Environmental Biology of Fishes (2005) 72: 429–441 

# Age and size at sexual maturity for the winter skate, *Leucoraja ocellata*, in the western Gulf of Maine based on morphological, histological and steroid hormone analyses

James A. Sulikowski[a], Paul C.W. Tsang[b] & W. Huntting Howell[a]
[a]*Department of Zoology, University of New Hampshire, Spaulding Hall, 46 College Road, Durham, NH 03824, U.S.A. (e-mail: jsulikow@hotmail.com)*
[b]*Department of Animal and Nutritional Sciences, University of New Hampshire, Kendall Hall, 129 Main Street, Durham, NH 03824, U.S.A.*

Received 18 December 2003 Accepted 29 July 2004

*Key words:* elasmobranch, life history, spermatogenesis, estradiol, testosterone

**Synopsis**

We determined age and size at sexual maturity in male and female winter skates, *Leucoraja ocellata*, from the western Gulf of Maine. Age estimated from vertebral band counts resulted in an Index of Average Percent Error (IAPE) of 5.6%, suggesting that this method represents an accurate approach to the age assessment of *L. ocellata*. Size at sexual maturity was assessed by evaluating three endpoints: steroid hormone concentrations, and morphological and histological criteria. Our results suggest that 50% maturity in males occurs at a total length of 730 mm and at 11 years of age. For females, our results suggest that 50% maturity occurs at a total length of 760 mm and between 11 and 12 years of age. Collectively, our study suggests that analyzing a combination of reproductive parameters offers an accurate estimation of sexual maturity in the winter skate. Moreover, our results indicate that *L. ocellata* is a late-maturing and long-lived species, characteristics which make it highly susceptible to over-exploitation by commercial fisheries.

## Introduction

The winter skate, *Leucoraja ocellata*, family Rajidae (Collette & Klein-MacPhee 2002, Robins & Ray 1986) is endemic to the inshore waters of the western Atlantic, from Newfoundland Banks and southern Gulf of St. Lawrence in Canada to North Carolina in the U.S.A. (Collette & Klein-MacPhee 2002). Traditionally, skates caught by ground fishing operations were discarded (Martin & Zorzi 1993, Junquera & Paz 1998, Sosebee 2000). However, new and expanding commercial markets for skates have made retention of these fish more lucrative in recent years (Sosebee 2000, Dulvey et al. 2000, New England Fishery Management Council 2001). This is particularly true for the *L. ocellata* collected in New England waters, as adults are processed for the flesh from their pectoral fins (New England Fishery Management Council 2001).

Recently, we reported age and growth (Sulikowski et al. 2003) and elucidated the reproductive cycle (Sulikowski et al. 2004) of winter skates inhabiting the western Gulf of Maine. According to characteristics outlined by Winemiller & Rose (1992) and the comparative analyses of Frisk et al. (2001), the winter skate, like other elasmobranchs, displays characteristics of equilibrium strategists ('k selected') because they grow slowly, are relatively long-lived and have a low fecundity. These characteristics, coupled with fisheries that selectively remove large individuals, especially those over 100 cm total length, make the winter skate highly susceptible to over-fishing (Hoenig & Gruber 1990, Dulvey et al. 2000, Frisk et al. 2001, 2002). Assessment

studies in the northeast U.S. (Northeast Fisheries Science Center 1999, New England Fisheries Management Council 2002), suggest over-exploitation of this resource may in fact be occurring, as the biomass of this species appears to be declining to levels that are at, or below, threshold levels mandated by the Sustainable Fisheries Act (SFA).

With the recent intensification in commercial landings of *L. ocellata,* the timely collection of their life history information is imperative to effectively monitor this population of fish in the western Gulf of Maine (Ryland & Ajayi 1984, Cailliet & Tanaka 1990, Dulvey et al. 2000, Frisk et al. 2002). One particular life history parameter that is essential to the management process for this or any other species is the age and size at which the animals become sexually mature. Information regarding sexual maturity in elasmobranchs is largely based on morphology. Past studies have used clasper length in males and ovary mass in females (Walmsley-Hart et al. 1999, Mollet et al. 2000, Francis et al. 2001, Conrath & Musick 2002), while others have utilized the gonadosomatic index (GSI) to help assess reproductive activity (Parsons 1983, Ryland & Ajayi 1984, Snelson et al. 1988, Loefer & Sedberry 2003). Using GSI assumes that relative gonad size and reproductive readiness are positively correlated. However, recent studies on gonadal development at the tissue level do not support this assumption, especially in males (Parsons & Grier 1992, Maruska et al. 1996, Sulikowski et al. 2004). Furthermore, few studies have linked histological changes in gonadal tissues with circulating steroid hormones when these fish become reproductively capable. Thus, in the present study, we present the first comprehensive determination of age and size at sexual maturity for a cartilaginous fish by correlating steroid hormone concentrations to morphological and histological characteristics. This information was then coupled with interpreting annular counts on vertebral centra to elucidate age at sexual maturity.

## Materials and methods

### *Sampling techniques*

We captured 184 winter skates (88 females and 96 males), *L. ocellata*, by otter trawl in a 5 800 sq km area centered at 42°15′N and 70°25′W in the Gulf of Maine. These locations varied from 1.6 to 32 km off the coast of New Hampshire, U.S.A. Approximate depths at these locations ranged from 9 to 107 m. Immediately after capture, we collected blood (5–10 ml) from skates by cardiac puncture using chilled, heparinized syringes with a 21 gauge needle, followed by centrifugation at $1300 \times g$ for 5 min. Then the separated plasma was placed in a shipboard cooler (4°C) for 4–8 h before storage at −20°C in the laboratory. Skates were maintained alive on board the vessel until transport to the University of New Hampshire's (UNH) Coastal Marine Laboratory (CML). We euthanized individual skates (in a bath of 0.05 MS222 g $l^{-1}$) before removing reproductive and vertebral tissues. The measurement of total length (TL in mm), disc width (DW in mm) and clasper length was previously described (Sulikowski et al. 2004).

### Assessment of sexual maturity

#### *Justification for sizes used*

We conducted a preliminary investigation on the reproductive tracts of 33 male and 62 female winter skates. Morphological examinations of males ranging in size from 147 to 574 mm TL, and ages 0–6 years, revealed undeveloped testes, epididymides, vas deferens and seminal vesicles. Likewise, morphological examinations of females ranging in size from 145 to 582 mm TL, and ages 0–7 years, revealed undeveloped shell glands and uteri as well as ovaries with no follicles greater than 1 mm in diameter. Based on these observations, we concentrated our efforts on males that were aged to 7 years and older and females that were aged to 8 years and older.

#### *Histology of the testis*

At the UNH CML, we removed whole testes, which were blotted dry and weighed to the nearest gram. Afterwards, we cut a single 2–3 mm thick segment from the central portion of a single lobe in the medial area of each testis (Maruska et al. 1996), which was fixed in 10% buffered formalin before processing for standard

hematoxylin and eosin staining at the UNH Veterinary Diagnostic Laboratory. In order to determine sexual maturity, we examined prepared slides to assess spermatogenic development, based on criteria outlined by Maruska et al. (1996). Specifically, we measured the mean proportion of the testes that were occupied by mature spermatocysts along a straight line distance across one representative full lobe cross section of the testes. Histologically, we identified mature spermatocysts by the organization of spermatozoa into tightly shaped packets that were arranged spirally along the periphery of the spermatocysts (Figure 1).

*Gross morphology of the female reproductive tract and enumeration of ovarian follicles*

We removed the ovaries, shell glands and uterus before blotting dry and weighing them to the nearest gram. Follicle development was measured with calipers and all eggs that were greater than or equal to 1 mm in diameter were counted (Martin 1982, Tsang & Callard 1987, Snelson et al. 1988, Sulikowski et al. 2004). Changes in mean ovary masss, mean shell gland masss and the presence of vitellogenic follicles in different age groups were used in determining sexual maturity.

*Criteria for determining sexual maturity in skates*

The reproductive system of female and male winter skates followed the morphological classification of rajid batoids as described by Pratt (1988). We considered females whose reproductive tracts contained follicles 20 mm in diameter or larger, with a shell gland mass of 22 g or larger, and estradiol concentrations of 1 000 pg $ml^{-1}$ or greater to be sexually mature. We deemed males with testosterone values of 30 000 pg $ml^{-1}$ or greater, calcified claspers with a length of 180 mm or greater, and a proportion of mature spermatocysts in the testes of 26% or greater as sexually mature. We adopted these criteria, for both female and male winter skates, from previous studies which reported similar characteristics for mature elasmobranch species (Koob et al. 1986, Heupel et al. 1999, Sulikowski et al. 2004). Accordingly, male and female skates that did not meet all three criteria were not considered sexually mature.

## Analysis of steroid hormones

*Thin layer chromatography*

We used thin layer chromatography (TLC) with the solvent system for $E_2$ (3 parts ether:1 part

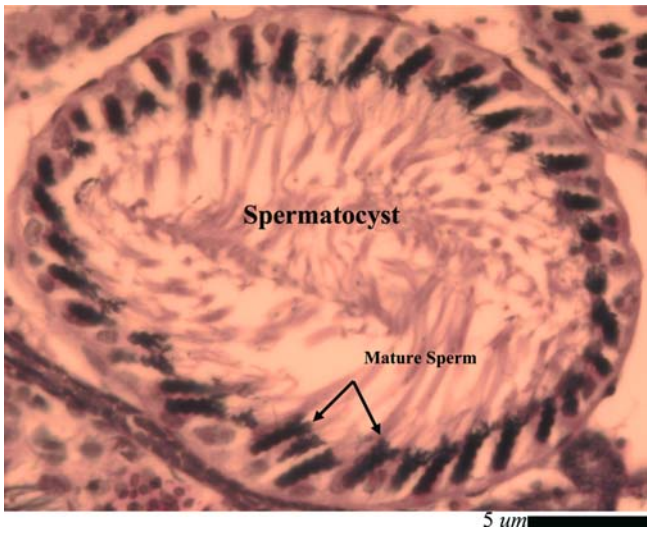


*Figure 1*. Histological section of an *L. ocellata* testis at 20 × magnification. Spermatocysts are characterized by mature sperm that are organized into cone-shaped packets arranged spirally along the outside of the spermatocysts.

hexane) and the solvent system for T (4 parts benzene:1 part acetone) to purify stock solutions of tritiated testosterone (T) and estradiol ($E_2$) (Amersham Biosciences, Piscataway, New Jersey). After extraction of steroids from the silica gel with methanol, the extracts were dried and reconstituted in absolute ethanol to yield stock concentrations of 5 μCi $ml^{-1}$ for T and 8 μCi $ml^{-1}$ for $E_2$ (Tsang & Callard 1987, Sulikowski et al. 2004). They were stored at −20°C.

*Solvent extraction of plasma steroid hormones*

We extracted each sample twice with 10 volumes of diethyl ether (anesthesia grade), which was evaporated under a stream of nitrogen before the dried extracts were reconstituted in phosphate-buffered saline with 0.1% gelatin (PBSG). All extracts were stored at −20°C until assay. To assess linearity and parallelism, the $R^2$ for standard curve/samples was calculated for T to be 0.98/0.97 and for $E_2$ to be 0.97/0.98. Furthermore, we added approximately 1 000 counts $min^{-1}$ (cpm) of an appropriate tritiated steroid into each plasma sample to account for procedural losses during the extraction. We determined the overall mean recoveries (i.e. how much tritiated steroid was not lost during extraction) for $E_2$ and T to be 76 and 74%, respectively, which were used to correct the steroid concentrations for each sample.

*Radioimmunoassay*

We determined plasma concentrations of testosterone and estradiol-17$\beta$ by radioimmunoassay modified from procedures of Tsang & Callard (1987). We obtained all nonradiolabeled steroids from Steraloids, Inc. (Wilton, New Hampshire). Duplicate aliquots of the reconstituted sample extracts received the appropriate tritiated steroid and anti-serum in a total volume of 0.4 ml. The final concentrations of the antibodies (from Gordon Niswender, Colorado State, Fort Collins, Colorado) were 1:64 000 for T (#250, anti-testosterone-11-BSA; Gay & Kerlan 1978) and 1:200 000 for $E_2$ (#244, anti-estradiol-6-BSA; Korenman et al. 1974). After an overnight incubation at 4°C, we separated free from bound steroids using a suspension of Norit A (0.2%) and Dextran T-70 (0.02%; Amersham Pharmacia Biotech, Piscataway, New Jersey), followed by centrifugation at 2500 × *g* for 10 min at 4°C. We then decanted the supernatants into minivials containing Ready Safe™ cocktail (Beckman Coulter, Somerset, New Jersey). Radioactivity was determined in a Beckman LS6000IC (Fullerton, California) liquid scintillation counter. The intra-assay coefficients of variance were 8.6% for T and 5.5% for $E_2$. The interassay coefficients of variance were 10.4% for T and 12.8% for $E_2$.

**Preparation and age analysis of vertebrae**

We previously validated the methods for the processing and age analyses of vertebrae (Sulikowski et al. 2003). Briefly, the vertebral centra were either cut or sanded (with a Dremel™ tool), mounted horizontally on glass microscope slides and ground with successively finer grits (#180, #400, #600) of wet–dry sandpaper to produce a thin (300 μm) 'hourglass' section. We conducted three nonconsecutive annulus counts for three vertebral sections from each specimen without prior knowledge of the skate's length or previous counts. If the variability between readings was more than 2 years, that particular specimen was eliminated from further analysis. We estimated count reproducibility by using the Index of Average Percent Error (IAPE) described by Beamish & Fournier (1981). The average of the mean counts for all three centra defined the age estimate for each specimen (Casey et al. 1985, Wintner & Cliff 1996). Finally, we investigated the annual periodicity of band pair formation using marginal increment analyses (MIA).

*Statistics*

We determined differences in morphological and histological parameters and hormone concentrations between age groups by using an analysis of variance (ANOVA) followed by a Tukey's *post-hoc* test. Statistical significance was accepted at $p < 0.05$. To estimate at what length and age winter skates reached sexual maturity, we fitted maturity ogives to length (at 20 mm intervals) and age (1 year intervals) by sex using probit analyses.

*Table 1.* Morphological measurements and reproductive parameters for the male winter skate, *Leucoraja ocellata*[*,a]

| Age | Sample size | Total length (mm) | Mass (kg) | Clasper length | % Mature spermatocytes | Average teste mass (g) | Testosterone (pg/ml) |
|---|---|---|---|---|---|---|---|
| 7 | 6 | 593 ± 15 | 1.6 ± 0.1 | 5.7 ± 0.6 | 0 ± 0 | 2.0 ± 0.3 | 10 ± 2[A] |
| 8 | 6 | 639 ± 2 | 1.9 ± 0.1 | 77.0 ± 0.3 | 0 ± 0 | 2.2 ± 0.3 | dnm |
| 9 | 5 | 660 ± 7 | 2.0 ± 0.2 | 90.0 ± 4 | 0 ± 0 | 2.6 ± 0.3 | 2602 ± 315[A] |
| 10 | 6 | 693 ± 4 | 2.3 ± 0.0 | 118 ± 3[*] | 0 ± 0 | 3.40 ± 0.3[*] | 10621 ± 2261 |
| 11 | 7 | 735 ± 6 | 2.6 ± 0.2 | 169 ± 11[*] | 13 ± 4[*] | 10.9 ± 1.9[*] | 19366 ± 4069 |
| 12 | 22 | 775 ± 3 | 3.8 ± 0.1 | 181 ± 7[*] | 29 ± 4[*] | 15.8 ± 1.3 | 32144 ± 4451 |
| 13 | 12 | 820 ± 2 | 4.1 ± 0.2 | 218 ± 3[*] | 42 ± 4[*] | 19.0 ± 1.0 | 46626 ± 7174 |
| 14 | 4 | 838 ± 2 | 4.9 ± 0.1 | 223 ± 5 | 44 ± 3 | 21.0 ± 1.2 | 49974 ± 1397 |
| 15 | 8 | 849 ± 1 | 4.5 ± 0.1 | 228 ± 0 | 44 ± 4 | 21.0 ± 1.0 | 50785 ± 6304 |
| 16 | 8 | 861 ± 3 | 4.9 ± 0.2 | 224 ± 3 | 44 ± 2 | 22.6 ± 1.0 | 47267 ± 6969 |
| 17 | 10 | 902 ± 5 | 5.7 ± 0.2 | 230 ± 6 | 41 ± 2 | 25.8 ± 1.2 | 46049 ± 6476 |
| 19 | 2 | 934 ± 7 | 5.8 ± 0.2 | 240 ± 0 | 43 ± 7 | 24.1 ± 3.3 | 47338 ± 562 |

Dnm = did not measure; values given as mean ± SEM.
[*]For each column, arrowed brackets and asterisks represent significant differences ($p < 0.05$; ANOVA followed by a Tukey's post hoc test) between skates in consecutive age groups.
[A]Value from two skates.

## Results

### *Reproductive parameters*

The TL and body mass of males (n = 96) ranged from 551 to 940 mm and 1.2 to 6.0 kg, respectively, while the TL and body mass of females (n = 88) ranged from 529 to 940 mm and 0.88 to 8.0 kg, respectively. To avoid any bias that might be associated with reproductive seasonality, we collected skates during the months of July and August (in 1999, 2000 and 2001) for this study. We recently reported (Sulikowski et al. 2004) that these months represent high reproductive activity in this species.

### *Males*

Overall, a steady and increasing trend in total length, clasper length, testis mass and circulating testosterone concentrations was observed until age 10 (Table 1). Notably, both the clasper length and testis mass increased ($p < 0.05$) between age 10 to age 11 (Table 1, Figure 2a), while the percent of mature spermatocysts increased ($p < 0.0\ 5$) between age 11 and 12 years (Table 1, Figure 2b). A second increase ($p < 0.05$) in clasper length and the percent of mature spermatocysts occurred in skates from age 12 to 13 years. Although testosterone concentrations increased dramatically from ages 7 to 13 years (Table 1, Figure 2b), no statistical differences were detected between any two consecutive age groups. However, testosterone concentration was correlated to clasper length ($r^2 = 0.62$), percent mature spermatocysts ($r^2 = 0.55$), and testis mass ($r^2 = 0.63$).

Maturity ogives predict that 50% maturity occurs at a total length of 730 mm and at age 11 years (Figure 3a and b). This is in overall agreement with our measured morphological parameters and testosterone concentrations, which suggest that 50% maturity occurs between total lengths of 735 and 775 mm and between the ages of 11 and 12 years (Table 1). In fact, the smallest sexually mature male within our sampling population measured 711 mm TL and was aged to 11 years.

### *Females*

We observed a steady and increasing trend in ovary mass, shell gland mass, average size of the largest follicle and $E_2$ concentration of skates until age 14, when values remained relatively constant in individuals between 15 and 18 years of age (Table 2, Figure 4a and b). Interestingly, the first and only significant ($p < 0.05$) change in ovary mass, shell gland mass, and average size of the largest follicle between consecutive age groups occurred among the years 8 and 9 age-classes. Similar to testosterone concentrations, no

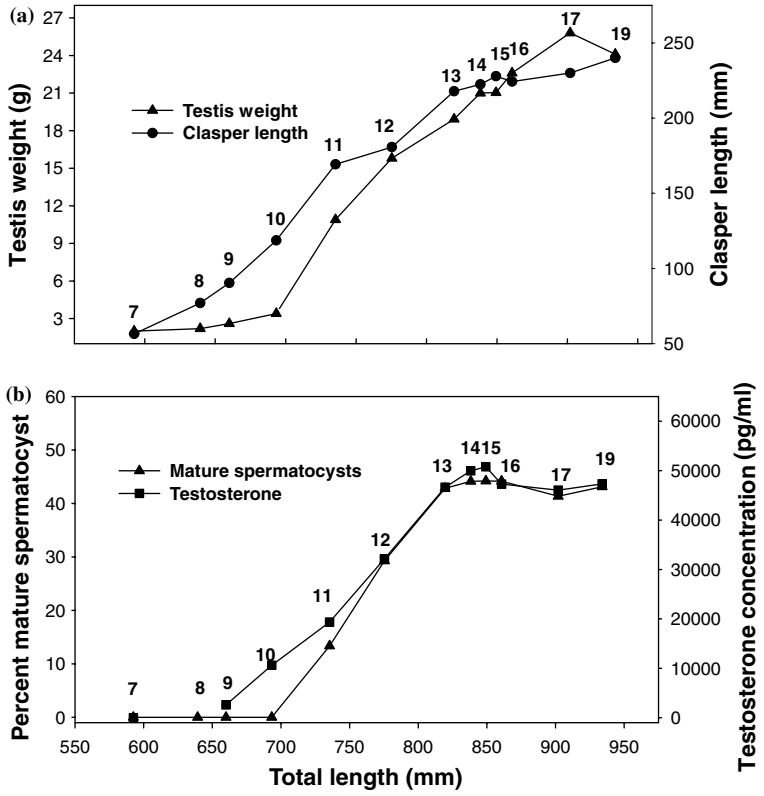


*Figure 2.* (a) The total length to clasper length and total length to testis mass as male winter skates, *L. ocellata,* progress through sexual maturity; (b) the proportion of mature spermatocysts and testosterone concentration as male winter skates, *L. ocellata,* progress through sexual maturity. Average age is given above each representative size class. Values are expressed as mean ± SEM. Refer to Table 1 for details.

statistical differences were detected for estradiol between any two consecutive age-groups. However, $E_2$ concentration was correlated to ovary mass ($r^2 = 0.68$), shell gland mass ($r^2 = 0.65$), and average size of the largest follicle ($r^2 = 0.64$).

We observed that follicles between 1 and 3 mm in diameter were predominant in ovaries of skates 8–14 years of age (Figure 5a–g). Furthermore, we noted three distinct patterns of follicular development in *L. ocellata.* In 8–10 year old skates, all ovarian follicles were less than 12 mm in diameter. Notably, starting at age 11, the pattern of follicle development changed dramatically, with the first appearance of follicles ranging in diameter from 13 to 25 mm. Thereafter, between 12 and 14 years of age, follicles greater than 25 mm in diameter appeared.

Maturity ogives for females predict that 50% maturity occurs at a total length of 760 mm and between 11 and 12 years of age (Figure 3a and b). This corroborates our reproductive tract size, circulating $E_2$ concentrations and follicle dynamics data, suggesting that 50% maturity occurs between 769 and 776 mm total length and between the ages of 12 and 13 years (Table 2, Figure 4a and b). Based on the increases in the measured reproductive parameters, particularly the pattern of follicle growth and $E_2$ levels (Figure 4b), the smallest mature female measured 735 mm TL and was aged to 11 years.

### *Vertebral analyses*

Results of the vertebral analyses were in agreement with those of Sulikowski et al. (2003). Briefly, all 184 processed vertebrae were readable. They (males = 96; females = 88) had annular count estimates that agreed within 2 years, resulting in

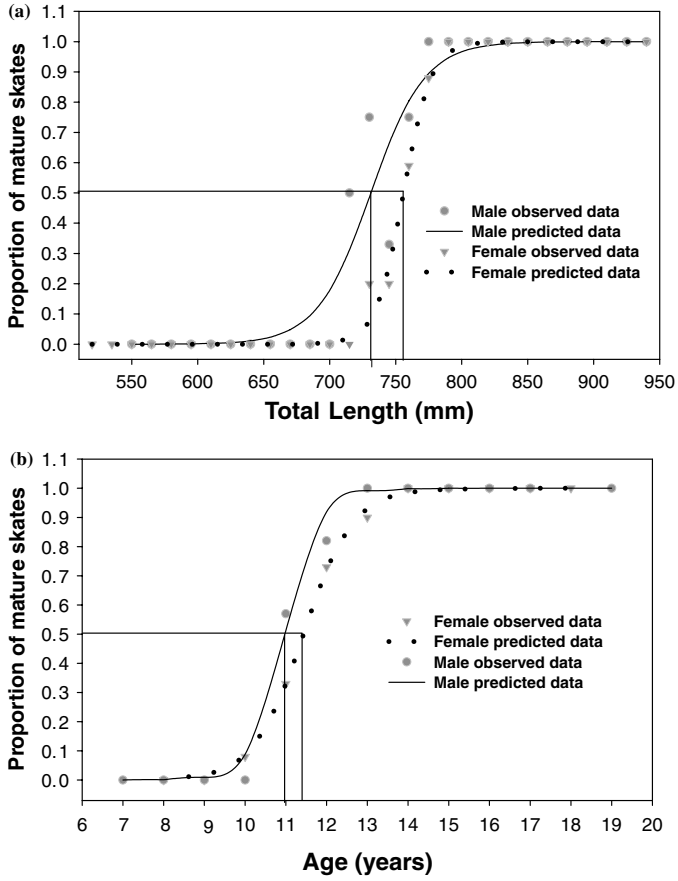


*Figure 3*. Maturity ogives for (a) total length of male and female *L. ocellata* given in 20 mm intervals, and (b) age of male and female *L. ocellata*.

an IAPE of 5.6%. Previous work by the authors suggests that the relationship between TL and centrum diameter is linear and that growth bands are formed annually within the vertebral centra of the winter skate.

## Discussion

Life history studies of elasmobranchs largely evaluate either morphological or biochemical changes. For example, some profiled steroid hormone concentrations to monitor reproductive cyclicity (Tsang & Callard, 1987, Rasmussen & Murru 1992, Manire et al. 1995, Snelson et al. 1997, Tricas et al. 2000, Sulikowski et al. 2004) while others described temporal patterns of gonadal activity in adult elasmobranchs (Parsons & Grier 1992, Maruska et al. 1996, Walmsley-Hart et al. 1999, Conrath & Musick 2002). In addition, studies that have evaluated age and size at sexual maturity are largely descriptive, addressing changes in GSI or morphology, such as clasper length in males and ovary mass in females (Walmsley-Hart et al. 1999, Mollet et al. 2000, Francis et al. 2001, Conrath & Musick 2002). To our knowledge, in the present study, we are the first to use a comprehensive approach by combining morphological and biochemical parameters to assess development in an elasmobranch as it matures. Furthermore, we coupled these parameters with age analyses to determine the age and size at which the winter skate reaches sexual maturity.

A variety of approaches have been used to estimate age and size at sexual maturity for winter

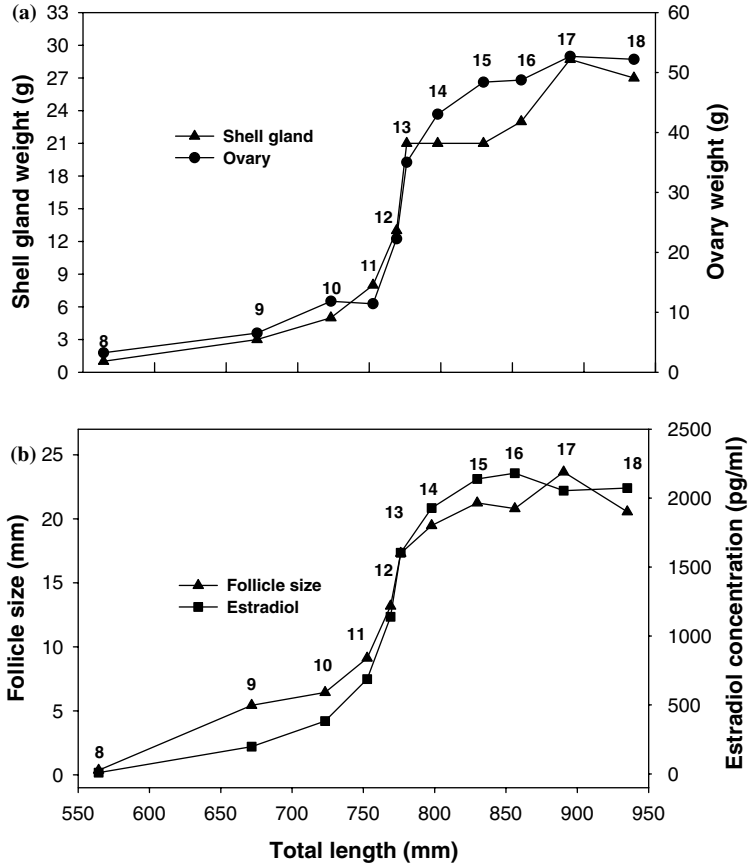


*Figure 4*. (a) Ovary mass and shell gland mass as female winter skates, *L. ocellata,* progress through sexual maturity; (b) follicle diameter and estradiol concentrations as female winter skates, *L. ocellata,* progress through sexual maturity. Average age is given above each representative size class. Values are expressed as mean ± SEM. Refer to Table 2 for details.

skates. Frisk et al. (2002) used empirical life history approaches while Sosebee (2002) employed methods developed for assessment of crustacean maturity (Somerton 1980) to arrive at estimates based on morphological measurements of clasper length in males and cloaca length in females collected during Northeast Fisheries Science Center research bottom trawl and scallop dredge surveys. When compared to our present study, their reported findings of age and size at 50% maturity differed, with males and females being younger and smaller than predicted by maturity ogives and our comprehensive approach using morphological and biochemical parameters. Interestingly, Simon and Frank's (1998) estimate of 750 mm for females using ovarian follicle characteristics is very close to the present studies' assessment. Whether these variations in age and size at sexual maturity are strictly due to methodological differences or to a geographic variable producing the disparity in estimated parameters between the studies need further attention. Collectively however, our findings from the present study suggest that analyzing a combination of reproductive parameters may perhaps offer a more accurate estimation of age and size at sexual maturity when compared to those calculated solely from empirical (Frisk et al. 2002) or morphological measurements (Sosebee 2000) other than follicle size (Simon & Frank 1998).

Several studies suggest that an abrupt increase in clasper length marks the onset of sexual maturity in elasmobranchs (Martin 1982, Ryland & Ajayi 1984, Zeiner & Wolf 1993, Mollet et al.

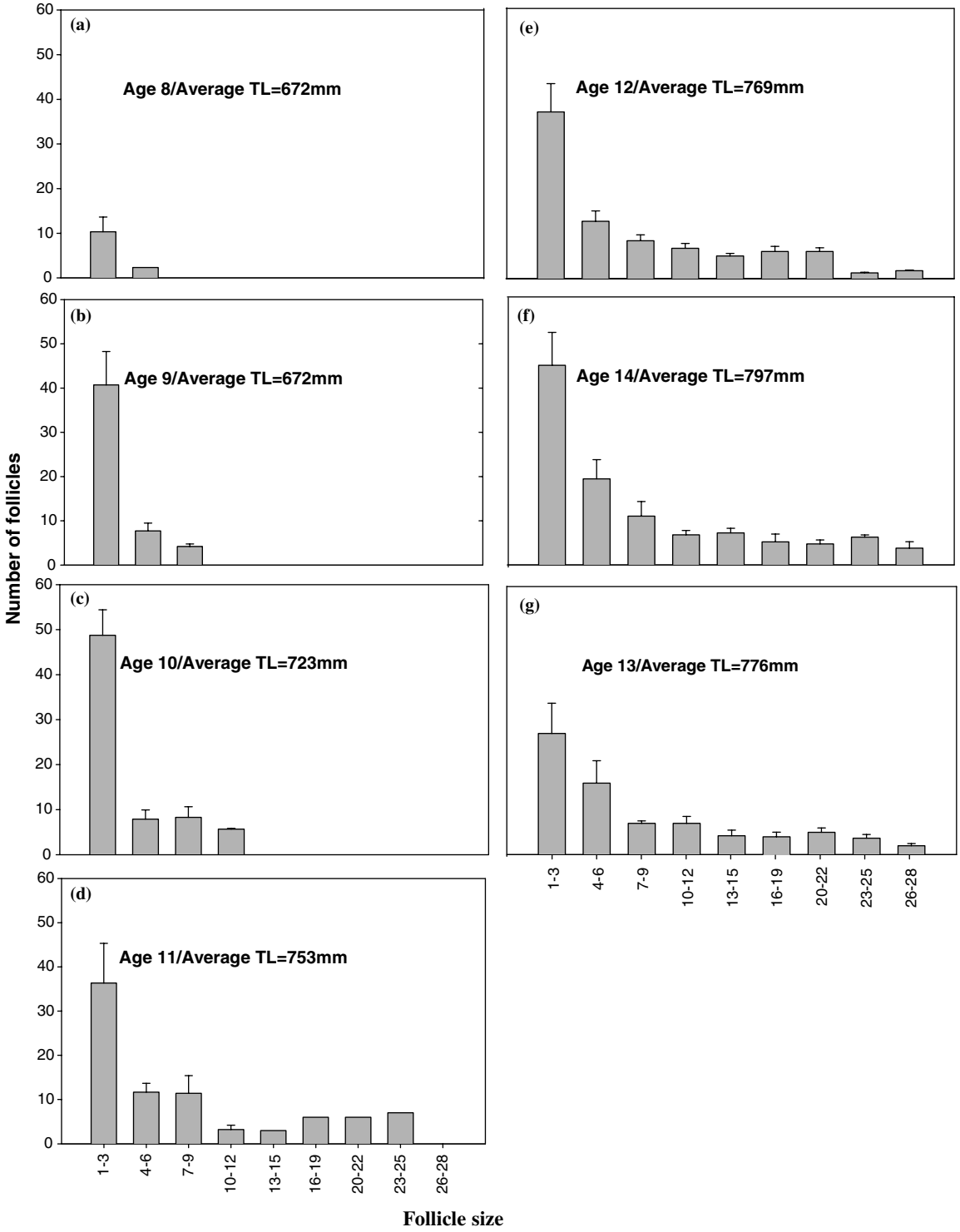


*Figure 5* (a–g). Follicle dynamics in winter skates. Patterns of follicular development (average follicle number versus average follicle size, in mm), as *L. ocellata* matures from age 8 (sexually immature; a) to age 14 (sexually mature; g). Average follicle number is expressed as mean ± SEM.

*Table 2.* Morphological measurements and reproductive parameters for the female winter skate, *Leucoraja ocellata*.

| Age | N | Total length | Mass (kg) | Ovary mass (g) | Shell gland mass (g) | Largest follicle size (mm) | $E_2$ (pg/ml) |
|---|---|---|---|---|---|---|---|
| 8 | 3 | 564 ± 18 | 1.3 ± 0.3 | 3 ± 1* | 1 ± 1* | 1 ≤ * | 9 ± 8 |
| 9 | 8 | 672 ± 4 | 2.3 ± 0.1 | 7 ± 1* | 3 ± 1* | 5 ± 1* | 198 ± 84 |
| 10 | 12 | 723 ± 2 | 3.1 ± 0.2 | 12 ± 5 | 5 ± 2 | 6 ± 2 | 383 ± 69 |
| 11 | 7 | 753 ± 4 | 3.8 ± 0.2 | 12 ± 3 | 8 ± 3 | 9 ± 1 | 687 ± 280 |
| 12 | 11 | 769 ± 1.0 | 4.1 ± 0.2 | 22 ± 4 | 13 ± 4 | 13 ± 3 | 1139 ± 287 |
| 13 | 10 | 776 ± 1 | 4.1 ± 0.3 | 35 ± 7 | 16 ± 3 | 17 ± 2 | 1604 ± 535 |
| 14 | 11 | 797 ± 2 | 4.7 ± 0.3 | 43 ± 9 | 21 ± 4 | 20 ± 2 | 1928 ± 435 |
| 15 | 11 | 830 ± 4 | 5.2 ± 0.3 | 48 ± 8 | 21 ± 2 | 20 ± 2 | 2139 ± 198 |
| 16 | 6 | 856 ± 3 | 5.9 ± 0.3 | 49 ± 4 | 23 ± 3 | 21 ± 1 | 2181 ± 352 |
| 17 | 7 | 890 ± 7 | 6.0 ± 0.3 | 53 ± 6 | 29 ± 1 | 24 ± 2 | 2054 ± 472 |
| 18 | 2 | 935 ± 04 | 7.7 ± 0.3 | 52 ± 5 | 27 ± 7 | 21 ± 1 | 2073 ± 194 |

Values given as mean ± SEM.
*For each column, arrowed brackets and asterisks represent significant differences ($p < 0.05$; ANOVA followed by a Tukey's *post hoc* test) between skates in consecutive age groups.

2000, Francis et al. 2001, Conrath & Musick 2002, Loefer & Sedberry 2003), corroborating our results in *L. ocellata* males, whose clasper length displayed a 35% ($p < 0.05$) increase as the fish matured from age 10 to 11 years. Furthermore, during this period of time, we also observed that testis mass, mature spermatocysts, and testosterone concentration concomitantly increased by 221, 120 and 82%, respectively, although the increase in testis mass was the only measured parameter of the three that was statistically significant ($p < 0.05$). Yoccoz (1991) discussed the relative merits of judging biological significance based on statistical tests, and suggested that it is not always associated with statistical significance. This may be the case in our study of sexual maturity in the male winter skate, since the abrupt and strongly correlated increases ($r^2 > 0.55$) in morphological and biochemical reproductive parameters appear to initiate a biologically significant shift towards sexual maturity as this species near age 10.

Determining the status of steroid hormones is another strategy to assess sexual maturity. Previously, Rasmussen and Murru (1992) determined that T concentrations in an immature sandbar, *Carcharhias plumbeus*, and an immature bull shark, *C. leucas*, were considerably lower than their adult counterparts that have reached sexual maturity. Similarly, in the winter skate, testosterone concentrations steadily increased until age 13, before leveling off and remaining constant throughout the rest of the sampled adult age classes. Testosterone is produced by the Sertoli cells of *Squalus acanthias* spermatocysts (Cuevas & Callard 1989, Du Bois et al. 1989) and the concentration of this hormone is associated with spermatocyst development in the winter skate (our present study), the epaulette shark (Heupel et al. 1999) and the Atlantic stingray (Tricas et al. 2000). However, besides T, it is also important to consider the possible action of other androgens not measured in this study, such as dihydrotestosterone (DHT) and 11-ketotestosterone (Borg 1994). Manire et al. (1999) found 11-ketotestosterone (the most active androgen in teleosts) in the serum of bonnet head sharks, while the pattern of DHT production in male *Dasyatis sabina* has been shown to closely follow that of T (Snelson et al. 1997).

For female elasmobranchs, several studies suggest that an abrupt increase in ovary mass and follicle size mark the onset of sexual maturity (Martin 1982, Zeiner & Wolf 1993, Mollet et al. 2000, Francis et al. 2001, Conrath & Musick 2002, Loefer & Sedberry 2003). When winter skates mature from 11 to 12 years of age, increases in ovary mass (95%) and follicle size (44%) were simultaneously accompanied by increases in shell gland mass (60%) and $E_2$ concentrations (66%). Although these changes were not statistically significant ($p > 0.05$), they were nonetheless strongly correlated ($r^2 > 0.65$), perhaps signaling a biologically significant (Yoccoz 1991) shift towards sexual maturity in female winter skates beginning at age 11 years.

Similar to males, steroid hormones are a good indicator of reproductive readiness in female elasmobranches. Rasmussen & Murru (1992) found $E_2$ concentrations in two immature grey nurse sharks, *C. taurus*, to be considerably lower ($p < 0.05$) than the sampled sexually mature adults of this species. Moreover, in the oviparous little skate, *L. erinacea*, (Koob et al. 1986), winter skate (Sulikowski et al. 2004), spotted catshark, *Scyliorhinus canicula* (Sumpter & Dodd 1979), aplacental viviparous dogfish, *Squalus acanthias* (Tsang & Callard 1987) and Atlantic stingray, *Dasyatis sabina*, (Snelson et al. 1997, Tricas et al. 2000), elevated concentrations of $E_2$ are associated with egg development during the follicular phase. Likewise, as winter skates reach sexual maturity, $E_2$ concentrations were correlated to follicle size ($r^2 = 0.64$) and shell gland mass ($r^2 = 0.65$), similar to observations made by Koob et al. (1986) and Tsang & Callard (1987). Thus, when the data for reproductive parameters are combined with patterns of follicular dynamics, especially the first emergence of follicles greater than 12 mm at age 11, our results collectively suggest that these observed changes at sexual maturity may be biologically relevant.

In summary, our results from the current study indicate that the coordinate examination of morphological and histological parameters, along with steroid hormone concentrations, is an accurate approach to determining age and size at sexual maturity for the winter skate. Furthermore, when these findings are combined with life history parameters from Sulikowski et al. (2003), our data collectively indicate that the winter skate is a late maturing and long-lived species, characteristics of elasmobranchs which make their populations highly susceptible to over-exploitation by commercial fisheries (Brander 1981, Kusher et al. 1992, Zeiner & Wolf 1993, Frisk et al. 2001, 2002).

## Acknowledgements

We thank Captain Joe Jurek of the F/V *Mystique Lady* for collection of the skates. We further extend our gratitude to Mike Morin and Holly Briggs for their help in animal dissections, Charles Walker for use of his equipment and to Noel Carlson for maintenance of the fish at the U.N.H. Coastal Marine Laboratory. This project was supported by a University of New Hampshire Hubbard Endowment Fund and the U.N.H. Center for Marine Biology.

## References

Beamish, R.J. & D.A. Fournier. 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38: 982–983.

Brander, K. 1981. Disappearance of common skate *Raia batis* from Irish Sea. Nature 290: 48–49.

Borg, B. 1994. Androgens in teleost fishes. Comp. Biochem. Physiol. 109C: 219–245.

Cailliet, G.M. & S. Tanaka. 1990. Recommendations for research needed to better understand the age and growth of elasmobranchs. pp. 505–508. *In:* H.L. Jr. Pratt, S.H. Gruber & T. Tanuichi (ed.), Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics and the Status of the Fisheries, NOAA Technical Report 90, NMFS, Washington, D.C.

Callard, G. & M.E. Cuevas. 1992. *In vitro* steroid secretion by staged spermatocysts (Sertoli/germ cell units) of dogfish (*Squalus acanthias* ) testis. Gen. Comp. Endocrinol. 88: 151–165.

Casey, J.G., H.L. Pratt & C.E. Stillwell. 1985. Age and growth of the sandbar shark (*Carcharhinus plumbeus* ) from the western North Atlantic. Can. J. Fish. Aqua. Sci. 42: 963–975.

Casey, J.M. & R.A. Myers. 1998. Near extinction of a large widely distributed fish. Science 28: 690–692.

Collette, B. & G. Klein-MacPhee (ed.). 2002. Bigelow and Schroeder's fishes of the Gulf of Maine, 3rd edition, Smithsonian Institution Press, 000 pp.

Conrath, C.L. & J.A. Musick. 2002. Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic Ocean. Environ. Biol. Fishes 64: 367–377.

Cuevas, M.E. & G.V. Callard. 1989. *In vitro* secretion by Sertoli/germ cell units (spermatocysts) derived from dogfish (*Squalus acanthias*) testis. Bull. Mount Desert Island Biol. Lab. 28: 30–31.

Du Bois W., P. Mak & G.V. Callard. 1989. Sertoli cell function during spermatogenesis: The shark testis model. Fish Physiol. Biochem. 7: 221–227

Dulvey, N.K., J.D. Metcalfe, J. Glanville, M.G. Pawson & J.D. Reynolds. 2000. Fishery stability, local extinctions, and shifts in community structure in skates. Conserv. Biol. 14: 283–293.

Francis, M., C.O. Maolagain & D. Stevens. 2001. Age, growth, and sexual maturity of two New Zealand endemic skates, *Dipturus nasutus* and *D. innominatus*. N. Z. J. Mar. Freshwater Res. 35: 831–842.

Frisk, M.G., T.J. Miller & M.J. Fogarty. 2001. Estimation and analysis of biological parameters in elasmobranch fishes: a comparative life history study. Can. J. Fish. Aqua. Sci. 58: 969–981.

Frisk, M.G., T.J. Miller & M.J. Fogarty. 2002. The population dynamics of little skate *Leucoraja erinacea*, winter skate

*Leucoraja ocellata*, and barndoor skate *Dipturus laevis*: Predicting over-exploitation limits using matrix analyses. ICES J. Mar. Sci. 59: 576–586.

Gay, V.L. & J.T. Kerlan. 1978. Serum LH and FSH following passive immunization against circulating testosterone in the intact male rat and in orchidectomized rats bearing subcutaneous silastic implants of testosterone. Arch. Androl. 1: 257–266.

Heupel, M.R., J.M. Whittier & M.B. Bennet. 1999. Plasma steroid hormone profiles and reproductive biology of the epaulette shark, *Hemiscyllium ocellatum*. J. Exp. Zool. 284: 586–594.

Hoenig, J.M. & S.H. Gruber. 1990. Life history patterns in the elasmobranchs: Implications for fisheries management. pp. 1–16. *In*: H.L. Jr. Pratt, S.H. Gruber & T. Tanuichi (ed.) Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics and the Status of the Fisheries, NOAA Technical Report 90, NMFS, Washington, D.C.

Junquera, S. & X. Paz. 1998. Non-traditional resources: Skate fishery and survey results in Division 3 NO. Northwest Atlantic Fisheries Organ. Dartmouth, NS [Canada]. Scientific Council Research Document NAFO, no. 98/26, 6 pp.

Koob, T.J., P. Tsang & I.P. Callard. 1986. Plasma estradiol, testosterone and progesterone levels during the ovulatory cycle of the little skate, *Raja erinacea*. Biol. Reprod. 35: 267–275.

Korenman, S.G., R.H. Stevens, L.A. Carpenter, M. Robb, G.D. Niswender & B.M. Sherman. 1974. Estradiol radioimmunoassay without chromatography: Procedure validation and normal values. J. Clin. Endocrinol. Metabol. 38: 718–720.

Kusher, D.I., S.E. Smith & G.M. Cailliet. 1992. Validated age and growth of the leopard shark, *Triakis semifasciata*, with comments on reproduction. Environ. Biol. Fish. 35: 187–203.

Loefer, J.K. & G.R. Sedberry. 2003. Life history of the Atlantic sharpnose shark
(*Rhizoprionodon terraenovae*) (Richardson, 1836) off the southeastern United States. Fish. Bull. 101: 75–88.

Manire, C.A., L.E.L. Rasmussen, D.L. Hess & H.E. Hueter. 1995. Serum steroid hormones and the reproductive cycle of the female bonnet head shark, *Sphyrna tiburo*. Gen. Comp. Endocrinol. 97: 366–376.

Manire C.A., L.E.L. Rasmussen & T.S. Gross. 1999. Serum steroid hormones including 11-ketotestosterone, 11-ketoandrostenedione, and dihydroprogesterone in juvenile and adult bonnethead sharks, *Sphyrna tiburo*. J. Exp. Zool. 284: 595.

Martin, L.K. 1982. Growth and reproduction of the bat ray, *Myliobatis californica*, Gill, in California. Masters Thesis, San Jose State University and Moss Landing Marine Laboratory, California. 82 pp.

Martin, L. & G.D. Zorzi. 1993. Status and review of the California skate fishery. Conserv. Biol. Elasmobr., NOAA Technical Report, NMFS. 115: 39–52.

Maruska, K.P., E.G. Cowie & T.C, Tricas. 1996. Periodic gonadal activity and protracted mating in elasmobranch fishes. J. Exp. Zool. 276: 219–232.

Mollet, H.F., G. Cliff, H.L. Pratt & J.D. Stevens. 2000. Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. Fish. Bull. 98: 299–318.

New England Fishery Management Council. 2001. 2000 Stock Assessment and Fishery Evaluation (SAFE) Report for the Northeast Skate Complex. Newburyport, MA. 179 pp.

New England Fishery Management Council. 2002. Skate Fisheries Newburyport, MA. 000 pp.

Northeast Fisheries Science Center. 1999. 30th Northeast Regional Stock Assessment Workshop. Woods Hole, MA. 00 pp.

Parsons, G.R. 1983. The reproductive biology of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Richardson). Fish. Bull. 81: 61–74.

Parsons, G.R. & H.J. Grier. 1992. Seasonal changes in the shark testicular structure and spermatogenesis. J. Exp. Zool. 261: 173–184.

Pratt, H.L. 1988. Elasmobranch gonad structure: A descriptive survey. Copeia, 719–729.

Rasmussen, L.E.L. & F.L. Murru. 1992. Long term studies of serum concentrations of reproductively related steroid hormones in individual captive carcharhinids. Aus. J. Mar. Freshwater Res. 43: 273–281.

Robins, C.R. & G.C. Ray. 1986. A Field Guide to Atlantic Coast Fishes of North America. Houghton Mifflin Company, Boston, U.S.A. 354 p.

Ryland, J.S. & T.O. Ajayi. 1984. Growth and population dynamics of three *Raja* species (Batoidei) in Carmarthen Bay, British Isles. J. Int. Council Explor. Sea 41: 111–120.

Simon, J.E. & K.T. Frank. 1998. Assessment of the winter skate fishery in Division 4VsW. DFO Atlantic Fisheries Research Document 98/145.

Snelson, F.F. Jr., S.E. Williams-Hopper & T.H. Schmid. 1988. Reproduction and ecology of the Atlantic stingray, *Dasyatis sabina*, in Florida coastal lagoons. Copeia 3: 729–739.

Snelson, F.F. Jr., L.E.L. Rasmussen, M.R. Johnson & D.L. Hess. 1997. Serum concentrations of steroid hormones during reproduction in the Atlantic stingray, *Dasyatis sabina*. Gen. Comp. Endocrinol. 108: 67–79.

Somerton, D.A. 1980. A computer technique for estimating the size of sexual maturity in crabs. Can. J. Fish. Aquat. Sci. 37: 1488–1494.

Sosebee, K. 2000. Skates. Status of fishery resources off the northeastern United States, NOAA Technical Memorandum. NMFS-NE 115: 114–115.

Sosebee, K. 2002. Maturity of skates in northeast United States waters. Northwest Atlantic Fisheries Organ., Dartmouth, NS. Scientific Council Research Document NAFO. no. 02/134. 17 pp.

Sulikowski, J.A., M.D. Morin, S.H. Suk & W.H. Howell. 2003. Age and growth of the winter skate, *Leucoraja ocellata*, in the Gulf of Maine. Fish. Bull. 101: 405–413.

Sulikowski, J.A., P.C.W. Tsang & W.H. Howell. 2004. Annual changes in steroid hormone concentrations and gonad development in the winter skate, *Leucoraja ocellata*. Mar. Biol. 144: 845–853.

Sumpter, J.P. & J.M. Dodd. 1979. The annual reproductive cycle of the female lessor spotted dogfish, *Scyliorhinus canicula*, and its endocrine control. J. Fish Biol. 15: 687–695.

Tricas, T.C., K.P. Maruska & L.E.L. Rasmussen. 2000. Annual cycles of steroid hormone production, gonad development,

and reproductive behavior in the Atlantic stingray. Gene. Comp. Endocrinol. 118: 209–225.

Tsang, P. & I.P. Callard. 1987. Morphological and endocrine correlates of the reproductive cycle of the aplacental viviparous dogfish, *Squalus acanthias*. Gen. Comp. Endocrinol. 66: 182–189.

Walmsley-Hart, S.A., W.H.H Sauer & C.D. Buxton. 1999. The biology of the skates *Raja wallacei* and *R. pullopunctata* (Batoidea: Rajidae) on the Agulhas Bank, South Africa. South African J. Mar. Sci. 21: 165–179.

Winemiller, K.O. & K.A. Rose. 1992. Patterns of life history diversification in North American fishes: implication for population regulation. Can. J. Fish. Aquat. Sci. 49: 2196–2218.

Wintner, S.P. & G. Cliff. 1996. Age and growth determination of the blacktip shark, *Carcharhinus limbatus*, from the east coast of South Africa. Fish. Bull. 94: 135–144.

Yoccoz, N.G. 1991. Use, overuse, and misuse of significance tests in evolutionary biology and ecology. Bull. Ecol. Soc. Am. 71: 106–111.

Zeiner, S.J. & P.G. Wolf. 1993. Growth characteristics and estimates of age at maturity of two species of skates (*Raja binoculata* and *Raja rhina*) from Monterey Bay, California. Conserv. Biol. Elasmobr., NOAA Technical Report NMFS. 115 pp.