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Size and age estimates at sexual maturity for the little skate *Leucoraja erinacea* from the western Gulf of Maine, U.S.A.

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Size and age estimates at sexual maturity were determined for 162 male and 273 female little skates *Leucoraja erinacea* collected from the western Gulf of Maine. Maturity ogives suggest that 50% maturity in females occurs at age 9·5 years and 480 mm total length (L_T), whereas 50% maturity in males occurs at a slightly younger age of 7·7 years and smaller size of 460 mm L_T. Age estimates were made from 389 *L. erinacea* ranging in size from 93 to 570 mm L_T. The index of average per cent error and age-bias plots indicated that the ageing methods were precise and non-biased. Additionally, annual periodicity of band formation was validated with oxytetracycline in eight individuals (three males and five females) ranging in age from 3 to 12 years. In conclusion, results from this study indicate that *L. erinacea* exhibits characteristics that make other elasmobranch populations highly susceptible to overexploitation.

Key words: elasmobranch; hormones; oxytetracycline; rajidae; reproduction.

INTRODUCTION

In the north-western Atlantic Ocean, seven species of skates occur in the coastal waters of the U.S.A., five of which are found within the Gulf of Maine (GOM) [New England Fisheries Management Council (NEFMC), 2003]. Historically, skates in this region were considered by-catch and discarded due to their low commercial value (NEFMC, 2003; Sulikowski *et al*., 2005*a*; Frisk & Miller, 2006); however, the escalating demand for the human consumption of skate wings and their use as lobster bait have made the retention of the thorny skate *Amblyraja radiata* (Donovan), winter skate *Leucoraja ocellata* (Mitchill) and little skate *Leucoraja erinacea* (Mitchill)

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more lucrative (NEFMC, 2003; Sulikowski *et al*., 2006). Despite recent intensification in commercial landings, federal management efforts to prevent overexploitation were not implemented until 2003. As a result, four of the five skate species in the GOM are either prohibited from commercial retention or labelled as overfished due to biomass estimates falling below or being near the mandatory threshold levels (NEFMC, 2005; NMFS, 2007).

To rebuild current populations and prevent future exploitation of GOM skates, the collection of accurate life-history information is imperative to managing populations effectively (Conrath *et al*., 2002; Oddone *et al*., 2007; Sulikowski *et al*., 2007*a*; Oddone & Amorim, 2008). Two life-history variables essential to the management process are the size and age at which a species reaches sexual maturity. For skates, and elasmobranches in general, information regarding sexual maturity has been primarily based on gross morphological changes in the reproductive tract and accessory organs (Walmsley-Hart *et al*., 1999; Driggers *et al*., 2004; Oddone *et al*., 2007; Ebert *et al*., 2008). Past studies have utilized clasper length (L_C) in males and shell gland mass (M_S) in females (Ebert *et al*., 2006; Oddone *et al*., 2007; Oddone & Amorim, 2008), whereas others have relied on the gonado-somatic index (I_G) to assess reproductive status (Parsons, 1983; Snelson *et al*., 1988; Loefer & Sedberry, 2003). The I_G assumes that gonad size and reproductive readiness are positively correlated; however, more recent studies on gonadal development do not always support this assumption, especially for male batoids (Maruska *et al*., 1996; Sulikowski *et al*., 2004, 2007*a*). More recently, studies have shown that the use of circulating steroid hormones and histological changes in gonadal tissues provide a more precise determination of sexual maturity in *L. ocellata* and *A. radiata* (Sulikowski *et al*., 2005*b*, 2006).

Leucoraja erinacea is the smallest and most common skate found in coastal waters (<100 m) of the GOM, the area where the majority of fishing effort in this region occur (McEachran, 2002; NEFMC, 2003, 2005). As a result, current stock estimates indicate that the population of *L. erinacea* has been declining over the past 5 years and is quickly approaching the mandatory threshold biomass levels set by the Sustainable Fisheries Act (NMFS, 2007). Due to increasing commercial value and declining biomass of *L. erinacea*, obtaining information concerning the size and age at sexual maturity is in critical need to revise and improve current management (Conrath *et al*., 2002; Oddone *et al*., 2007; Sulikowski *et al*., 2007*a*; Oddone & Amorim, 2008). Thus, the present study represents the first comprehensive determination of size at sexual maturity for *L. erinacea* by correlating steroid hormone concentrations to morphological and histological characteristics. This information was then coupled with interpreting annular counts on vertebral centra to estimate age at sexual maturity.

MATERIALS AND METHODS

During May and July 2007, *L. erinacea* were collected from nearshore waters in the western GOM, along the coasts of New Hampshire (NH) and Massachusetts (MA) (Fig. 1). Specimens were captured using an otter trawl by commercial fishermen or the Massachusetts Department of Marine Resources (DMR). Immediately after capture, *L. erinacea* were placed in a live well until external morphological measurements and blood samples could be obtained. Fish were euthanized in a 0·3 g l^{-1} bath of MS-222 and total length (L_T, mm), mass (M, kg) and L_C

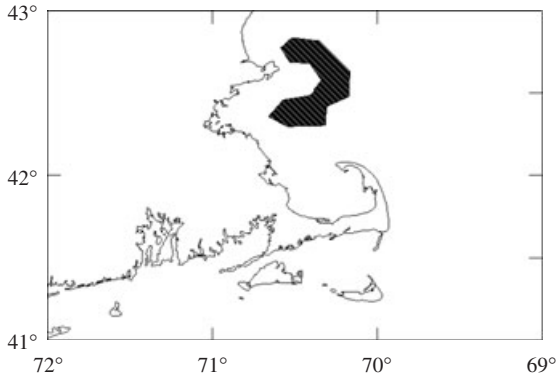

FIG. 1. Sampling area within the western Gulf of Maine. ⊡, area is where the majority of *Leucoraja erinacea* were collected.

(mm) were recorded. Once external data were documented, 5–10 ml of blood were collected from a sub-sample of individuals (37 males and 34 females) by cardiac puncture using chilled, heparinized syringes with a 21 gauge needle. Blood samples were then centrifuged at 1500 **g** for 5 min. The separated plasma was placed in a cooler for 4–8 h before storage at −20° C. At the conclusion of the cruise, the remaining fish were transported on ice back to the University of New England's (UNE) Marine Science Center (MSC), where specimens were dissected within 2 days of capture.

Additionally, nine males, ranging between 315 and 517 mm L_T, and nine females, ranging between 375 and 547 mm L_T, were kept alive and transported back to UNE's MSC in a 1219 mm × 1118 mm × 914 mm insulated container equipped with an aerator. Eleven days after arrival, *L. erinacea* were injected with; 0·25 mm kg^{-1} body mass oxatetracycline (OTC) to validate annual periodicity of vertebral band formation (Natanson, 1993).

SIZES USED FOR HORMONE ANALYSES

To determine size and age estimates at 50% maturity, samples were collected over a broad range of size classes. Based on previous studies, maturity in skates is known to occur at *c*. 80 to 90% of their maximum L_T (Ebert, 2005; Sulikowski *et al*., 2007*a*). As a result, blood samples were collected from specimens starting at *c*. 50% (300 mm) of their maximum L_T and sampling concentrated on size classes where the transition toward sexual maturity was thought to occur. In total, 71 blood samples were collected: 34 from females ranging in size from 333 to 570 mm L_T and 37 from males ranging in size from 353 to 535 mm L_T.

HISTOLOGY OF TESTES

Histological examination of testes followed the protocol of Sulikowski *et al*. (2005*b*). Whole testes were removed, blotted dry and weighed to the nearest g (M_T). A single 2–3 mm thick segment of the central portion of a single lobe in the medial area of each testis (Maruska *et al*., 1996) was fixed in 10% buffered formalin before processing for standard haemotoxylin and eosin staining. To determine sexual maturity in males, prepared slides were examined to assess spermatogenic development, based on criteria outlined by Maruska *et al*. (1996). Specifically, the mean proportion of a testis occupied by mature spermatocysts along a straight line distance across one representative full lobe cross-section of the testis was measured. Histologically, mature spermatocytes were identified by the organization of spermatozoa into tightly shaped packets that were arranged spirally along the periphery of the spermatocytes (Sulikowski *et al*., 2005*b*, 2006).

GROSS MORPHOLOGY OF FEMALE REPRODUCTIVE TRACT

Ovaries (M_O) and shell glands (M_S) were removed, blotted dry and weighed to the nearest g. Follicle development was assessed by measuring the width of all oocytes >1 mm in size (Tsang & Callard, 1987; Snelson *et al*., 1988; Sulikowski *et al*., 2006). Changes in the mean M_O and M_S and the mean diameter of the largest follicle were used to determine the transition towards sexual maturity (Walmsley-Hart *et al*., 1999; Sulikowski *et al*., 2005*b*; Ruocco *et al*., 2006; Oddone & Amorim, 2008).

ANALYSIS OF STEROID HORMONES

Stock solutions of radiolabelled testosterone (T) and oestradiol (E_2) (GE Healthcare; http://www.gehealthcare.com/usen/index.html) were purified by thin layer chromatography (TLC) on silica gel plates (HLF, scored 20 cm × 20 cm, 250 μm; Analtech Inc.; www.analtech.com). The solvent systems and specific procedures for TLC were identical to those described in Sulikowski *et al*. (2004).

Individual plasma samples were extracted twice with 10 volumes of diethyl ether and then snap frozen. The supernatant was evaporated under a stream of nitrogen and dried extracts were reconstituted in phosphate-buffered saline with 0·1% gelatine (PBSG). All extracts were stored at −20° C until assay. To account for procedural loss during the extractions, *c*. 1000 counts min^{-1} (cpm) of the appropriate tritiated steroid hormone was added into each plasma sample. The overall mean recoveries (*e.g*. how much tritiated steroid was not lost during extraction) for E_2 and T were 68 and 78% respectively, which were used to correct for the appropriate steroid concentration in each sample.

Plasma concentrations of T and E_2 were determined by radioimmunoassay, modified from procedures of Sulikowski *et al*. (2004, 2005*b*, 2006). All non-radiolabelled steroids were obtained from Steraloids, Inc. (http://www.bioportfolio.com/search/Steraloids,_Inc..html). The specifics of the radiolabelled steroids, the antibody characteristics and titres are found in Sulikowski *et al*. (2004). Radioactivity was determined in a Perkin Elmer Tri-Carb 2900 PR liquid scintillation counter (http://las.perkinelmer.com). Intra-assay coefficients of variance were 12% for T and 14% for E_2. Interassay coefficients of variance were 13% for T and 4% for E_2.

CRITERIA USED FOR DETERMINING SEXUAL MATURITY

Criteria for determining sexual maturity of *L. erinacea* followed the protocol of Sulikowski *et al*. (2005*b*, 2006). Females were considered mature when the reproductive tract contained vitellogenic follicles ≥14 mm in diameter, M_S ≥3·25 g and E_2 concentrations ≥1000 pg ml^{-1}. Males were considered mature when T concentrations were ≥30 000 pg ml^{-1}, claspers were calcified and ≥120 mm L_C, testes contained a proportion of mature spermatocyst ≥20% and M_T was ≥4 g. Male and female *L. erinacea* that did not meet all the criteria were considered immature.

PREPARATION AND AGE ANALYSIS OF VERTEBRAE

Age estimates were determined by gross vertebral band counts, processed following the protocol of Sulikowski *et al*. (2003, 2005*b*). Briefly, a sagittal cross-section of three vertebrae from each specimen was cut using a gem saw. Each section was then affixed horizontally on a glass microscope slide and polished with successively finer grits (#400 and #600) of wet-dry sandpaper. Two non-consecutive band counts were made independently by two readers for each specimen without knowledge of L_T or previous counts. If the variability between readings was >2 years, that specimen was eliminated from further analysis. Count reproducibility was estimated by using the index of average per cent error (IAPE) and the coefficient of variation (c.v.) (Beamish & Fournier, 1981; Cailliet & Goldman, 2004). Age determination bias between readers was assessed using an age-bias plot (Chang, 1982; Campana *et al*., 1995; Campana, 2001). Growth parameters were then determined using the von Bertalanffy growth function (VBGF) (von Bertalanffy, 1938).

STATISTICS

Differences in morphological and histological variables and hormone concentrations among age groups were determined using ANOVA, followed by a Tukey's *post hoc* test. Statistical significance was accepted at $P < 0{\cdot}05$. To determine whether a relationship existed among the measured morphological, histological and hormone variables, a Pearson correlation analysis (r) was performed. To determine size and age estimates at 50% maturity, sex-specific maturity ogives were fitted to L_T (at 20 mm intervals) and age (1 year intervals) data using probit analyses.

To test for differences among von Bertalanffy model parameters between collection site (NH or MA) and sex, a modified sum-of-squares reduction test (SSRT) was employed (Schabenberger *et al*., 1999). This was accomplished using PROC NLIN in SAS (v 9.1.3) (www.sas.com). Statistical significance was accepted at $P < 0{\cdot}05$.

RESULTS

A total of 435 *L. erinacea* were utilized to assess maturity. Males ($n = 162$) ranged between 98 and 559 mm L_T and between 4 g and 1·80 kg. Females ($n = 273$) ranged between 93 and 570 mm L_T and between 9 g and 1·27 kg. Of these specimens, vertebrae from 405 individuals were processed for age determination.

MALES

Overall, there was an increasing trend among L_C, M_T, per cent mature spermatocytes and circulating T as fish increased in L_T and age [Table I and Fig. 2(a), (b)]. Both L_C and M_T significantly increased ($P < 0{\cdot}05$) between ages 6 and 7 years. A second significant increase ($P < 0{\cdot}05$) in L_C, M_T and percentage mature spermatocytes occurred in fish from 7 to 8 years. Although T doubled in concentration between ages 6 and 7 years, no statistical difference ($P > 0{\cdot}05$) was observed. Despite the lack of statistical difference, T concentration was strongly correlated to changes in L_C ($r = 0{\cdot}69$), per cent mature spermatocytes ($r = 0{\cdot}81$) and M_T ($r = 0{\cdot}80$) as males matured.

Maturity ogives indicated that 50% maturity occurs at 460 mm L_T and an estimated age of 7·7 years [Fig. 3(a), (b)]. This agrees with the measured morphological variables and T concentration, which suggest that 50% maturity occurs between 450 and 460 mm L_T and between 7 and 8 years. Thus, maturity in males occurs at *c*. 81% of their maximum observed L_T and 53% of their maximum observed age. The smallest mature male was 438 mm L_T and 7 years old, whereas the largest immature male was 497 mm L_T and 10 years old.

FEMALES

An increasing trend in M_O, M_S, follicle diameter and E_2 concentration was observed as fish increased in size and age until they reached age 13 [Table II and Fig. 4(a), (b)]. Interestingly, there was no significant difference ($P > 0{\cdot}05$) found among M_O, M_S, follicle diameter or E_2 concentration between any two consecutive age groups. Similar to T in males, observed increases in morphological variables were correlated (r values) to the increase in E_2 concentration as females progressed through maturity. For example, r values of 0·80, 0·78 and 0·89 were observed for

TABLE I. Morphological measurements and reproductive variables for male *Leucoraja erinacea**

Age (years)	N	L_T (mm)	L_C (mm)	M (kg)	M_T (g)	Testosterone concentration (pg ml^{-1})	% Mature spermatcysts
0	4	103 ± 2		< 0·00			
1	2	170 ± 11	11 ± 0 (1)	0·02 ± 0·00			
2	4	265 ± 11	20 ± 1	0·08 ± 0·01	0·2 ± 0·0 (3)		
3	6	324 ± 3	32 ± 1	0·19 ± 0·01	0·4 ± 0·1		0·0 ± 0·0 (3)
4	10	336 ± 5	34 ± 1	0·22 ± 0·01	0·5 ± 0·0 (8)	4244 ± 0 (1)	0·0 ± 0·0 (7)
5	12	366 ± 2	41 ± 1	0·28 ± 0·01	0·5 ± 0·1	13 107 ± 9026 (3)	0·0 ± 0·0 (7)
6	14	401 ± 4	53 ± 3 ←	0·36 ± 0·02	0·6 ± 0·0 (13) ←	15 253 ± 2183 (3)	0·0 ± 0·0 (10)
7	25	444 ± 5	92 ± 7 ←	0·50 ± 0·02	2·1 ± 0·3 (24) ←	30 735 ± 6701 (7)	4·4 % ± 2·0 (16) ←
8	25	465 ± 4	124 ± 6 ←	0·61 ± 0·02	3·8 ± 0·4 ←	35 190 ± 7694 (9)	23·.3 % ± 3·4 (14) ←
9	17	485 ± 7	127 ± 7	0·69 ± 0·04	4·6 ± 0·6	38 775 ± 6083 (8)	22·1 % ± 4·5 (9)
10	18	502 ± 3	148 ± 4	0·77 ± 0·02	5·6 ± 0·4	51 333 ± 4351 (3)	28·.4 % ± 2·6 (8)
11	14	511 ± 5	156 ± 4	0·85 ± 0·03	6·1 ± 0·5	46 403 ± 14194 (2)	29·8 % ± 1·4 (7)
12	7	524 ± 4	159 ± 5	0·88 ± 0·03	6·4 ± 0·3	70 312 ± 0 (1)	38·5 % ± 3·2 (2)
13	4	541 ± 6	170 ± 2	0·98 ± 0·16	6·8 ± 0·5		33·3 % ± 0·0 (3)

n, sample size; L_T, total length; L_C, clasper length, M, fish mass; M_T, testes mass.

*For each column, arrowed bracket represent significant differences ($P < 0·05$; ANOVA followed by a Tukey's *post hoc* test) among *L. erinacea* in consecutive age groups. Values given as mean ± S.E. Numbers in parenthesis represent sample size when different from n.

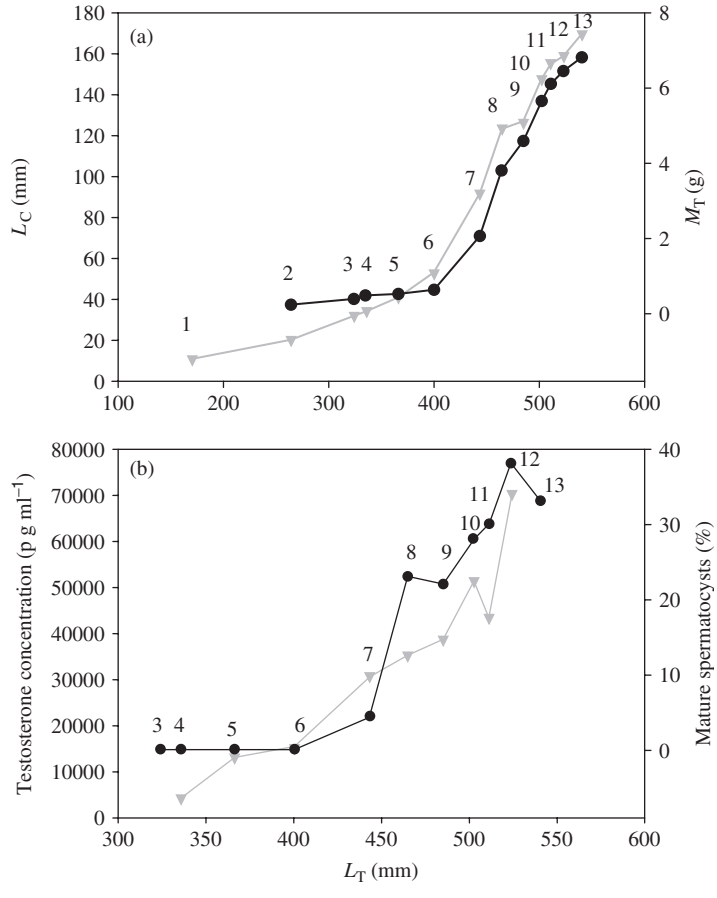

FIG. 2. Relationships between (a) total length (L_T) and clasper length (L_C; ▼) and L_T and testis mass (M_T; ●) and (b) testosterone concentration (▼) and L_T and proportion of mature spermatocysts (●) as male *Leucoraja erinacea* progress through sexual maturity. The mean age (years) is shown above each representative size class. Values are expressed as mean ± S.E. (see Table I).

the morphological variables of M_O, shell gland mass and diameter of the largest follicle, respectively.

Maturity ogives indicated 50% maturity occurred at an L_T of 480 mm and an estimated age of 9·5 years [Fig. 3(a), (b)]. These values agreed with the observed morphological measurements and E_2 concentrations, which suggested that 50% maturity occurs between 480 and 500 mm L_T and between 9 and 10 years of age. Thus, female maturity occurred at *c*. 86% of their maximum observed L_T and 68% of their maximum observed age. Based on increases in reproductive variables, the smallest mature female measured 452 mm L_T and was 9 years old, whereas the largest immature female measured 520 mm L_T and was 12 years old. Interestingly, the majority of female *L. erinacea* aged 14 years displayed a decrease in all morphological variables [Table II and Fig. 4(a), (b)]. Decreases of 38, 55 and 53% were observed in M_O, M_S and follicle size, respectively.

TABLE II. Morphological measurements and reproductive variables for female *Leucoraja erinacea**

Age (years)	*n*	L_T (mm)	*M* (kg)	M_O (g)	M_S (g)	Largest follicle size (mm)	Oestradiol (pg ml^{-1})
0	4	97 ± 2	0·01 ± 0·00				
2	1	186 ± 0	0·03 ± 0·00				
3	13	312 ± 5	0·15 ± 0·01	0·4 ± 0·0 (8)	0·1 ± 0·0 (8)	1·0 ± 0·0 (8)	
4	9	347 ± 3	0·25 ± 0·02	0·6 ± 0·0 (1)	0·6 ± 0·6 (3)	1·0 ± 0·0 (3)	0 ± 0 (1)
5	6	376 ± 4	0·30 ± 0·03	0·7 ± 0·1 (4)	0·1 ± 0·0 (5)	1·0 ± 0·0 (4)	
6	21	411 ± 4	0·42 ± 0·02	0·9 ± 0·1 (18)	0·2 ± 0·1 (19)	1·3 ± 0·1 (17)	94 ± 22 (6)
7	40	447 ± 3	0·55 ± 0·02	1·6 ± 0·2 (34)	0·9 ± 0·2 (33)	2·8 ± 0·8 (32)	41 ± 23 (4)
8	32	465 ± 4	0·64 ± 0·03	3·3 ± 0·5 (31)	2·6 ± 0·4	9·8 ± 1·6 (31)	483 ± 412 (3)
9	31	476 ± 4	0·68 ± 0·02	3·8 ± 0·6	3·1 ± 0·5	10·8 ± 1·6 (30)	1046 ± 640 (7)
10	50	499 ± 2	0·80 ± 0·02	5·6 ± 0·4 (48)	4·7 ± 0·3 (48)	16·0 ± 1·2 (47)	1432 ± 214 (6)
11	33	510 ± 3	0·80 ± 0·02	6·5 ± 0·6	5·3 ± 0·5	16·9 ± 1·4	1780 ± 469 (4)
12	15	530 ± 4	0·94 ± 0·03	6·7 ±1·0 (13)	5·9 ± 0·7 (13)	15·3 ± 1·8 (13)	2445 ± 293 (3)
13	13	545 ± 3	0·97 ± 0·02	9·8 ±1·4 (11)	6·9 ± 0·5 (11)	23·6 ± 1·1 (11)	
14	5	551 ± 2	0·94 ± 0·04	6·1 ±1·5	3·1 ± 0·7	11·7 ± 4·0	

n, sample size; L_T, total length; *M*, fish mass; M_O, ovary mass; M_S, shell gland mass.

*Values given as mean ± S.E. Numbers in parenthesis represent sample size when different from *n*.

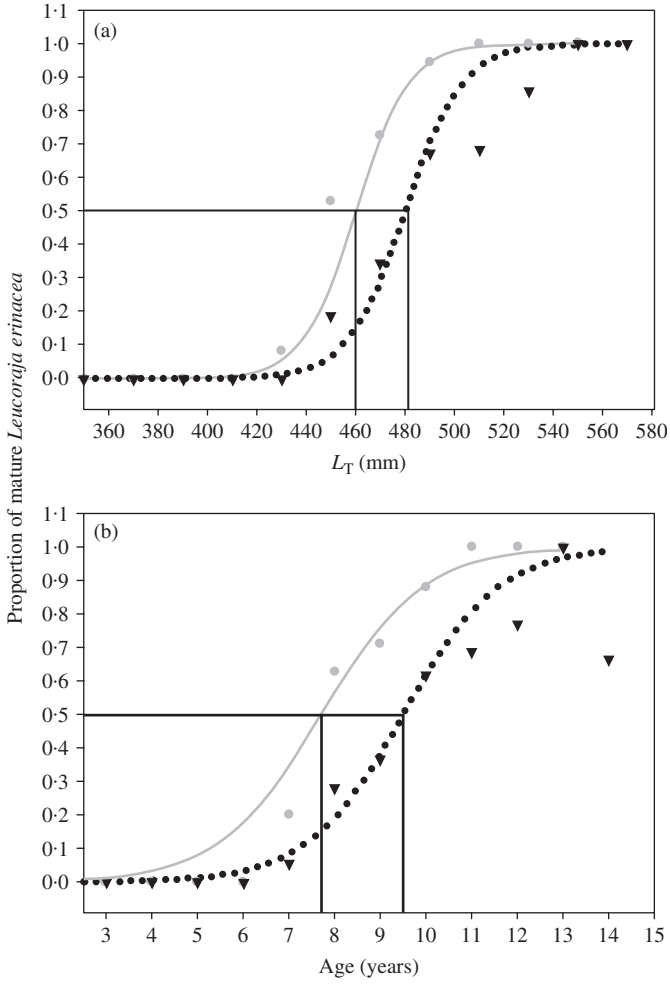

FIG. 3. Maturity ogives based on morphological, histological and steroid hormone analyses for male and female *Leucoraja erinacea*. (a) Total length of male and female *L. erinacea* given in 20 mm intervals and (b) age of male and female *L. erinacea* given in one year increments (●, male observed data; —, male predicted data; ▼, female observed data; •••, female predicted data).

VERTEBRAL ANALYSIS

The relationship between L_T and centrum diameter was linear for both males ($r^2 = 0{\cdot}88$) and females ($r^2 = 0{\cdot}83$). Since no significant differences were observed between L_T and centrum diameter for either sex ($P > 0{\cdot}05$), the data were combined ($r^2 = 0{\cdot}84$).

Of the 405 vertebra processed, 389 (96%) were readable. These vertebra (males = 146; female = 243) were aged by both readers within 2 years, resulting in an IAPE of 1·7% and a C.V. of 1·2%. Moreover, the comparison of these counts also suggested no appreciable bias existed between readers (Fig. 5). Since this level of precision

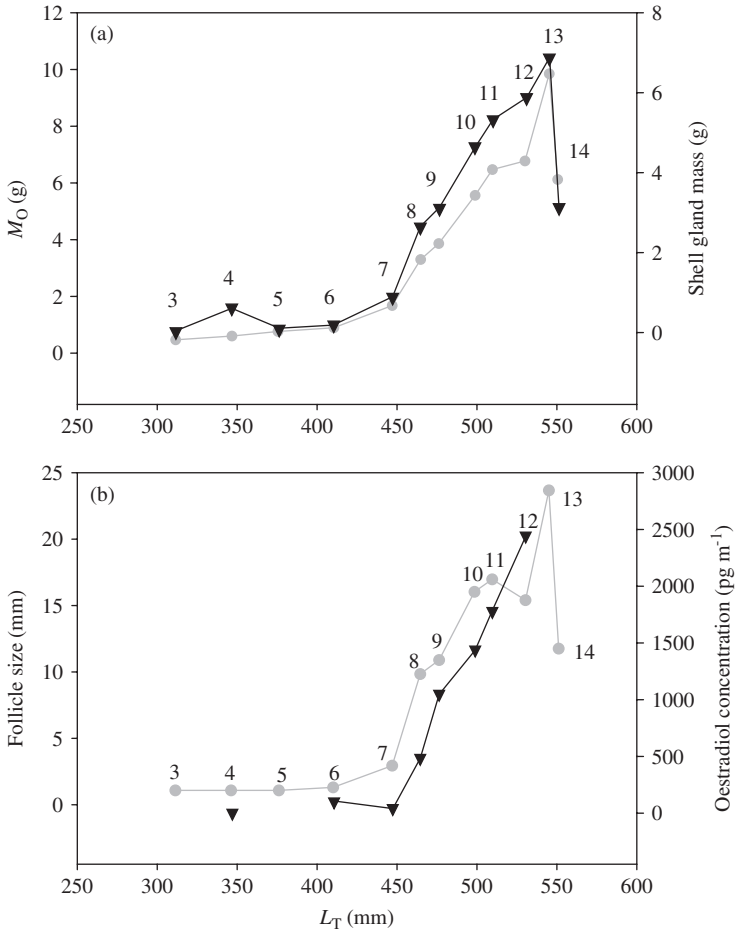

FIG. 4. The relationship between (a) total length (L_T) and shell gland mass (M_S; ▼), and L_T and ovary mass (M_O; ●), and (b) L_T and oestradiol concentration (▼), and L_T and largest follicle (●) as female *Leucoraja erinacea* progress through sexual maturity. The mean age (years) is indicated above each representative size class. Values are means ± S.E. (see Table II).

is considered acceptable (Cailliet & Goldman, 2004), the ages obtained from both readers were averaged and used for subsequent analyses.

Of the 18 *L. erinacea* injected with OTC in June 2007, four males and two females died prior to the conclusion of the experiment in June 2008. Vertebra processed from all fish showed absorption of OTC within a translucent band and four were followed by opaque growth (Table III). After the full year, three *L. erinacea* were killed (one male 348 mm L_T and two females ranging in size from 399 to 530 mm L_T) and vertebra processed confirmed the assumption that a single opaque and translucent band is formed annually in specimens between ages 3–5 and 11–12 years [Fig. 6]. Additionally, observed annual growth of captive individuals was comparable with growth rates predicted by the VBGF in all aforementioned age classes. Moreover,

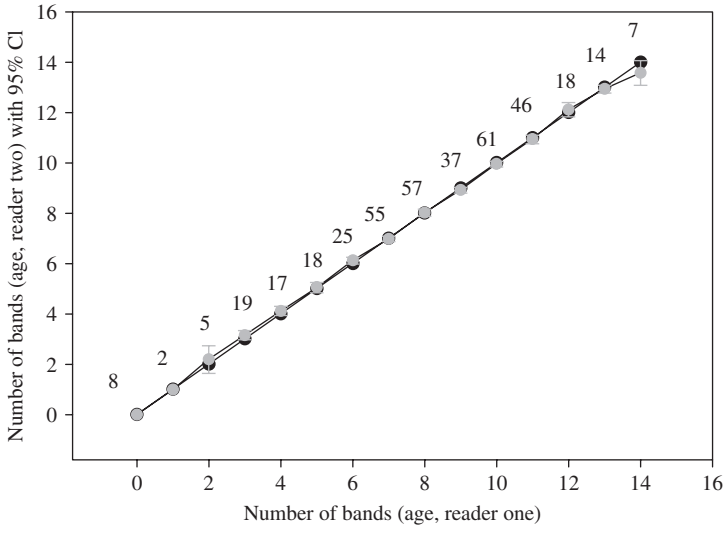

FIG. 5. Age-bias graph for pair-wise comparison of 389 *Leucoraja erinacea* vertebral counts by two independent readers. Each error bar represents the 95% CI for the mean age assigned by reader two for all fish assigned a given age by reader one. The diagonal line represents the one-to-one equivalence line. Sample sizes are given above each corresponding age.

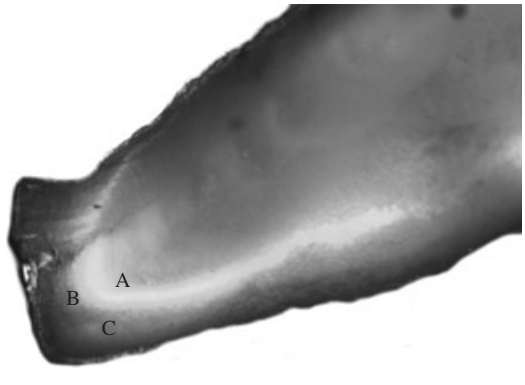

FIG. 6. Representative oxytetracycline (OTC) banding pattern observed in eight captive *Leucoraja erinacea* killed over the course of the study. The sectioned vertebra displays the florescent OTC (A) marker 1 year after injection (June 2007). Note the OTC mark is preceded by an opaque (B) and translucent band (C).

the annual periodicity of formation appears to occur between June and July in this species.

Based on age estimates from vertebral band counts, males ranged in age from 0 to 13·5 years and females ranged in age from 0 to 14 years. No significant difference ($P > 0{\cdot}05$) among VBGF parameters existed between collection site (MA or NH) and sex. Thus, the data were combined for analysis. A three parameter VBGF was fitted to L_T -at-age data (Fig. 7) and provided a good fit for males, females and sexes combined.

TABLE III. Change in total length (L_T), mass (M) and observed vertebral growth for male (M) and female (F) *Leucoraja erinacea* injected with oxytetracycline (OTC)

Specimen number	Initial L_T (mm)	Change in L_T (mm)	Initial M (kg)	Change in M (kg)	Sex	Date of OTC injection	Date died or killed	Observed vertebral growth proceeding OTC marker
997	482	0	0·57	0·02 (+)	F	May 2007	July 2007	OTC marker on marginal edge
993	493	3 (−)	0·63	0·08 (+)	F	May 2007	July 2007	OTC marker on marginal edge
977	442	1 (−)	0·34	0·11 (+)	M	May 2007	August 2007	Beginning of opaque growth
995	451	11 (+)	0·58	0·00	M	May 2007	September 2007	Opaque growth
987	479	3 (+)	0·52	0·10 (−)	M	May 2007	October 2007	Opaque growth
980	462	29 (+)	0·73	0·13 (+)	M	May 2007	October 2007	Opaque growth
991	522	8 (+)	0·97	0·10 (+)	F	May 2007	May 2008	Single opaque and translucent band
LEF1	375	24 (+)	0·26	0·08 (+)	F	May 2007	May 2008	Single opaque and translucent band
LEM1	315	33 (+)	0·16	0·04 (+)	M	May 2007	May 2008	Single opaque and translucent band

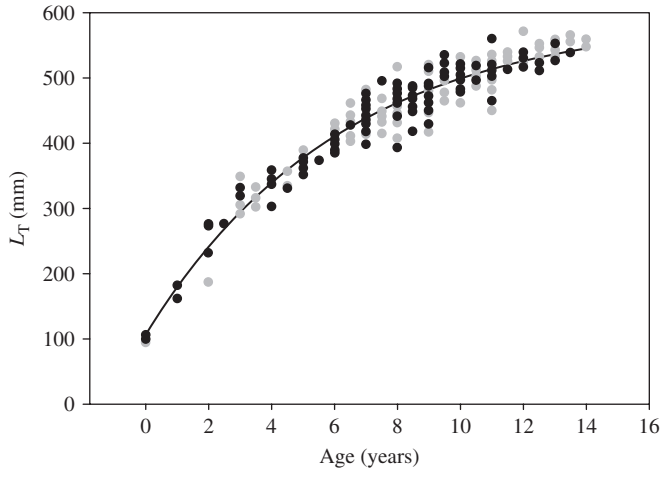

FIG. 7. von Bertalanffy growth model (—) generated from combined vertebral data for male (●) and female (●) *Leucoraja erinacea* from the western Gulf of Maine. Corresponding growth parameters for combined male and female data resulting in $K = 0{\cdot}16$, $L_\infty = 600$ mm.

DISCUSSION

This study represents the first comprehensive investigation into size and age estimates at maturity of *L. erinacea* in the western GOM. Sulikowski *et al*. (2005*b*, 2006, 2007*b*) suggested using a combination of gross reproductive morphology, histological examination of reproductive tissue and steroid hormone concentrations. This provided a more accurate estimation of sexual maturity in both *L. ocellata* and *A. radiata*, than relying only on morphological changes in reproductive organs or I_G. For instance, the inclusion of biochemical and histological variables in estimates of maturity determined that functional–physiological maturity can lag behind observed morphological maturity in male batoids >1 year (Sulikowski *et al*., 2005*b*, 2006, 2007*a*). Thus, the addition of the histological and biochemical components to the current study increased the accuracy of maturity estimates.

The size at sexual maturity has been reported for *L. erinacea* (Sosebee, 2005). That study, however, was not specific to the GOM and included samples from throughout their geographical range (Nova Scotia, Canada to Cape Hatteras, U.S.A.; Sosebee, 2002). Additionally, Sosebee (2005) employed methods developed for assessment of crustacean maturity (Somerton, 1980) to arrive at estimates based on morphological measurements of L_C in males and cloaca length in females. When compared with the present study, the reported maturity findings of Sosebee (2005) differed, with males and females being considerably smaller than predicted by the present comprehensive approach using morphological and biochemical variables. For example, males began maturing at an L_T of 390 mm, whereas females matured between 400 and 480 mm L_T (Sosebee, 2005). Additionally, no estimates of age or L_{50} was given for either sex (Sosebee, 2005). Based on size alone, all male and most female *L. erinacea* in Sosebee's (2005) study would be considered immature using the present criteria. Whether these variations in size at sexual maturity are strictly due to methodological differences or to a geographic variable producing the disparity in estimated variables

between the studies, needs further attention. Collectively, however, the findings from the present study, along with those of Sulikowski *et al*. (2005*b*, 2006), suggest that analysing a combination of reproductive variables may perhaps offer a more accurate estimation of size at sexual maturity when compared with those calculated solely from morphological measurements (Sosebee, 2002).

In male elasmobranchs, maturity is most often marked by an abrupt increase in L_C and calcification (Francis *et al*., 2001; Oddone *et al*., 2007), which was consistent with the present results. Male *L. erinacea* showed a 35% increase in L_C as these fish matured from 7 to 8 years. Furthermore, during this period there was an observed 81% increase in M_T, 442% increase in mature spermatocysts and 12% increase in T. Although an increase in T was observed, it was the only measured variable that did not display a significant difference between any consecutive age groups. Yoccoz (1991) suggested that statistical analyses might not always be relevant to patterns observed in biology, and that changes that display a biologically relevant shift might not always be associated with statistical significance. This appears to be the case in the present study as a biologically significant shift towards maturity appeared to take place between ages 7 and 8 years. In addition, similar to the findings of Sulikowski *et al*.(2005*b*, *c*; 2006), an abrupt and strongly correlated increase (average $r = 0{\cdot}77$) was observed among T and the other measured variables, further indicating that these changes were linked to a biologically significant event between ages 7 and 8 years.

No lag period was observed between the morphological, histological and biochemical variables in male *L. erinacea*. This is in contrast to previous studies on other skates (Sulikowski *et al*., 2005*b*, 2006, 2007*a*), and suggests a high degree of variability in the co-ordination of reproductive events leading to maturity exists among males of different skate species. This variability gives further support for the use of more than one variable for determining sexual maturity, especially in male elasmobranchs. Functional maturity in male *L. erinacea* occurred once they reached 81% of their maximum observed size and 53% of their maximum observed age, suggesting that the majority of growth occurs prior to the onset of maturity and then slows considerably. This observation is consistent with maturity and growth patterns exhibited by males of other skate species, with maturity occurring at 80–90% of maximum length (Francis *et al*., 2001; Ebert, 2005; Sulikowski *et al*., 2007*a*).

In female elasmobranchs, maturity is often marked by an abrupt increase in shell gland, ovary and follicle size (Ebert, 2005; Oddone & Vooren, 2005; Ruocco *et al*., 2006; Sulikowski *et al*., 2007*a*). Between the ages 9 and 10 years, female *L. erinacea* displayed a 48% increase in M_O, a 53% increase in M_S, a 60% increase in follicle size and a 37% increase in E_2 concentration. Although these changes were not statistically significant ($P > 0{\cdot}05$), they were strongly correlated (average $r = 0{\cdot}82$). This signalled a biologically significant shift towards sexual maturity occurring between ages 9 and 10 years (Yoccoz 1991; Sulikowski *et al*., 2005*b*, 2006). Similar patterns were observed by Sulikowski *et al*. (2005*b*, 2006) in *L. ocellata* and *A. radiata*. These studies indicated a strong correlation between E_2 concentrations, M_O, M_S and diameter of the largest follicle existed as females progressed towards sexual maturity. Additionally, other studies have linked increasing E_2 concentrations to development and maturation of follicles in several other elasmobranchs species (Tsang & Callard, 1987; Heupel *et al*., 1999; Tricas *et al*., 2000), further demonstrating the importance of this hormone in elasmobranch reproduction.

Based on maturity ogives, females reached sexual maturity at a larger size and older age than males (females = 480 mm L_T and 9–10 years; male = 460 mm L_T and 7–8 years). It has been suggested that females require a longer period for their reproductive tract to become functional (Oddone *et al*., 2007; Sulikowski *et al*., 2007*a*). Larger size at maturity in females could be connected to a higher energy demand associated with the production of follicles and enlargement of the shell gland. Additionally, female *L. erinacea* reach functional maturity at 86% of their maximum observed size and 68% of their maximum observed age. Interestingly, a marked decline in ovary, shell gland and follicle size was observed in females aged 14 years. This contrasts with maturity studies conducted on other skate species, which reported a constant increase in the size of reproductive organs, which then stabilized at sexual maturity (Francis *et al*., 2001; Ebert, 2005; Sulikowski *et al*., 2007*a*). Although an increasing trend in morphological variables was observed in females until age 13 years, reproductive development did not plateau; instead, a large per cent decrease was observed in M_O, M_S and largest follicle size. The abrupt decline in reproductive variables suggests that older females could be displaying senescence and are incapable of reproduction (Ebert, 2005; Oddone *et al*., 2007). Further investigation, especially of larger and presumably older individuals, is needed to verify this observed trend.

Another phenomenon observed in female *L. erinacea* was the inconsistent trends in sexual maturity by age and size class. This is in contrast with previous studies conducted on other skate species, which indicate that after attaining a certain size and age, larger and older individuals were always identified as sexually mature (Ebert, 2005; Sulikowski *et al*., 2005*b*, 2006; Oddone *et al*., 2007). In the current study, increasing trends were observed in reproductive variables as *L. erinacea* grew in L_T and age. Based on these trends, all females aged >10 years should have reached functional maturity. In contrast, 39, 31 and 23% of *L. erinacea* in the 10, 11 and 12 year age classes displayed underdeveloped ovaries, shell glands and follicles, and were considered sexually immature. One possible explanation for this phenomenon is that the observed females were sexually mature but out of synchronization with the population's reproductive cycle (Oddone & Amorim, 2008). Johnson (1979) and Fahey (1993) suggest the *L. erinacea* is capable of reproducing throughout the year but has two peaks in their reproductive cycle, one in the spring and other in the autumn. Johnson (1979) also observed that between spawning peaks shell glands regressed to 1–2% of their body mass until females were ready to reproduce again. *Leucoraja ocellata* displays a partially defined reproductive cycle with one annual peak. When this species is not in a mating peak, up to a 70% regression in shell gland mass and 60% regression in follicle size were observed (Sulikowski *et al*., 2004). In contrast, *A. radiata* possesses a continuous reproductive cycle, and as a result minimal fluctuation in M_S and follicle size is observed (Sulikowski *et al*., 2005*c*). In order to determine whether seasonal regression could account for the observed anomalies in the reproductive organs of *L. erinacea* in the 10, 11 and 12 year age classes, a 70% reduction in M_S and 60% reduction in follicles size were applied to female *L. erinacea* that were identified as mature (Sulikowski *et al*., 2004). Even after the new minimums were applied, however, females with the observed anomalies still possessed an immature reproductive tract (Fig. 8). As far as is known, this observation has not been documented in any species within the GOM and should be further examined.

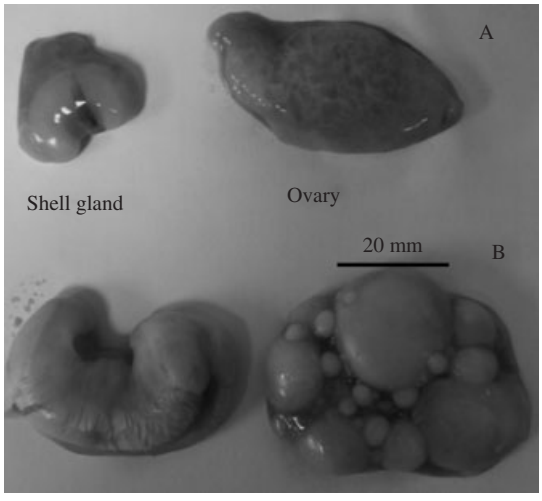

FIG. 8. Observed differences in the reproductive biology of two female *Leucoraja erinacea* approximately the same size and age. The reproductive tract of female A is immature (total length, $L_T = 506$ mm; age = 10 years), displaying an underdeveloped shell gland and ovary with no mature or maturing (non-vitellogenic) follicles present. The reproductive tract of female B is considered mature ($L_T = 503$ mm; age = 10 years), and displays a functional shell gland and ovary containing vitellogenic follicles.

The linear relationship ($r^2 = 0{\cdot}83$) between centrum diameter and L_T indicates centra grew proportionally in length for all size classes, and justifies the use of these structures for age estimation in *L. erinacea* (Cailliet & Goldman, 2004). Growth bands were easily distinguishable, and precision estimates along with the age-bias plot indicated that vertebral bands represented a precise and non-biased method of ageing this species (Campana, 2001). The corroboration of the OTC results in the current study to those observed by Natanson (1993) confirmed the annual periodicity of band formation within the vertebra of juvenile and adult *L. erinacea* from 3 to 5 and 11 to 12 years of age. Additionally, the incorporation of an OTC band on the marginal edge in June and July suggest this species follows a similar band deposition trend as both *L. ocellata* and *A. radiata* captured in the GOM (Sulikowski *et al*., 2003, 2005*a*). Additionally, VBGF parameters determined in the current study ($L_\infty = 595$ mm L_T, $K = 0{\cdot}16$ and $t_0 = -1{\cdot}23$) were similar to those reported by Frisk & Miller (2006) ($L_\infty = 593$ mm, $K = 0{\cdot}18$ and $t_0 = -1{\cdot}15$), further supporting the accuracy of the ageing technique used in this investigation.

In conclusion, the results of the current study indicate that the combined examination of morphological, histological and steroid hormone concentration provides an accurate approach for determining sexual maturity of *L. erinacea*. The 50% maturity ogives for L_T and age indicate both male and female *L. erinacea* mature at a late age and large size, which are characteristics that have made other skate species highly susceptible to overexploitation by commercial fisheries (Zeiner &Wolf, 1993; Frisk *et al*., 2001; Sulikowski *et al*., 2005*b*, 2006; Oddone & Amorim, 2008).

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