Received: 30 March 2019 | Accepted: 17 September 2019
DOI: 10.1111/jfb.14141

REGULAR PAPER

JOURNAL OF FISH BIOLOGY fsbi

Vertebral growth and band-pair deposition in sexually mature little skates *Leucoraja erinacea*: is adult band-pair deposition annual?

Kelsey C. James

Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, Kingston, Rhode Island, USA

Correspondence
Kelsey C. James, Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, Kingston, RI, 02881, USA.
Email: kelsey.c.james@gmail.com

Funding information
American Elasmobranch Society Student Research Award; Marine Anglers for Research and Conservation Memorial Fund

Abstract

Mature male and female little skates *Leucoraja erinacea* were injected with oxytetracycline and maintained in captivity for 13 months to assess centrum growth and the frequency of band-pair deposition. Sixty per cent of the individuals analysed did not deposit a full band pair over the 13 month period. Thus, a majority of captive skates did not exhibit annual band-pair deposition. Previous research confirms annual band-pair deposition in all juvenile and most adult *L. erinacea*, therefore sexual maturation may lead to decreased frequency of band-pair formation. Age underestimation of larger, older elasmobranchs is being identified in an increasing number of elasmobranch species including *L. erinacea* as demonstrated in this study. The effect of age underestimation from band-pair counts on studies that use age-based characteristics needs to be addressed.

KEYWORDS
age, batoids, decreased centrum growth, *Leucoraja erinacea*, oxytetracycline

1 | INTRODUCTION

In fishes, accurate age-based characteristics, such as age at sexual maturity and longevity, are important for many fields of study (*e.g.*, demography and stock assessments (Goldman *et al.*, 2012) and contaminant studies (Mull *et al.*, 2012)). One particular use, accurately estimating population size and productivity, is crucial to monitor and maintain sustainable fisheries (Goldman *et al.*, 2012; Ricker, 1979). Age is generally determined by counting growth zones on a hard structure that grows in proportion to body size or weight (Goldman *et al.*, 2012; Lagler, 1952). 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 elasmobranchs. The centra have characteristic band pairs, each of which is composed of one opaque and one translucent band (Cailliet *et al.*, 2006; Lagler, 1952). The assumption has been that one band pair is deposited per year (Ishiyama, 1951), but it is difficult to validate this assumption throughout the entire lifespan of a species. Age validation studies (confirming the accuracy of age estimates with a determinate method; Cailliet, 1990) support the assumption of annual band-pair deposition in immature individuals of many species (Casey & Natanson, 1992; Natanson *et al.*, 2014) and mature individuals of some species (Ardizzone *et al.*, 2006; Campana *et al.*, 2002; Harry, 2017; Kneebone *et al.*, 2008), while other studies have shown that band-pair deposition slows or stops at older ages (Francis *et al.*, 2007; Harry, 2017; Kalish & Johnston, 2001).

While most of the research illustrating a decreased frequency of band-pair deposition (less than one band pair per year) has been in large-bodied sharks, this has also been documented in several batoid species (McPhie & Campana, 2009; Natanson, 1993; Pierce & Bennett, 2009). McPhie and Campana (2009) used bomb radiocarbon dating to validate band-pair counts of two skate species. Winter skate *Leucoraja ocellata* (Mitchill 1815) was validated to 19 years (longevity estimated at 24 years), however, band-pair counts of the thorny skate *Amblyraja radiata* (Donovan 1808) underestimated age by 5 years (McPhie & Campana, 2009). Pierce and Bennett (2009) used chemical mark–recapture to validate band-pair periodicity of the blue-spotted maskray *Neotrygon kuhlii* (Müller & Henle 1841) and four of the five recaptured rays at liberty for more than 250 days exhibited band-pair deposition consistent with time at liberty, while the fifth individual was at liberty for 651 days and exhibited no bands distal to the chemical mark (Pierce & Bennett, 2009). Little skate *Leucoraja erinacea* (Mitchill 1825) has been investigated in three captive studies that documented annual band-pair deposition in juveniles and most adults (Cicia *et al*., 2009; Natanson, 1993; Sagarese & Frisk, 2010). However, Natanson (1993) noted two mature, ovipositing females that only deposited a partial opaque band in one year of captive growth rather than a full band pair and concluded that for little skates 'annual banding may not occur when the females are reproductively active' (Natanson, 1993). This suggests that reproductive state may affect the rate of band-pair deposition in *L. erinacea*.

Sexual maturity marks a change in growth resource allocation from only to somatic growth to both somatic growth and reproduction (Jobling, 1993). Based on available resources for somatic growth, body growth and centrum growth should be reduced after sexual maturation. It is well documented that as an individual approaches maximum total length (L_T), 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). These decreases in growth and band-pair frequency may start as early as sexual maturation (Casey & Natanson, 1992; Natanson *et al*., 2014; Natanson *et al*., 2018).

The goal of the present study was to investigate band-pair deposition and centrum growth in captive sexually mature individuals. *Leucoraja erinacea* was used as a model organism based on its use in previous research and its resilience in captivity. In southern New England it attains sexual maturity between 39 and 44 cm L_T and the age at 50% maturity is 7.5 years old (Frisk & Miller, 2009; Sosebee, 2005). 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 the chemical marker oxytetracycline (OTC). The characterization of centrum growth in adults is critical to assess the validity of using band-pair counts for accurate age estimates of sexually mature individuals and their subsequent use for population monitoring, life history and ontogenetic studies, stock assessments, and fisheries management.

2 | MATERIALS AND METHODS

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).

Forty-four sexually mature *L. erinacea* (22 males (range 43.9–52.3 cm L_T) and 22 females (range 44.5–51.1 cm L_T)) were collected from Narragansett Bay, Rhode Island (RI), USA in February and May–November 2015 (water temperature < 19°C) using an otter trawl with a tow duration of 30 min. Healthy individuals, indicated by an active defence response to handling, were transported in seawater-filled coolers to the aquarium facility at the Narragansett Bay Campus, University of Rhode Island. Total length was measured as the straight-line-distance from snout tip to tail tip to the nearest 0.1 cm. Fish were weighed (total mass, M_T) to the nearest 0.5 g upon capture, and monthly thereafter to track health of each skate. Pectoral fin clips, notching the fin in strategic locations, were used to identify individuals. Holding tanks were 2.44 m in diameter by 0.5 m deep with flow-through water at a flow rate of 8 l min^{-1}. Skates were evenly distributed between two tanks with males and females housed together. 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. Egg deposition was recorded daily and reproductive activity was confirmed in females when the horns of an egg case were observed protruding from the cloaca.

Skates were acclimated for at least 2 weeks. On 4 December 2015, each skate was injected intramuscularly in the thickest part of the pectoral fin muscle with a 25 mg kg^{-1} body mass dosage of OTC. Skates were fed every other day by offering 2 g pieces of herring *Clupea harengus* L. 1758 (and occasionally squid) to each individual until food was refused. Food consumption was recorded at each feeding for each individual. Food consumption per individual was summed for the entire experimental period (13 months). Differences between males and females of ten growth variables: food consumption, monthly growth rate, total centrum growth, change in L_T, initial L_T, final L_T, initial M_T, final M_T, total band-pair count, and band pairs distal to OTC were analysed using *t*-tests in R (www.r-project.org).

At the end of 13 months (4 January, 2017) skates were killed with an overdose of 400 mg l^{-1} MS-222 and measured (L_T) and weighed (M_T). Monthly growth rate (g month^{-1}) was calculated as ($M_{T\text{final}}$ – $M_{T\text{initial}}$)/13. Maturity status was determined by visually inspecting the condition of the gonads (Ebert, 2005).

Two adjacent vertebral centra between vertebrae #10 and #20 (Figure 1a) were extracted from each skate. One centrum used to identify the OTC mark (hereafter referred to as the OTC section) was embedded in TAP Clear-Lite casting resin (TAP Plastics; www.tapplastics.com), and sectioned horizontally through the focus (Figure 1b) with a low-speed saw (Buehler Isomet; www.buehler.com) with paired diamond-edged blades separated by a 0.2 or 0.4 mm spacer. The section was viewed with a dissecting microscope (Nikon model SMZ1500; www.nikon.com) using UV light (366 nm) to identify and measure the OTC mark. Images were captured with a digital camera (Nikon model DSR12) and image processing software (NIS Elements, version 4.40; Nikon Corp.). Total centrum growth was measured as the distance from the beginning of the OTC mark to the centrum edge (to the nearest 0.01 mm) using Adobe Photoshop® (www.adobe.com). The other centrum extracted from each individual (hereafter referred to as the histology section) was processed histologically to enhance and count band pairs (Natanson *et al*., 2007) and photographed with

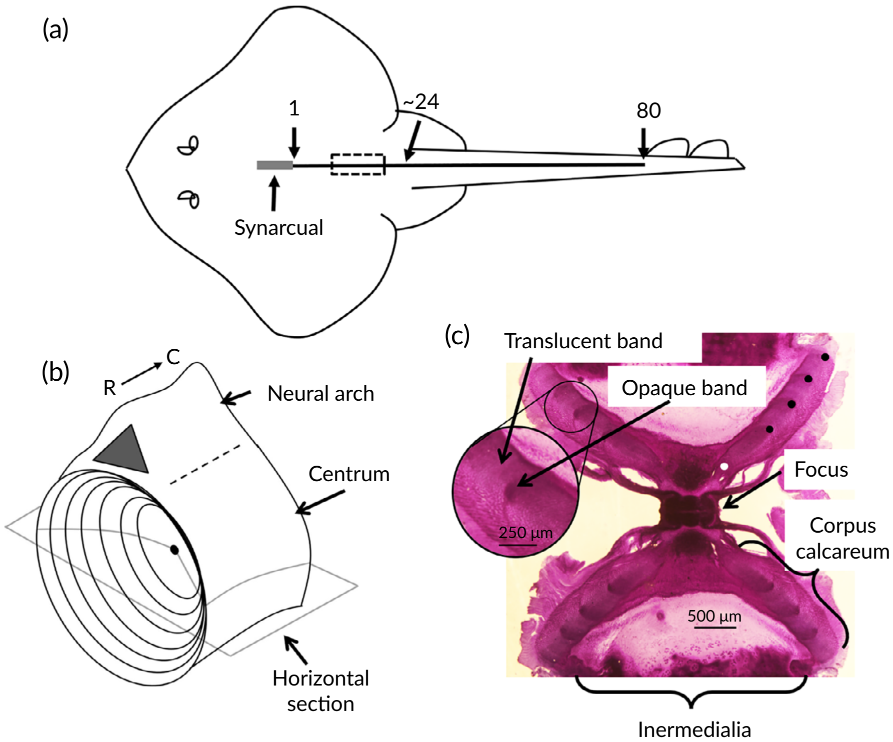

FIGURE 1 (a) Outline diagram of a *Leucoraja erinacea* indicating the position of vertebrae 1, *c*. 24, and 80. The transition between abdominal and caudal vertebrae varies among individuals but occurs at *c*. vertebra 24, Centra 10–20 where vertebrae were sampled. (b) Parts of a vertebra and the horizontal plane through which section were cut. R, rostral; C, caudal. (c) The resulting horizonal section used for staining and ageing, The birth band; ●, subsequent opaque bands

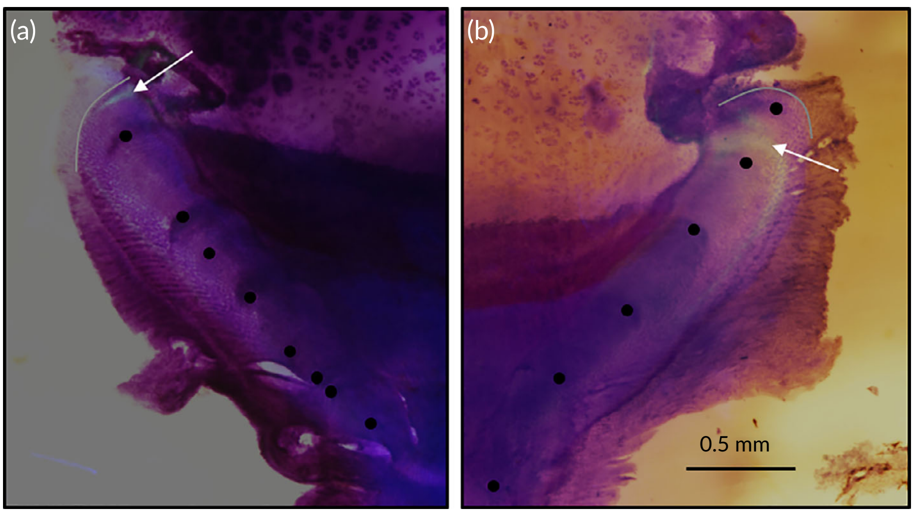

FIGURE 2 Sample histological sections with oxytetracyline (a) in the ultimate band (green mark; ↓) and (b) with a distally formed band pair. Oxytetracycline was deposited in a translucent band in both images. ∩, The edge of the corpus calcareum

reflected light using the camera system described above (Figure 1c). A superimposition of the OTC and the histology section photographs allowed the identification of which band type the OTC was incorporated into and the number of band pairs located distal to the OTC mark (new deposition; Figure 2). The effect of total band-pair count and L_T on band type that incorporated the OTC mark was analysed using logistic regressions in R. The effect of each of seven growth variables (food consumption, monthly growth rate, centrum growth, change in L_T, final L_T, final M_T, and total band-pair count) on number of band pairs distal to the OTC mark was analyzed using logistic regressions in R.

Total band-pair counts were made from 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 corpus calcareum (CC; Figure 1c). 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 CV (Chang, 1982). A CV < 10% was interpreted as acceptable intra and inter-reader precision. Bias, as a result of either systematic or random error, was assessed with the Evans-Hoenig test of symmetry (Evans and Hoenig, 1998). Final band-pair counts were assigned from the primary reader's second count or from consensus between readers if the band-pair counts differed by more than two band pairs.

3 | RESULTS

Forty-one skates (21 females and 20 males) were used for analysis; three individuals were eliminated due to procedural error and

TABLE 1 Comparison of ten growth variables in male and female *Leucoraja erinacea* at the end of the 13 month experimental period

Variable	Females (mean ± SE)	Males (mean ± SE)	*t*	*df*	*P*
Food consumption (g)	2640 ± 51.6	1723 ± 57.7	11.866	39	< 0.001
Monthly growth (g month^{-1})	4.8 ± 0.8	2.6 ± 1.8	2.485	31	< 0.05
Centrum growth (mm)	0.05 ± 0.005	0.04 ± 0.007	1.085	32	> 0.05
Change in L_T (cm)	−1.1 ± 0.4	−1.1 ± 0.4	0.060	39	> 0.05
Initial L_T (cm)	48.1 ± 0.4	48.0 ± 0.6	0.185	31	> 0.05
Final L_T (cm)	47.0 ± 0.3	46.9 ± 0.5	0.272	39	> 0.05
Initial M_T (cm)	651.0 ± 10.3	599.9 ± 18.1	2.457	30	< 0.05
Final M_T (cm)	713.0 ± 12.3	633.7 ± 20.0	3.381	32	< 0.01
Total band pair count	9 ± 0.5	9 ± 0.5	1.416	32	> 0.05
Band Pairs Distal to OTC	0.7 ± 0.06	0.6 ± 0.05	0.079	39	> 0.05

Note: L_T: Total length; M_T: total body mass; OTC: oxytetracycline.

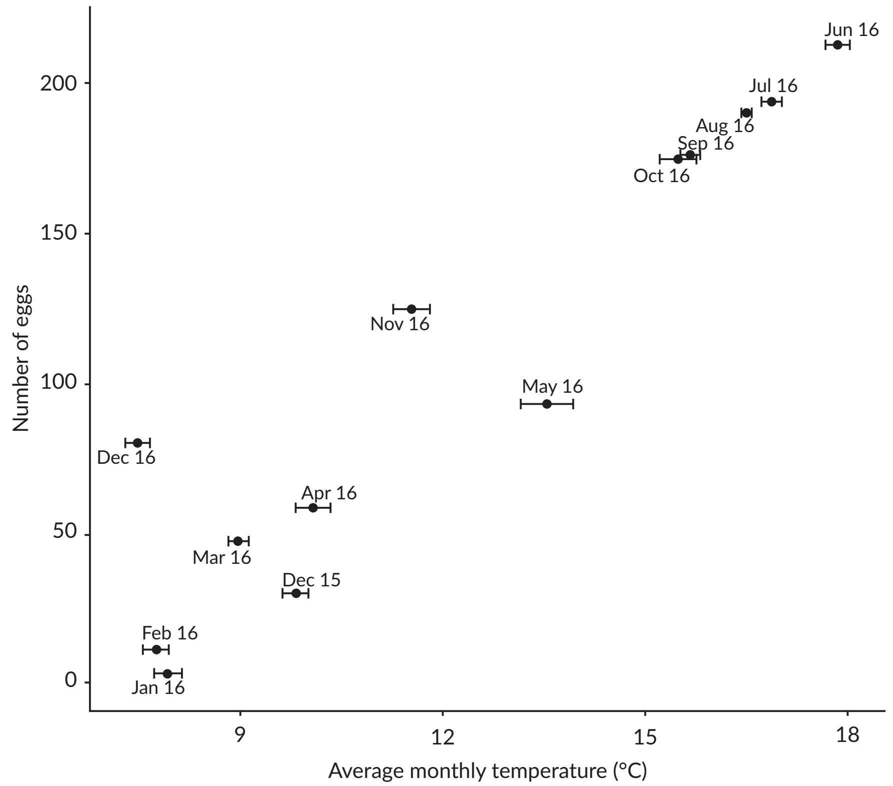

FIGURE 3 Relationship between monthly total number of eggs laid by 20 *Leucoraja erinacea* and mean (± SE) monthly water temperature in 2015 and 2016

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^{-1}. Total 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 1). All individuals were found to be sexually mature after a visual inspection of the condition of the gonads (Ebert, 2005).

All but one female laid at least one egg during the experimental period. This was tracked by the observation of horns protruding from the cloaca. 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 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 oviducal glands, ovaries, and uteri, but no eggs were present in either ovary. In the remaining females, each ovary had 1–6 eggs ≥10 mm in diameter.

A mean (± SE) of 2192 ± 81.8 g of food was consumed per individual during the experimental period. Females consumed significantly more food and had a higher mean monthly growth rate than males (Table 1 and Figure 4a,b).

Total band-pair counts ranged from 5 to 15 for all 41 individuals (Table 2). The number of band pairs were not significantly different between males and females (Table 1). The within-reader precision of the primary reader was good (CV = 8.68%), while the within-reader precision of the secondary reader was greater than 10% (CV = 12.33%). Comparing the second counts between readers, between-reader CV was 20.30% with 20 centra differing by more than two band pairs. These 20 centra were re-examined by both readers and a consensus band-pair count was reached. The Evans-Hoenig test of symmetry detected within-reader bias for the primary reader (χ^2 = 14.31, *df* = 4, *P* < 0.01), and between-reader bias (χ^2 = 30.33, *df* = 8, *P* < 0.001). Within-reader bias was not detected for the secondary reader (χ^2 = 3.12, *df* = 3, *P* > 0.05). Total band-pair counts were not the primary objective of this study therefore no individuals were removed from the study based on bias and precision. Band pairs at the edge of the CC and distal to the OTC mark were interpreted the same between readers in 40 out of 41 individuals. The band pairs on the edge of the 41st individual were agreed upon by both readers and included in further analysis.

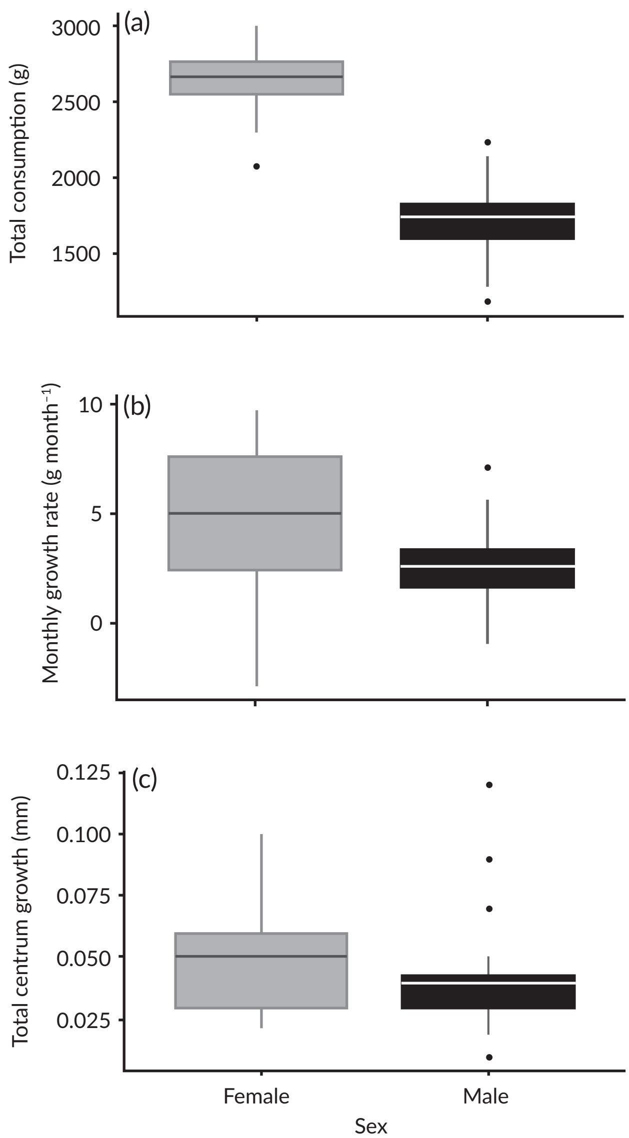

FIGURE 4 Boxplots (–, median; □, interquartile range; ꟾ, 95% range; ●, outliers) of *Leucoraja erinacea* (a) total food consumption, (b) mean monthly growth rate by sex (21 females, 20 males) and (c) mean total centrum growth (21 females, 15 males) over 13 months

Oxytetracycline was observed in some part of the vertebrae for all 41 individuals. Six individuals (15%; 1 female, 5 males) did not have an OTC mark across the CC (where band pairs are visible; Figure 5), therefore the number of band pairs distal to the OTC mark and total centrum growth was only measured in 35 individuals. OTC was present on the outer edge of CC in 32 of 41 individuals (78%), diffuse throughout the CC in 14 of 41 individuals (34%), in the arch tissue surrounding the centrum in 24 of 41 individuals (58.5%), at the focus in eight of 41 individuals (19.5%), and diffuse in the intermedialia in five of 41 individuals (12%, 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 < 0.01) and did not significantly differ between males and females (Table 1 and Figure 4c).

Oxytetracycline was incorporated into the translucent band in 71% of individuals (15 females and 10 males) while in the remaining 29% of individuals (five females and five males) OTC was incorporated into the opaque band (Table 2). There were no significant differences between total band-pair count and which band (opaque or translucent) the OTC mark was incorporated into (average ± SE band-pair count with OTC in translucent = 8.7 ± 0.5, band-pair count with OTC in opaque = 9.9 ± 0.7, *z*-value = –1.366, *df* = 34, *P* > 0.05), nor between final L_T and which band the OTC mark was incorporated into (average ± SE final L_T with OTC in translucent = 47.0 ± 0.4, final L_T with OTC in opaque = 47.2 ± 0.5, *z*-value = –0.288, *df* = 34, *P* > 0.05).

Sixty per cent of individuals had OTC in the ultimate band having formed 0.5 band pairs (10 females and 11 males) and 37% had formed one band pair (10 females and three males). One male (#10) had formed 1.5 band pairs during the experimental period (Table 2 and Figure 2) 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 3). The number of band pairs deposited (0.5 or 1) was not significantly different between males and females (Table 1), or with reference to any of the following growth variables: food consumption, monthly growth rate, change in L_T, final M_T, or total band-pair count (Table 3).

4 | DISCUSSION

The current study adds more evidence to the growing body of literature showing that some elasmobranchs do not demonstrate annual band-pair deposition throughout their entire lifespan (Francis *et al.*, 2007; Kalish & Johnston, 2001; Natanson *et al.*, 2018). A recent meta-analysis investigated 58 elasmobranch age validation studies and concluded that age was probably underestimated in 30% of 53 elasmobranch populations examined (Harry, 2017). All but two of these populations validated annual band-pair deposition in younger ages,

TABLE 2 Total length (L_T), sex (M, male; F, Female), location of oxytetracycline mark (OTC), total body mass (M_T), and total band-pair count of captive *Leucoraja erinacea* used in this study

Final L_T (cm)	Sex	OTC[a]	Individual[b]	Initial L_T (cm)	Initial M_T (g)	Final M_T (g)	Band–pair Count
44.3	M	O	26	46.7	550.0	592.0	9
44.5	M	T	23	45.2	481.0	498.0	10
44.6	M	T	99	43.9	464.0	490.5	8
45.6	F	T	33	46.1	564.0	590.5	9
46.4	F	T	52	48.1	631.0	730.0	10
46.6	F	O	11	47.0	683.0	743.0	10
46.8	M	O	88	46.8	581.0	615.0	7
47.0	F	T	18	47.9	558.0	685.0	8
47.1	M	T	6	49.6	569.0	618.0	12
47.7	M	T	66	48.1	660.0	674.5	9
47.9	F	T	PCP	49.8	631.5	698.0	8
48.0	F	T	15	50.4	612.0	715.0	9
48.2	F	T	50	49.6	632.0	646.5	7
48.5	M	T	4	48.1	590.0	682.5	5
48.5	F	T	60	47.7	687.0	667.0	8
48.9	M	T	3	52.2	691.0	713.5	9
49.1	M	T	70	48.9	665.0	704.0	7
49.5	F	T	82	49.6	662.0	777.5	11
49.9	F	O	69	48.1	673.0	766.5	15
50.1	M	O	120	50.0	639.5	676.5	11
51.5	M	T	39	52.2	808.0	865.5	15
44.0	F	T, O	RRRF	49.2	709.5	672.0	8
44.1	M	T, O	31	45.3	524.0	513.5	8
44.2	F	T, O	16	45.7	598.5	647.0	5
45.2	F	T, O	LEO	47.7	686.0	718.0	7
46.2	F	O, T	14	44.5	608.0	653.0	9
46.5	F	O, T	36	48.0	643.5	729.5	9
46.6	F	O, T	40	46.6	610.0	702.5	9
46.6	F	T, O	CRC	47.8	679.0	699.5	13
46.7	M	T, O	1	48.9	579.0	604.0	6
46.8	F	T, O	92	51.1	674.0	789.0	7
47.0	F	T, O	7	50.4	722.5	788.5	9
47.5	M	O, T	100	48.2	624.5	650.5	10
47.8	F	T, O	13	47.4	707.0	742.5	7
47.6	M	O, T, O	10	47.8	580.0	591.0	10
42.9	M	–	44	45.5	524.0	597.0	8
45.1	M	–	8	44.9	566.0	599.0	10
45.6	M	–	90	44.1	559.0	555.5	7
47.5	M	–	29	51.1	682.0	717.5	5
47.6	M	–	9	52.3	661.5	715.5	10
47.9	F	–	55	48.0	699.5	813.0	9

[a]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.

[b]Numeric or alphabetic code for individual skates.

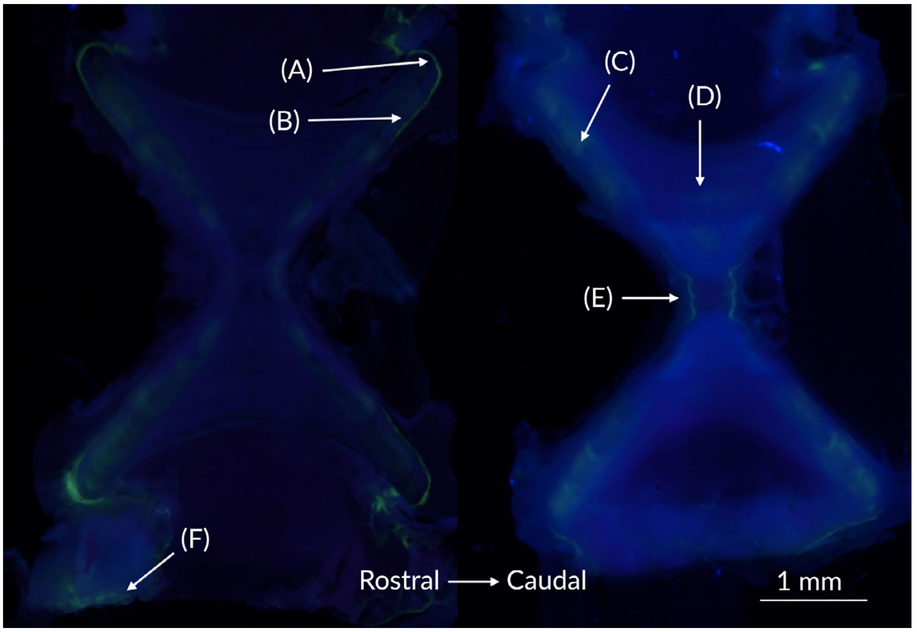

FIGURE 5 Locations of oxytetracycline mark in *Leucoraja erinacea* centra. Oxytetracycline was expected to be across the corpus calcareum (A), but also occurred on the outer edge of the corpus calcareum (B), diffuse in the corpus calcareum (C), diffuse in the intermedialia (D), at the focus (E), and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

TABLE 3 The effect of seven growth variables on the number of band pairs present distal to the oxytetracycline mark (0.5, 1) in males and females at the end of 13 month experimental period for *Leucoraja erinacea* analysed using logistic regressions

Variable	0.5 Band Pair (mean ± SE)	1 Band Pair (mean ± SE)	*z*	*df*	*P*
Food Consumption (g)	2177 ± 120.0	2371 ± 135.9	0.830	32	> 0.05
Monthly Growth (g month^{-1})	4.1 ± 0.7	3.1 ± 0.9	−0.379	32	> 0.05
Centrum growth (mm)	0.04 ± 0.003	0.06 ± 0.007	2.108	32	< 0.05
Change in L_T (cm)	−0.7 ± 0.3	−1.5 ± 0.5	−1.159	32	> 0.05
Final L_T (cm)	47.7 ± 0.4	46.0 ± 0.4	−2.263	32	< 0.05
Final M_T (g)	673.8 ± 19.2	676.8 ± 19.9	−0.076	32	> 0.05
Total Band-pair Count	10 ± 0.5	8 ± 0.6	−1.454	32	> 0.05

Note: L_T: Total length; M_T: total body mass.

even in cases where decreased band-pair deposition frequency was observed after sexual maturity (Harry, 2017). Previous captive growth experiments documented annual band-pair deposition for juvenile *L. erinacea* and adults except two mature females (Cicia *et al*., 2009; Natanson, 1993; Sagarese & Frisk, 2010). The present study shows decreased frequency of band-pair deposition in 60% of sexually mature *L. erinacea* of either sex and supports a link between sexual maturation and decreased frequency of band-pair deposition.

Sexually mature *L. erinacea* in southern New England range from 39 to 56 cm L_T (Frisk & Miller, 2006; Sosebee, 2005). The skates in this study are representative of the mature size range and include a wide range of band-pair counts. Total band-pair count did not have an effect on the number of bands distal to the OTC mark or which band type the OTC mark was incorporated into. This suggests that decreased frequency of band-pair deposition is equally likely across all sexually mature *L. erinacea*, independent of size, sex, or band-pair count. An explanation for this decreased frequency of band-pair deposition is that band-pair deposition is correlated with somatic growth rather than age (Natanson & Cailliet, 1990; Tanaka, 1990). This correlation has been demonstrated in 15 species across six families (Chidlow *et al*., 2007; Huveneers *et al*., 2013; Natanson *et al*., 2008; Natanson *et al*., 2018; Natanson & Cailliet, 1990; Tanaka, 1990) with the most recent research demonstrating a link between band-pair count and the structural requirements of the skeleton (Natanson *et al*., 2018).

A traditional problem with captive growth studies is that captive growth rates are faster than wild growth rates (Wass, 1971). Gruber and Stout (1983) observed growth rates nine times faster than wild estimates for juvenile lemon sharks, *Negaprion brevirostris* (Poey 1868). Huveneers *et al*. (2013) also observed faster growth rates for juvenile captive wobbegongs *Orectolobus* spp. than wild ones, however the two captive adults had similar growth rates (2.24 cm L_T year^{-1}) to wild adults. *Leucoraja erinacea* grew a maximum of 1.66 cm L_T year^{-1} in this study. Wild growth rates estimated from von Bertalanffy growth functions for adult *L. erinacea* (> 40 cm L_T) range from 0.9–4.4 cm L_T year^{-1} (Frisk & Miller, 2006;Johnson, 1979 ; Waring, 1984). *Leucoraja erinacea* individuals in this study did not have faster growth rates in captivity compared to wild growth rates.

The band-type (opaque or translucent) in which OTC was deposited was not synchronous among individuals in this study. Oxytetracycline was observed in either a translucent band (71% of individuals) or an opaque band (29% of individuals), despite all individuals being injected with OTC on the same day. An assumption of annual band-pair formation is that all individuals, regardless of age, are synchronised in depositing alternating opaque and translucent bands in an annual cycle. The appearance of OTC in different band types across individuals could be explained by the timing of injection (December), which was suggested to be a transition period between deposition of opaque and translucent bands in *L. erinacea* (Johnson, 1979; Waring, 1984) therefore the presence of both band types is expected. On the other hand, Natanson (1993) suggested that opaque bands are formed in autumn–winter in *L. erinacea*. Another potential effect on band deposition is diet; the experimental diet (herring and some squid) did not reflect wild diet of *L. erinacea* (Bigelow & Schroeder, 1953). However, since all skates received the same diet, any potential effect of diet on band deposition would not be detected. Nevertheless, since initial band type deposition and rate of band-pair deposition varied among individuals in this experiment, it is not likely that synchronous seasonal switching of band types is occurring in adult *L. erinacea*. The inconsistencies in previous studies (Johnson, 1979; Natanson, 1993; Waring, 1984) 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 oxyrhinchus* Rafinesque 1810; Wells *et al*., 2013; Kinney *et al*., 2016) are probably the result of decreased frequency of band-pair deposition 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 (Huveneers *et al*., 2013; Sagarese & Frisk, 2010; Smith, 1984). 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% (Sagarese & Frisk, 2010). Mark–recapture studies of wild elasmobranchs had OTC mark failure rates from 6.38% (Walker *et al*., 2001) to 66% (McFarlane & Beamish, 1987). Oxytetracycline is deposited at sites of active mineralisation of bone and cartilage (Frost *et al*., 1961; Milch *et al*., 1957). Smith (1984) attributed the absence of OTC to insufficient mineralisation of the vertebral centra directly after injection. Oxytetracycline deposited outside of the CC was common in the current study indicating active mineralisation at these different sites (*e.g*., outer edge of CC) at the time of injection. Oxytetracycline was also present diffusely in both the intermedialia and throughout the CC in several individuals. Holden and 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 mineralising at the time of OTC injection while the CC, where band pairs are counted for *L. erinacea*, is not actively mineralising. 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 (Huveneers *et al*., 2013; Tanaka, 1990). Variable deposition of OTC warrants careful examination. Further investigation into cases where OTC is not present or is deposited in unexpected areas is crucial (Smith, 1984). Careful planning of OTC studies is necessary as individuals must be depositing material in their vertebral centra during the time of OTC injection for these studies to be valid.

The higher energetic cost of reproduction for females over males is well documented in many invertebrate and vertebrate taxa (Haywood & Gillooly, 2011). Female elasmobranchs demonstrate the high cost of reproduction through larger livers relative to L_T than males (Garcia-Garrido *et al*., 1990; Lucifora *et al*., 2002). Elasmobranch livers produce vitellogenin which is directly related to yolk production in the ovary (Koob & Callard, 1999). Female skates in this study consumed 1.5 times the 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 probably allocated energy from food to egg production, females also grew more in body mass (Table 1). A captive breeding group of *L. erinacea* from the Gulf of Maine documented 324 egg cases for seven females resulting in an average of 46.2 eggs per female with peak deposition in June (*c*. 18°C) and a minimum in March (*c*. 7°C; Palm *et al*., 2011). The fecundity observed in this study was higher, an average of 65.2 eggs per female with similar seasonal patterns (Figure 3). The higher average fecundity may be attributed to the differences in food ration; Palm *et al*., (2011) provided daily equal rations while this study fed until food was refused. There are probably many factors that would influence annual fecundity. Captive fecundity of several other skate species ranges from 40.5 to 117 eggs per year with sample sizes from one to 55 adult females (Koop, 2005; Luer & Gilbert, 1985; Parent *et al*., 2008).

The increasing instances of decreased frequency of band-pair deposition and age underestimation in elasmobranchs raises concerns for population monitoring and all uses of age data in science. Although some recent studies have validated absolute ages for part of the lifespan of elasmobranch populations, Harry (2017) documented 21 elasmobranch populations with possible or likely age underestimation. The extent of age underestimation throughout all elasmobranchs is currently unknown and will only be determined through continued efforts to validate elasmobranch ages. Regardless of the extent of age underestimation, the systematic under-ageing of larger, older individuals will lead to biased growth parameters with specific implications for stock assessments (Harry, 2017). Some stock-assessment methods used for teleosts do incorporate ageing bias and imprecision (Methot, 1990; Punt *et al*., 2008; Reeves, 2003), which reveal increases in the variability of assessment results that are particularly sensitive to the magnitude of the biases (Bertignac & de Pontual, 2007; Bradford, 1991; Reeves, 2003). 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. If age underestimation is identified in more elasmobranch species, the effects of biased growth parameters on population monitoring, life history and ontogenetic studies, and stock assessment results must be better understood.

ACKNOWLEGEMENTS

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.

ORCID

Kelsey C. James https://orcid.org/0000-0002-9906-4789

REFERENCES

Ardizzone, D., Cailliet, G. M., Natanson, L. J., Andrews, A. H., Kerr, L. A., & Brown, T. A. (2006). Application of bomb radiocarbon chronologies to shortfin mako (*Isurus oxyrinchus*) age validation. *Environmental Biology of Fishes*, *77*, 355–366.

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.

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.

Campana, S. E., Natanson, L. J., & Myklevoll, S. (2002). Bomb dating and age determination of large pelagic sharks. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*, 450–455.

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., & Sulikowski, J. A. (2009). 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.

Frisk, M. G., & 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.

Frisk, M. G., & Miller, T. J. (2009). Maturation of little skate and winter skate in the western Atlantic from Cape Hatteras to Georges Bank. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, *1*, 1–11.

Frost, H. M., Villanueva, A. R., Roth, H., & Stanisavljevic, S. (1961). Tetracycline bone labelling. *Journal of New Drugs*, *1*(5), 206–216.

Garcia-Garrido, L., Munoz-Chapuli, R., & De Andres, A. V. (1990). Serum cholesterol and triglyceride levels in *Scyliorhinus canicula* (L.) during sexual maturation. *Journal of Fish Biology*, *36*, 499–509.

Goldman, K. J., Cailliet, G. M., Andrews, A. H., & Natanson, L. J. (2012). Assessing the age and growth of Chondrichthyan Fishes. In J. C. Carrier, J. A. Musick, & M. R. Heithaus (Eds.), *Biology of sharks and their relaives II* (pp. 423–451). New York, NY: CRC Press.

Gruber, S. H., & Stout, R. G. (1983). Biological materials for the study of age and growth in a tropical marine elasmobranch, the lemon shark, *Negaprion brevirsotris*. In E. D. Prince & L. M. Pulos (Eds.), *Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks. NOAA Technical Report 8* (pp. 193–205). Miami, Florida: National Oceanic and Atmospheric Administration.

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

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.

Huveneers, 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.

Jobling, M. (1993). Bioenergetics: Feed intake and energy partitioning. In J. C. Rankin & F. B. Jensen (Eds.), *Fish Ecophysiology. Fish and Fisheries Series 9*. London: Chapman & Hall.

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 J. M. Kalish (Ed.), Final Report, FDRC Project 93/109 *Use of bomb radiocarbon chronometer to validate fish age* (pp. 116–122). Canberra, Australia: Fisheries Research and Development Corporation.

Kinney, M. J., Wells, R. J. D., & Kohin, S. (2016). Oxytetracycline age validation of an adult shortfin mako shark *Isurus oxyrhinchus* after 6 years at liberty. *Journal of Fish Biology*, *89*, 1828–1833.

Kneebone, J., Natanson, L. J., Andrews, A. H., & Howell, W. H. (2008). Using bomb radiocarbon analyses to validate age and growth estimates for the tiger shark, *Galeocerdo cuvier*, in the western North Atlantic. *Marine Biology*, *154*, 423–434.

Koob, T. J., & Callard, I. P. (1999). Reproductive endocrinology of female elasmobranchs: Lessons from the little skate (*Raja erinacea*) and spiny dogfish (*Squalus acanthias*). *Journal of Experimental Zoology.*, *284*, 557–574.

Koop, J. H. (2005). Reproduction of captive *Raja* spp. in the Dolfinarium Harderwijk. *Journal of Marine and Biological Association U.K.*, *85*, 1201–1202.

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

Lucifora, L. O., Menni, R. C., & Escalante, A. H. (2002). Reproductive ecology and abundance of the sand tiger shark, *Carcharias taurus*, from the southwestern Atlantic. *ICES Journal of Marine Science*, *59*, 553–561.

Luer, C. A., & Gilbert, P. W. (1985). Mating behavior, egg deposition, incubation period, and hatching in the clearnose skate, *Raja eglanteria*. *Environmental Biology of Fishes*, *13*(3), 161–171.

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.

Milch, R. A., Rall, D. P., & Robie, J. E. (1957). Bone localisation of the tetracyclines. *Journal of the National Cancer Institute*, *19*, 87–93.

Mull, C. G., Blasius, M. E., O'Sullivan, J. B., & Lowe, C. G. (2012). Heave metals, trace elements, and organochlorine contaminants in muscle and liver tissue of juvenile white sharks, *Carcharodon carcharias*, from the Southern California Bight. In M. L. Domeier (Ed.), *Global Perspectives on the Biology and Life History of the White Shark* (pp. 59–75). New York, NY: CRC Press.

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. 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*, *69*(9), 1440–1452.

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., … Wedderburn-Maxwell, A. (2008). Ontogenetic vertebral growth patterns in the Basking Shark, *Cetorhinus maximus*. *Marine Ecology Progress Series*, *361*, 267–278.

Palm, B. D., Koester, D. M., Driggers, W. B., III, & Sulikowski, J. A. (2011). Seasonal variation in fecundity, egg case viability, gestation, and neonate size for little skates, *Leucoraja erinacea*, in the Gulf of Maine. *Environmental Biology of Fishes*, *92*, 585–589.

Parent, S., Pepin, S., Genet, J.-P., Misserey, L., & Rojas, S. (2008). Captive breeding of the Barndoor skate (*Dipturus laevis*) at the Montreal Biodome, with comparison notes on two other captive-bred skate species. *Zoo Biology*, *27*, 145–153.

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.

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* (Vol. 3, pp. 677–743). New York, NY: Academic Press.

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*, 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.

Wass, R.C. (1971). *A comparative study of the life history, distribution and ecology of the sandbar shark and the grey reef shark in Hawaii*. *PhD Thesis*, University of Hawaii, Honolulu. 219 pp.

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.

How to cite this article: James KC. Vertebral growth and band-pair deposition in sexually mature little skates *Leucoraja erinacea*: is adult band-pair deposition annual? *J Fish Biol*. 2020;96:4–13. https://doi.org/10.1111/jfb.14141