estimates for *Carcharhinus obscurus* in the northwestern Atlantic Ocean, with pre- and post management growth comparisons. Environmental Biology of Fishes. 97: 881-896.

Natanson, L.J., and Cailliet, G.M. 1990. Vertebral growth zone deposition in Pacific angelsharks. Copeia. 4: 1133-1145.

Natanson, L.J., Hamady, L.L., and Gervelis, B.J. 2015. Analysis of bomb radiocarbon data for common thresher sharks, *Alopias vulpinus*, in the northwestern Atlantic Ocean with revised growth curves. Environmental Biology of Fishes. 99: 39-47.

Natanson, L.J., Skoma, G.B., Hoffmann, S.L., Porter, M.E., Goldman, K.J., and Serra, D. 2018. Age and growth of sharks: do vertebral band pairs record age? Marine and Freshwater Research. <https://doi.org/10.1071/MF17279>

National Marine Fisheries Service (NMFS). 2007. 44<sup>th</sup> SAW (Stock Assessment Workshop) Assessment Report. B. Assessment of Northeast skate species complex. p. 285-547.

New England Fishery Management Council (NEFMC). 2009. Final Amendment 3 to the Fishery Management Plan (FMP) for the Northeast Skate Complex and Final Environmental Impact Statement with an Initial Regulatory Flexibility Act Analysis. 459 p.

Northeast Skate Complex. 2018.

<https://www.greateratlantic.fisheries.noaa.gov/sustainable/species/skate/index.html>

Passerotti, M.S., Andrews, A.H., Carlson, J.K., Wintner, S.P., Goldman, K.J., and Natanson, L.J. 2014. Maximum age and missing time in the vertebrae of sand tiger shark (*Carcharias taurus*): validated lifespan from bomb radiocarbon dating in the western North Atlantic and southwestern Indian Oceans. *Marine and Freshwater Research*. 65: 674-687.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Reeves, S.A. 2003. A simulation study of the implications of age-reading errors for stock assessment and management advice. *ICES Journal of Marine Science*. 60: 314-328.

Ricker, W.E. 1979. Growth rates and models. In. W.S. Hoar, D.J. Randall, J.R. Brett (eds.) *Fish Physiology*, Volume III. Pp. 677-743. New York: Academic Press.

Smith, S.E., Au, D.A., and Show, C. 1998. Intrinsic rebound potentials of 26 species of Pacific sharks. *Marine and Freshwater Research*. 49: 663-678.

Stevens, J.D., Bonfil, R., Dulvy, N.K. and Walker, P.A. 2000. The effects of fishing on sharks, rays and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science*. 57: 476-494.

Tanaka, S. 1990. Age and growth studies on the calcified structures of newborn sharks in laboratory aquaria using tetracycline. In: Pratt Jr., H.L., S.H. Gruber, and T. Taniuchi. (eds.). Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of Fisheries. NOAA Technical Report 90: 189-202.

Vannuccini, S. 1999. Shark utilization, marketing and trade. FAO Fisheries Technical Paper. No. 389. Rome, FAO. 470 p.

von Bertalanffy, L. 1938. A quantitative theory of organic growth (inquires on growth laws, II). Human Biology. 10:181-213.

Wells, R.J.D., Smith, S.E., Kohin, S., Freund, E., Spear, N., and Ramon, D.A. 2013. Age validation of juvenile shortfin mako (*Isurus oxyrinchus*) tagged and marked with oxytetracycline off southern California. Fishery Bulletin. 111: 147-160.

## CONCLUSIONS

The banding pattern in elasmobranch vertebrae which alternates opaque and translucent, has been used to estimate elasmobranch age for over 35 years (Haskell 1949; Ishiyama 1951). However, there are still outstanding questions about how and why these bands form and whether they accurately reflect the age of the individual. An increasing number of studies show exceptions to a pattern of annual band-pair formation, so that band-pair counts cannot accurately reflect age, and this is particularly true for older individuals (Kalish and Johnston 2001; Francis et al. 2007; Harry 2017). Many of these studies validate annual band-pair formation up to the approximate age of maturity, but the ages of older, mature individuals are underestimated (Casey and Natanson 1992; Andrews et al. 2011; Hamady et al. 2014; Natanson et al. 2014; Passerotti et al. 2014; Natanson et al. 2015; Natanson al. 2018). This dissertation addressed several questions regarding opaque and translucent bands in elasmobranch vertebrae, whether they accurately reflect age, and how inaccurate ages may affect fisheries management.

Elasmobranch vertebral centra fit the criteria for an appropriate ageing structure because they are a permanent record of growth and grow in proportion to body size (Cailliet et al. 2006). However, not all centra within individuals are the same size (Figures 1.1-1.5). There are two explanations for variation in the alternating opaque and translucent banding pattern between centra and along the column of an individual: (1) the widths of the opaque and translucent bands are thinner in smaller centra, but every centrum along the column of an individual contains the same number of bands or, (2) band width is approximately the same among centra, but smaller

centra contain fewer bands. The second possibility aligns with the hypothesis that band pair number is related to somatic growth and/or the structural needs of the individual rather than to age (Natanson and Cailliet 1990; Tanaka 1990; Natanson et al. 2008; Natanson et al. 2018). This hypothesis poses a problem for age and growth estimates because band-pair counts that vary along the vertebral column of an individual cannot accurately reflect a single age estimate. Variable band-pair counts have been documented in several species of sharks (Natanson et al. 2018). In this dissertation, band-pair counts were found to vary along the vertebral column in five batoid species (Figure 1.1-1.5). This finding extends the issue from sharks to all elasmobranchs. Since different centra have different numbers of band pairs within an individual, the mechanism of band-pair formation cannot be directly linked to an annual cycle.

Sexual maturation may be a key transition point in the timing of deposition of opaque and translucent bands. Species with successful validation of annual band-pair formation have only been validated up to the approximate age at maturity (Casey and Natanson 1992; Andrews et al. 2011; Hamady et al. 2014; Natanson et al. 2014; Passerotti et al. 2014; Natanson et al. 2015; Natanson al. 2018). Natanson (1993) showed that two captive adult female *L. erinacea* did not deposit a full band pair in one year. In this dissertation, 63% of adult male and female *L. erinacea* held in captivity for 13 months did not deposit a full band pair. While it was expected (based on Natanson, 1993) that only sexually mature females would exhibit decreased frequency of band-pair deposition, this trend in both sexes suggests a link between maturation and decreased frequency of band-pair deposition.

The inability to accurately age elasmobranchs throughout their lifespan using current techniques raises concerns for current stock-assessment practices. The systematic under-ageing of larger, older individuals will lead to biased growth parameters with implications for stock assessments (Harry 2017). The only research on ageing bias to date has been on teleosts, where incorporating ageing bias into stock assessments increases the variability of assessment results, which are particularly sensitive to the magnitude of the biases (Bradford 1991; Reeves 2003; Bertignac and de Pontual 2007; Catalano and Bence 2012). In this dissertation, intentionally biased age estimates also introduced variability into stock assessment results but, the direction of bias (negative or positive) did not have a predictable effect (i.e. consistent over- or under-estimation) on the estimated parameters. If age estimates are inaccurate at any point in the lifespan of a fish, the consequences will be seen in the stock assessment of the population.

The explanation of the difference between opaque and translucent bands is that they have different amounts of mineralization, but results conflict on which band type is more mineralized. Some studies document higher amounts of Ca and P in the opaque band (Cailliet and Radtke 1987; Hale et al. 2006) while other studies claim the translucent bands are hypermineralized (Richards et al. 1963; Johnson 1979; Officer et al. 1997). Previous research correlated peaks and troughs of Ca and P to opaque and translucent bands, but the peaks and troughs are variable with some troughs having similar values as other peaks (Jones and Geen 1977; Cailliet and Radtke 1987; Hale et al. 2006; Christensen 2011). In this study, bulk chemical composition did not differ between band types (opaque and translucent) within *L. erinacea* and among 11 other

elasmobranch species. The variation of each element among bands of the same type (opaque vs. opaque) along a centrum may preclude the detection of differences between different band types (opaque vs. translucent). Nonetheless, this dissertation showed that the optical differences between band types was not based on a difference in basic chemical composition, but a satisfactory explanation for the difference between the two band types is still unknown.

The results of this dissertation have wide-ranging impacts on the fields of age and growth and fisheries management. The major conclusion here is it is unlikely that band pairs are deposited annually throughout the entire lifespan of the fish, therefore an alternate method to estimate elasmobranch age must be found. The mechanism that controls band-pair deposition may approximate an annual cycle for a portion of the lifespan (e.g. up to sexual maturity). In this case including uncertainty in band pair counts particularly for sexually mature individuals may mitigate the effects that inaccurate ages have on stock assessments. The next step is to confirm the mechanism behind band-pair deposition so it can be predicted. If band-pair deposition is based on somatic growth then age can be predicted based on body size and annual growth. In the meantime, the effects that inaccurate ages have on stock assessments must be explored and incorporated into fisheries management.

## References

- Andrews, A.H., Natanson, L.J., Kerr, L.A., Burgess, G.H., and Cailliet, G.M. 2011. Bomb radiocarbon and tag-recapture dating of sandbar shark (*Carcharhinus plumbeus*). Fishery Bulletin. 109: 454-465.
- Beamish R.J. and McFarlane G.A. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society. 112: 735-743.
- Bertignac, M., and de Pontual, H. 2007. Consequences of bias in age estimation on assessment of the northern stock of European hake (*Merluccius merluccius*) and on management advice. ICES Journal of Marine Science. 64: 981-988.
- Bradford, M.J. 1991. Effects of ageing errors on recruitment time series estimated from sequential population analysis. Canadian Journal of Fisheries and Aquatic Sciences. 48: 555-558.
- Cailliet, G.M. and R.I. Radtke. 1987. A progress report on the electron microprobe analysis technique for age determination and verification in elasmobranchs. In. The Age and Growth of Fish. (eds.) R.C. Summerfelt and G.E. Hall. Iowa State University Press, Iowa. 359-369 pp.

Cailliet, G.M., Smith, W.D., Mollet, H.F. and Goldman, K.J. (2006). Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. *Environmental Biology of Fishes* 77, 211-228.

Casey, J.G. and Natanson, L.J. (1992). Revised estimates of age and growth of the Sandbar Shark (*Carcharhinus plumbeus*) from the western North Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* 49, 1474-1477.

Catalano, M.J., and Bence, J.R. 2012. The sensitivity to assumed ageing error of the stock assessment used to recommend lake whitefish yield for 2008 from management unit WFH01 of Lake Huron. QFC Technical Report 2011-01.

Christensen, H.M. 2011. Developing and applying elemental composition of shark vertebrae as a tool for quantifying life history characteristics over ontogeny. University of Windsor, Ontario, Canada. M.S. Thesis.

Francis, M.P., Campana, S.E., and Jones, C.M. 2007. Age under-estimation in New Zealand porbeagle sharks (*Lamna nasus*): is there an upper limit to ages that can be determined from shark vertebrae? *Marine and Freshwater Research*. 58: 10-23.

Hale, L.F., J.V. Dudgeon, A.Z. Mason, and C.G. Lowe. 2006. Elemental signatures in the vertebral cartilage of the round stingray, *Urobatis halleri*, from Seal Beach, California. Environmental Biology of Fishes. 77: 317-325.

Hamady, L.L., Natanson, L.J., Skomal, G.B., and Thorrold, S.R. 2014. Vertebral bomb radiocarbon suggests extreme longevity in white sharks. PLoS ONE 9(1): e84006.

Harry, A.V. 2017. Evidence for systemic age underestimation in shark and ray ageing studies. Fish and Fisheries. 2017: 1-16.

Haskell, W.L. 1949. An investigation of the possibility of determining the age of sharks through annuli as shown in cross-sections of vertebrae. Annual Report of the Marine Laboratory of the Texas Game, and Fish Commission FY 1948-49: 212-217.

Ishiyama, R. 1951. Studies on the rays and skates belonging to the family Rajidae, found in Japan and adjacent regions. 2. On the age-determination of Japanese Black-Skate *Raja fusca*. Bulletin of the Japanese Society for the Science of Fish 16: 112-118.

Johnson, G.F. 1979. The biology of the Little Skate, *Raja erinacea*, in Block Island Sound, Rhode Island. Master's Thesis. University of Rhode Island, Kingston, RI, USA.

Jones, B.C. and G.H. Geen. 1977. Age determination of an elasmobranch (*Squalus acanthias*) by x-ray spectrometry. Journal of the Fisheries Research Board of Canada. 34: 44-48.

Kalish, J. and Johnston, J. 2001, Determination of school shark age based on analysis of radiocarbon in vertebral collagen. In: Use of the bomb radiocarbon chronometer to validate fish age. Final Report FRDC Project 93/109. Pp. 116-129.

Natanson, L.J. (1993). Effect of temperature on band deposition in the Little Skate, *Raja erinacea*. Copeia 1993: 199-206.

Natanson L.J., Cailliet G.M. 1990. Vertebral growth zone deposition in Pacific Angel Sharks. Copeia 1990: 1133-1145.

Natanson, L.J., Gervelis, B., Winton, M.V., Hamady, L.L., Gulak, S.J.B., and Carlson, J.K. 2014. Validated age and growth estimates for *Carcharhinus obscurus* in the northwestern Atlantic Ocean, with pre- and post management growth comparisons. Environmental Biology of Fishes. 97: 881-896.

Natanson, L.J., Hamady, L.L., and Gervelis, B.J. 2015. Analysis of bomb radiocarbon data for common thresher sharks, *Alopias vulpinus*, in the northwestern Atlantic Ocean with revised growth curves. Environmental Biology of Fishes. 99: 39-47.

Natanson, LJ., Skomal, G.B., Hoffmann, S.L., Porter, M.E., Goldman, K.J., and Serra, D. 2018. Age and growth of sharks: do vertebral band pairs record age? Marine and Freshwater Research. <https://doi.org/10.1071/MF17279>

Natanson LJ, Wintner S, Johansson F, Piercy AN, Campbell P, De Maddalena A, Gulak SJ, Human B, Fulgosi FC, Ebert DA, Hemida F, Mollen FH, Vanni S, Burgess GH, Compagno LJV, and Wedderburn-Maxwell A. 2008. Ontogenetic vertebral growth patterns in the Basking Shark, *Cetorhinus maximus*. Marine Ecological Progress Series 361: 267-278.

Officer, R.A., R.W. Day, J.G. Clement, and L.P. Brown. 1997. Captive gummy sharks, *Mustelus antarcticus*, form hypermineralized bands in their vertebrae during winter. Canadian Journal of Fisheries and Aquatic Sciences. 54: 2677-2683.

Passerotti, M.S., Andrews, A.H., Carlson, J.K., Wintner, S.P., Goldman, K.J., and Natanson, L.J. 2014. Maximum age and missing time in the vertebrae of sand

tiger shark (*Carcharias taurus*): validated lifespan from bomb radiocarbon dating in the western North Atlantic and southwestern Indian Oceans. *Marine and Freshwater Research*. 65: 674-687.

Reeves, S.A. 2003. A simulation study of the implications of age-reading errors for stock assessment and management advice. *ICES Journal of Marine Science*. 60: 314-328.

Richards, S.W., Merriman, D., and Calhoun, L.H. 1963. Studies on the marine resources of southern New England, IX. The biology of the little skate, *Raja erinacea*, Mitchell. *Bulletin of the Bingham Oceanographic Collection Yale University* 18: 5-67.

Tanaka, S. 1990. Age and growth studies on the calcified structures of newborn sharks in laboratory aquaria using tetracycline. In *Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of Fisheries* NOAA Technical Report 90: 189-202.