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Age, growth, and reproductive biology, of the winter skate, Leucoraja ocellata, in the western Gulf of Maine

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AGE, GROWTH, AND REPRODUCTIVE BIOLOGY, OF THE WINTER SKATE,

Leucoraja ocellata, IN THE WESTERN GULF OF MAINE

BY

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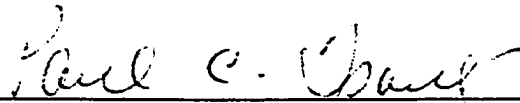All rights reserved.

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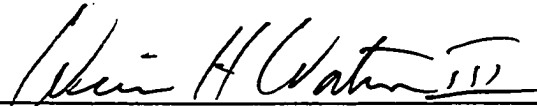
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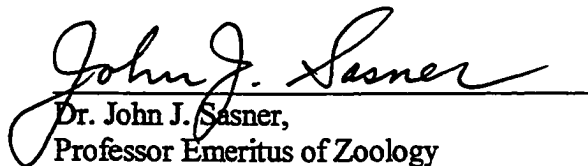
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DEDICATION

It is with my greatest pleasure that I dedicate this dissertation to Sandy Sulikowski, my wife and best friend. I will always be grateful for her love and support while completing this study. But most of all, for all the sacrifices she has made in order for me to fulfill this dream.

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Leucoraja ocellata, IN THE WESTERN GULF OF MAINE

by

James Antoni Sulikowski

University of New Hampshire, May, 2003

This study describes the age, growth and reproductive biology of the winter skate, *Leucoraja ocellata,* in the Western Gulf of Maine. Age was estimated by enumerating annular bands within the vertebral centra. Precision of the age estimates was evaluated using the Index of Average Percent Error and the annual nature of growth band formation was documented using marginal increment analyses. Growth was assessed with the use of the von Bertalanffy growth equation. Age and size at maturity was estimated by measuring morphological and histological changes in the reproductive tract and circulating steroid hormone concentrations. Maturity ogives for males predict that 50% maturity occurs at a total length of 730 mm and at 11 years. For females, maturity ogives predict that 50% maturity occurs at a total length of 760 mm and between 11 and 12 years of age. To elucidate the reproductive cycle of the winter skate, plasma concentrations of the sex steroids testosterone (T), 17ß-estradiol (E_2), and progesterone (P_4) were determined by radioimmunoassay and compared to morphological and histological changes occurring in the reproductive tract over a complete reproductive cycle for mature individuals. Overall, when the results of my dissertation are combined,

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they indicate that the winter skate is a late maturing, slow growing, long lived species, with an apparent succinct reproductive cycle. Like many other elasmobranchs, these characteristics make *L. ocellata's* populations highly susceptible to exploitation by commercial fisheries.

INTRODUCTION

The Northeast skate complex consists of seven species indigenous to the Gulf of Maine (Sosebee, 1998; New England Fishery Management Council, 2002; Collette and Klein-MacPhee, 2002). Remarkably, little biological data exists for six of these species (New England Fishery Management Council, 2001), including important parameters such as age and growth, age at maturity, reproductive cycles and annual fecundity. Due to many variables, such as their large size, high mobility, and minor commercial value makes such studies difficult and in some respects impractical (Cailliet *et al.* 1983; Cailliet *et al.*, 1986). Understanding the life history is necessary for the advancement of general elasmobranch biology, but more importantly, for the conservation of these fish. Elasmobranchs display characteristics of equilibrium strategists (Frisk *et al.*, 2001; 1992) and as such grow slowly, are relatively long-lived, and have low fecundities. Furthermore, after oviposition, skate egg gestation rates can range from one to six years depending on skate size and ambient water temperature (Johnson, 1979; Berestovskii, 1994). These characteristics, and the practice of selective removal of large individuals, make skates especially susceptible to exploitation by commercial fisheries (Hoenig and Gruber, 1990; Dulvey *et al.*, 2000). For example, larger species appear to mature late (at least 11 years) and it is highly probably that immature individuals will be caught before they are able to reproduce (Casey and Myers 1998). Limited life history knowledge and the failure to implement a proper fishery management plan has resulted in the near

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extinction of the common skate, *R. batis*, in the Irish Sea (Brander 1981) and the barndoor skate, *Raja laevis*, in the northwest Atlantic (Casey and Myers 1998).

The winter skate, *Leucoraja ocellata*, is a large species (total length over 100 cm) of skate of the family Rajidae (Bigelow and Schroeder, 1953; Robins and Ray, 1986). It 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. (Bigelow and Schroeder, 1953). Despite this wide range, information on the biology and ecology is very limited for this species (Simon and Frank, 1996; Casey and Myers, 1998, Frisk, 2000). These skates are taken during commercial ground fishing operations and in the past were usually discarded (Junquera 1998; Sosebee 1998). However, the rapidly expanding markets for human consumption of skate wing and for use as lobster bait has made the winter skate commercially more viable in recent years (Sosebee 1998). Despite an increasing commercial importance, there are no regulations governing the harvesting of *L. ocellata* or any other of skate species in the U.S. (Sosebee 1998). Recent assessment studies in the northeast U.S. (Northeast Fisheries Science Center 2001), suggest the biomass of the winter skate may be below threshold levels mandated by the Sustainable Fisheries Act (SFA). Moreover, there is little data on age or growth to determine fishing mortality rates, or propose SFA fishing mortality reference points (Northeast Fisheries Science Center, 2001). Thus, the level at which sustainable fisheries can be maintained for these species is unknown.

According to the New England Fishery Management Council, adequate and comprehensive scientific information about this species is lacking, and is hindering the

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effective development of a Skate Fishery Management Plan. Moreover, the current biomass threshold levels necessitate the development of management measures to end overfishing and rebuild these stocks in accordance with the Magnuson-Stevens Fishery Conservation and Management Act. However, the development and implementation of a successful fisheries management plan requires in-depth analyses of appropriate biological information. The goal of this research is to provide the first comprehensive study of the winter skate by investigating three important biological parameters fundamental to this species conservation. This research will asses the age and growth, size at sexual maturity, and define the reproductive cycle for *L. ocellata* in the western Gulf of Maine.

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CHAPTER 1

AGE AND GROWTH ESTIMATES OF THE WINTER SKATE, *leucoraja ocellata*, IN THE WESTERN GULF OF MAINE.

Introduction

Little is known about the biology of many elasmobranchs, including important parameters such as validated age, growth, age at maturity, reproductive cycles and annual fecundity (Frisk *et al.*, 2001). Difficulty in obtaining samples, their large size, high mobility, and minor commercial value are just a few of the problems that make such studies complicated and in some respects impractical (Cailliet *et al.*, 1983; Cailliet *et al.*, 1986). The recent intensification in commercial fishing of elasmobranchs (Cailliet et al., 1983; Brown and Gruber, 1988; Kusher et al., 1992; Dulvey et al., 2000) has made the collection of their life history information essential to the realistic management of their populations (Cailliet *et al.*, 1983; Ryland and Ajayi, 1984; Dulvey *et al.*, 2000). Historically, batoids have been of minimal commercial value (Otwell and Lanier, 1979; Sosebee, 1998), hence the majority of research on elasmobranchs has focused on commercially valuable sharks (e.g. Holden, 1977; Natanson *et al.*, 1995; Walmsley-Hart *et al.*, 1999). According to the characteristics outlined by Winemiller and Rose (1992) and the comparative analyses of Frisk *et al.*, (2001), skates, like other elasmobranchs, fall into the category of equilibrium strategists and as such, reach sexual maturity at a late age, have a low fecundity and are relatively long-lived. These characteristics, coupled with fisheries

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that select for the removal of large individuals (especially those over 100 cm total length), makes these particular fish highly susceptible to overfishing (Hoenig and Gruber, 1990; Dulvey *et al.*, 2000; Frisk *et al.*, 2001).

Traditionally, skates caught by ground fishing operations were discarded (Martin and Zorzi, 1993; Junquera and Paz, 1998; Sosebee, 1998). New and expanding markets for skate wings have made retention of these fish commercially more lucrative in recent years (Sosebee, 1998; New England Fishery Management Council, 2001). Skate harvests in the U.S. portion of the western north Atlantic are currently unregulated. Moreover, biological information on skate life histories is almost non-existent (Frisk, 2000). This combination of factors is believed to have lead to a depletion of common skates, *Raja batis*, in the Irish sea (Brander, 1981).

The winter skate, *Leucoraja ocellata*, is a large species (total length over 100 cm) of skate of the family Rajidae (Bigelow and Schroeder, 1953; Robins and Ray, 1986; New England Fishery Management Council, 2001). It 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. (Bigelow and Schroeder, 1953). Despite this wide range, little direct biological data is available for this species (Simon and Frank, 1996; Casey and Myers, 1998, Frisk, 2000). Recent assessment studies in the northeast U.S. (Northeast Fisheries Science Center, 1999), suggest the biomass of the winter skate may be below threshold levels mandated by the Sustainable Fisheries Act (SFA). To add insight into the life history of this species, and the status of the stock (Simpfendorfer, 1993; Frisk *et al.*, 2001), we estimated age and growth rates of *L. ocellata* by interpreting annular counts and marginal

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increments on vertebral centra from specimens collected in the western Gulf of Maine.

Materials and methods

Sampling

A total of 304 winter skates were captured by otter trawl between November 1999 and May 2001 at locations that ranged from 1.6 to 32 km off the coast of New Hampshire. Approximate depths at these locations ranged between 9 and 107 m. Skates were maintained alive on board the vessel until transport to the University of New Hampshire's Coastal Marine Laboratory (CML). There, individual fish were euthanized (0.05g/L bath of MS222). We measured total length (TL in mm) as a straight line distance from the tip of the rostrum to the end of the tail, and disc width (DW in mm) as a straight line distance between the tips of the widest portion of pectoral fins. Total wet weight (kg) was also recorded. In order to differentiate between the small, immature specimens of little skates, *Leucoraja erinacea*, (a congener species also found in the Gulf of Maine) and winter skates, rows of teeth in the upper jaw were counted. Skates whose number of teeth ranged between 72 and 110 per row were identified as *L. ocellata* while skates whose number of teeth ranged between 38 and 64 per row were identified as *L. erinacea* (Bigelow and Schroeder, 1953). To reduce any uncertainty in species identification, skates having between 38 and 71 teeth per row were not used in this study.

Preparation of vertebral samples

Vertebral samples, taken from above the abdominal cavity, were removed from 132 females and 98 males, labeled, and stored frozen. After defrosting, three

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centra from each specimen were freed from the vertebral column, stripped of excess tissue and air dried. Large centra were cut sagitally with a Dremel™ tool fitted with a mini-saw attachment while held within a vise. Smaller centra were sanded with a Dremel™ tool to replicate a sagittal cut. Processed vertebrae were mounted horizontally on glass microscope slides and ground with successively finer grits (#180, #400, #600), of wet-dry sandpaper. Each vertebra was then remounted and the other side ground to produce a thin (300 micrometer) "hourglass" section.

<u>Counts of annuli</u>

Vertebral sections were viewed through a compound microscope (25 to 40X) using reflected light (Figure 1.1). A growth ring (annulus) was defined as an opaque and translucent band pair that traversed the intermedialia and that clearly extended into the corpus calcareum (Casey *et al.*, 1985; Brown and Gruber, 1988). The birth mark (age zero) was defined as the first distinct mark distal to the focus that coincided with a change in the angle of the corpus calcareum (Casey *et al.*, 1985; Wintner and Cliff, 1996).

Three nonconsecutive annulus counts were made for the 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 two years, that particular specimen was eliminated from further analyses. Count reproducibility was estimated using the Index of Average Percent Error (IAPE) described by Beamish and Fournier (1981):

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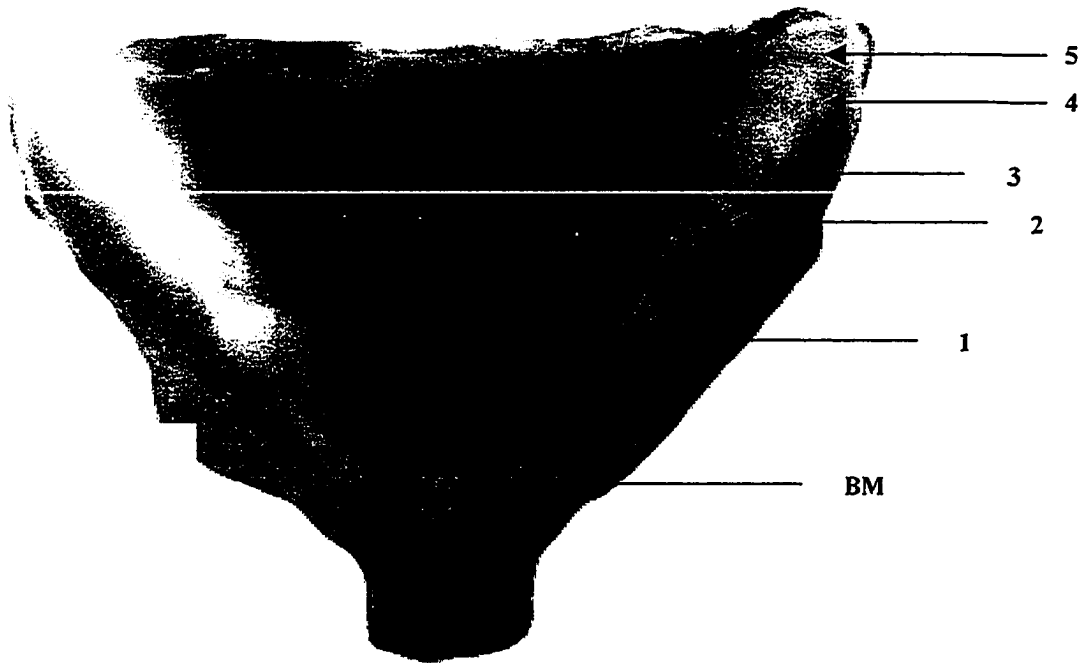
Figure 1.1. Longitudinal cross-section of vertebral centra from a 422 mm TL male caught in July and estimated to be 5 years. BM= birth mark; Arrows represent age in years.

$$IAPE = 1/N\sum(1/R\sum(|X_{ij}-X_j|/X_j)100$$

where

N= the number of skates aged
R= the number of readings
X_{ij} =the ith age determination of the jth fish
X_j = the average calculated for the jth fish

An upper limit for the IAPE was arbitrarily set at 15% for each vertebra. Vertebrae with statistically acceptable IAPE indexes were used for estimation of asymptotic growth rates (Brown and Gruber, 1988; Cailliet and Tanaka, 1990). The average of the mean counts for all three centra defined the age estimate for each specimen (Casey *et al.*, 1985; Wintner and Cliff, 1996).

A Von Bertalanffy growth function (VBGF) was fitted to the data with the following equation (von Bertalanffy, 1938):

$$L_t = L_\infty(1-e^{-k(t-to)})$$

where

L_t = total length at time t (age in years)

L_∞ =theoretical asymptotic length

k= Brody growth constant

t_0= theoretical age at zero length

Growth in length data were analyzed by using FISHPARM, a computer program for parameter estimation of nonlinear models with Marquardt's (1963) algorithm for least-square estimation of non-linear parameters (Prager et al., 1987) .

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<u>Marginal increment analyses</u>

The annual periodicity of band pair formation was investigated using marginal increment analyses (MIA). Since the annuli in older specimens were closer together, marginal increments were calculated from five specimens per month whose centra contained either four or five annuli. For MIA determination, the distance of the final opaque band and the penultimate opaque band, from the centrum edge were measured using an occular micrometer. The marginal increment was calculated as the ratio of the distance between the last and penultimate bands (Branstetter and Musick, 1994; Cailliet, 1990; Simpfendorfer, 1993; Simpfendorfer, 2000). Mean average increments by month of capture were plotted to identify trends in band formation using a Kruskal-Wallis one-way analysis of variance on ranks. (Simpfendorfer, 1993; Simpfendorfer, 2000).

<u>Results</u>

<u>Morphological measurements</u>

A total of 230 specimens were used for this study. Males (n=98) ranged between 147-932 mm TL, 82-601 mm DW and 0.015–6.2 kg. Females (n=132) ranged between 145-940 mm TL, 82-635 mm DW and 0.015–7.5 kg. A linear relationship existed between the total length, disk width and mass relationships for male, female and the sexes combined (all R^2 values were greater than 0.85). Two skates (one male: TL = 147 mm, DW = 82 mm, weight. = 0.015 kg; and one female TL = 145 mm, DW = 82 mm, weight. = 0.015 kg) hatched from egg cases during May 2001 in the CML after gestating 18 months.

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Vertebral analyses

No difficulty was encountered in estimating the age of *L. ocellata*. False bands (bands which do not completely encircle the centra) were easily distinguished from complete bands. Of the 230 processed vertebrae, 209 (91%) were readable. These 209 vertebrae (males = 88; females = 121) had annular count estimates that agreed within two years, resulting in an IAPE of 5.8%. Mean total length and disk width at age for male, female and sexes combined are given in Table 1.1. The relationship between TL and centrum diameter was linear (R^2=0.92; P< 0.05; Figure 1.2) and there were no significant differences (ANCOVA, P<0.05) between males and females. Since no significant difference existed for TL and centrum diameter between the sexes, the data were combined (Figure 1.2).

Marginal increments were averaged from five specimens for each month, except June when skates belonging to the 4 and 5 year age classes were unavailable. Marginal increments were significantly different between months (Kruskal-Wallis p< 0.001) with a distinct trend of increasing monthly increment growth beginning in July (Figure 1.3). Maximum marginal increment measurement occurred in May. Minimum marginal increment measurement occurred in July. Two recently hatched males (one from the laboratory (147 mm TL) and one from field collections (175 mm TL) had opaque zones on the distal edge of their vertebral centra. Based on this information, we suspect that a single opaque band may be formed annually on the vertebral centra during June/July in the winter skate.

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Table 1.1: Average total length, TL, and disc width, DW, at age for winter skates, *L. ocellata*, by sex and combined sexes. Mean ± 1 SEM; Sample size in parentheses.

Age	Male TL	Female TL	Sexes Combined	Male DW	Female DW	Sexes Combined
0	161 (2) ± 14	145 (1)	156 (3) ± 10	93 ± 7	81	89 ± 7
1	228 (1)	--	228 (1)	139	--	139
2	264 (5) ± 14	268 (4) ± 21	266 (9) ± 11	153 ± 5	158 ± 8	155 ± 5
3	340 (4) ± 20	317 (9) ± 9	324 (13) ± 9	198 ± 6	188 ± 6	191 ± 6
4	379 (12) ± 8	392 (25) ± 8	388 (37) ± 6	223 ± 8	233 ± 5	230 ± 4
5	435 (4) ± 19	429 (16) ± 13	430 (20) ± 6	264 ± 11	259 ± 13	260 ± 6
6	536 (5) ± 13	501 (7) ± 16	516 (12) ± 12	338 ± 8	310 ± 15	322 ± 11
7	609 (1)	551 (12) ± 6	556 (13) ± 7	392	342 ± 6	346 ± 6
8	651 (1)	565 (11) ± 13	570 (12) ± 13	401	352 ± 10	356 ± 10
9	658 (9) ± 24	632 (9) ± 20	645 (18) ± 15	420 ± 18	403 ± 16	411 ± 11
10	690 (12) ± 20	704 (8) ± 18	696 (20) ± 14	441 ± 22	447 ± 17	444 ± 11
11	735 (10) ± 17	761 (5) ± 22	744 (15) ± 14	479 ± 24	498 ± 20	485 ± 14
12	743 (5) ± 24	763 (5) ± 19	753 (10) ± 15	488 ± 12	501 ± 15	494 ± 8
13	830 (3) ± 7	772 (3) ± 16	801 (6) ± 15	495 ± 6	506 ± 8	500 ± 5
14	838 (4) ± 10	803 (3) ± 23	821 (7) ± 13	530 ± 9	527 ± 24	529 ± 10
15	841 (4) ± 12	--	841 (4) ± 12	541 ± 19	--	541 ± 13
16	860 (4) ± 4	842 (1) ± 0	857 (5) ± 5	565 ± 16	542	560 ± 10
17	921 (1)	--	921 (1)	579	--	579
18	--	940 (2) ± 0	940 (2) ± 0	--	623 ± 13	623 ± 13
19	932 (1)	--	932 (1)	601	--	601

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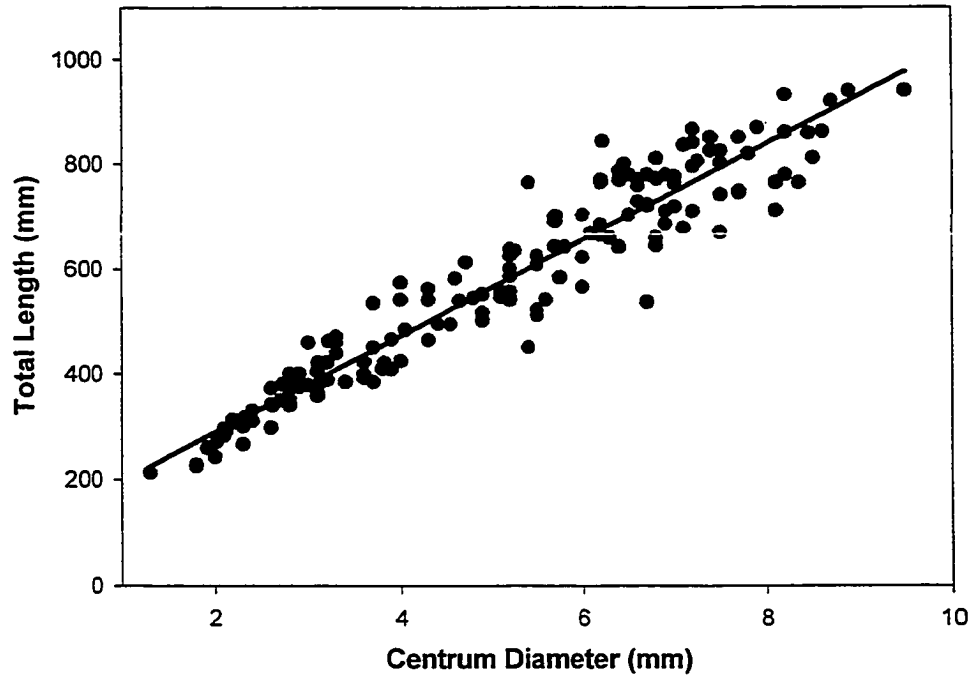
Figure 1.2. The relationship of total length (mm) to centrum diameter (mm) for combined sexes of winter skate.

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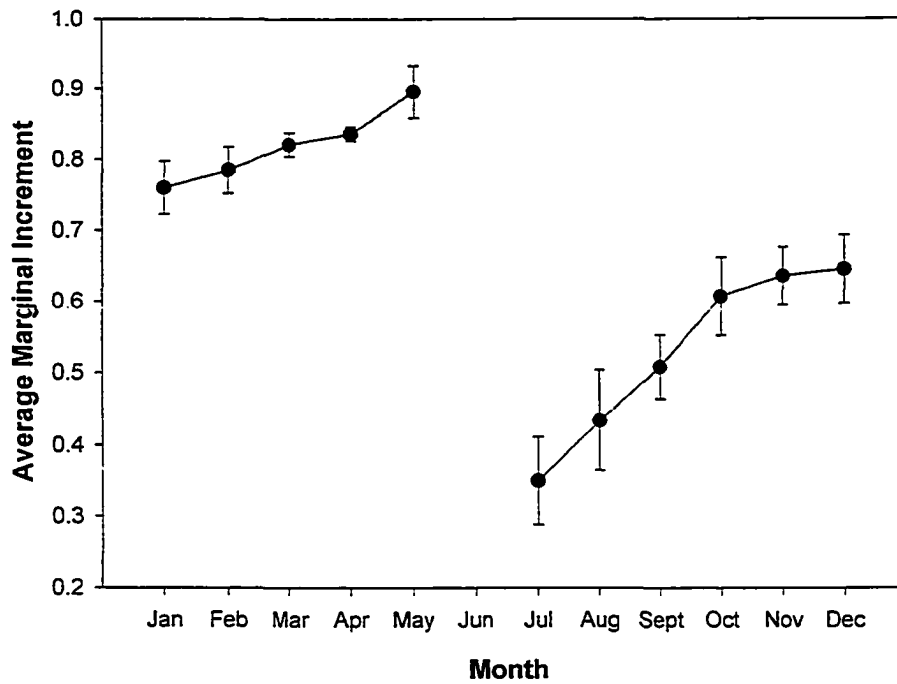
Figure 1.3. Mean monthly marginal increments of opaque bands for *L. ocellata* from the Gulf of Maine. Marginal increments were calculated from five specimens per month whose centra contained either 4 or 5 annuli. Error bars represent 1 ± SEM.

<u>Age and growth estimates</u>

We assumed that opaque-translucent band pairs were formed annually, and we fit von Bertalanffy growth curves (VBGC) to total length at age data (Figure 1.4A-C). The VBGC provided a good fit with a low standard error for males, females and both sexes combined (Table 1.2). The t_0 values (−1.4 to −1.6) compared favorably with gestation rates for the two skates hatched in captivity (1.5 years) (Table 1.2). The von Bertalanffy growth parameters for males, females and the sexes combined were similar but k values higher for males and sexes combined, than for females.

<u>Discussion</u>

The relationship between TL and centrum diameter was linear and significant, indicating that the centra grew proportionally to skate length for all size classes, and thus making this structure useful for age analyses (Kusher *et al.*, 1992). The 5.8% IAPE index suggests that our ageing method represents a precise approach to the age assessment of *L. ocellata*. Minimal width of the marginal increment for winter skates captured in May supports the hypothesis of annual band formation in this species. Moreover, these results compare favorable to cycles in marginal increments for other skates found in temperate waters whose vertebral bands are formed annually (Holden and Vince, 1973; Waring, 1984; Natanson, 1993).

Von Bertalanffy parameters, as determined by our study, suggest that females attain a slightly larger asymptotic TL_∞ (1374 mm) than males (1218 mm), and grow slower (k=0.059 and 0.074, respectively). This trend follows a common pattern in batiods. Holden (1977), Waring (1984), Ryland and Ajayi (1984), Brander (1981) and Walmsley-Hart et al., (1999) found similar tendencies in several species of

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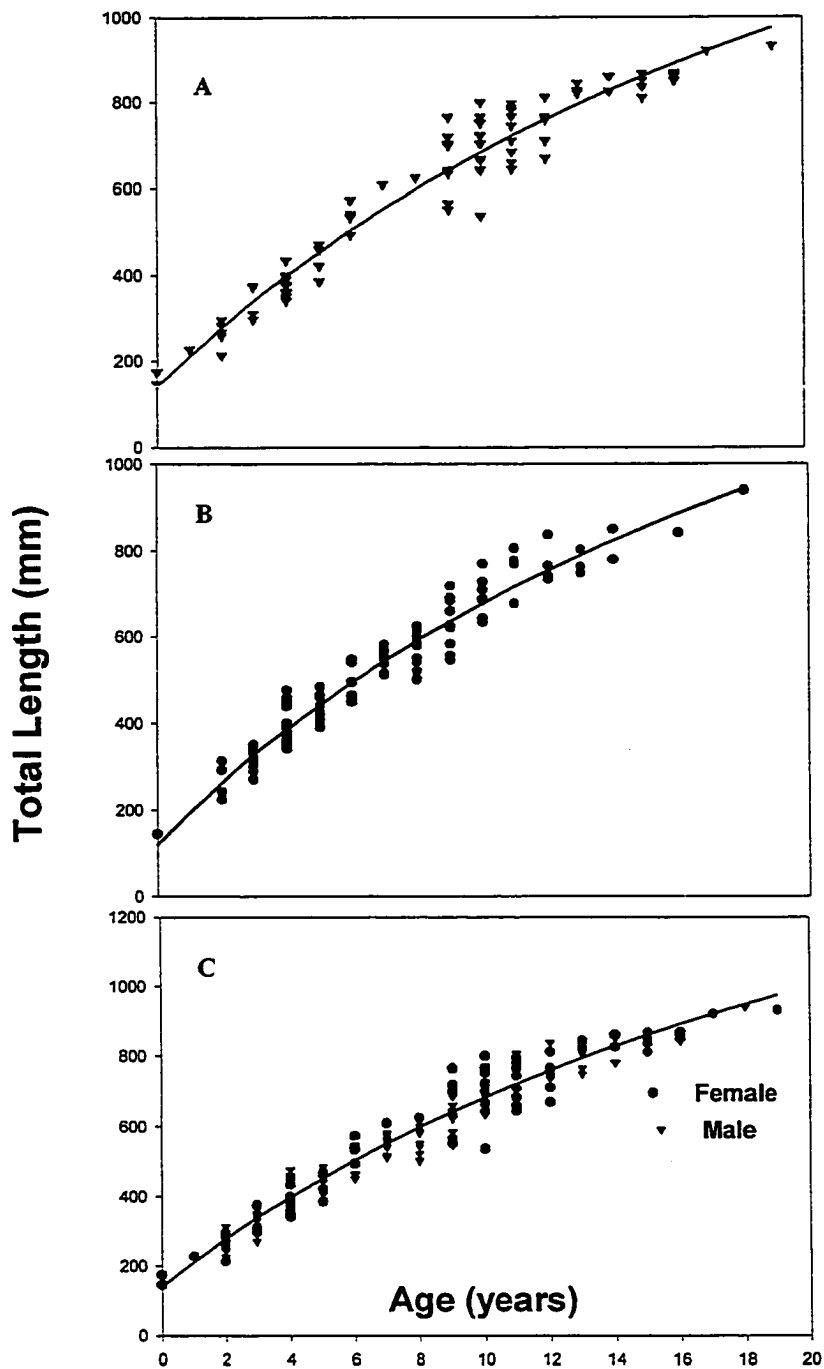
Figures 1.4. Von Bertalanffy growth curves generated from vertebral data for (A) male, (B) female and (C) combined sexes of winter skate , *L. ocellata*, from the western Gulf of Maine. Individual VBGC parameters are given in Table 1.2.

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Table 1.2: Calculated von Bertalanffy parameters for male, female and combined

sexes of *L. ocellata*.

L_∞ (mm TL)	1218	1374	1314
k (year^{-1})	0.074	0.059	0.064
t_0 (year)	-1.418	-1.609	-1.531
R^2	0.946	0.939	0.946
SE	0.01	0.01	0.001
n	88	121	209

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skates, while Martin and Cailliet (1988) found comparable results in the bat ray, *Myliobatis californica*.

Our estimates of L_∞ exceed the largest specimens in our field collections (940 mm for females and 932 mm for males). Nevertheless, extensive trawl surveys in the western Gulf of Maine and the Mid-Atlantic offshore region spring and autumn bottom trawl surveys from 1967-2000 (Northeast Fisheries Science Center 1999) found mean TL not to exceed 1000 mm. Thus, we suspect that our von Bertalanffy equation produces an accurate estimation of L_∞ for winter skate. Walmsley-Hart *et al.*, (1999) over estimated L_∞ for *R. pullopunctata* and suggested that small sample size and rareness of large individuals were most likely responsible. Since fishing gear was not biased towards a specific marketable skate size and all size classes of *L. ocellata* were represented, it is quite possible that the rareness of large individuals led to the augmented L_∞ in combined and individual sexes in this study. Possibly, a larger sample size of winter skates would produce significant and divergent results with regard to von Bertalanffy parameters. However, the close fit of the data to the VBGC for *L. ocellata* indicates this is an appropriate model for this species.

Preliminary estimates of age and growth parameters are available for winter skate in Canadian waters (eastern Scotian Shelf) from Simon and Frank (1996), who reported the results of a study conducted at St. Mary's University by R. Nearing. Combined sexes of winter skates (*n*=242) with TL ranging from 120 to 1060 mm and ages from 0 to 16 years provided von Bertalanffy parameters of L_∞ = 114.1 cm, k = 0.14405, and t_0 = 0.00315. However, these data should be viewed with caution

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since no IAPE values nor validation of the annual nature exist for these estimates, and it is likely that the older specimens had been under-aged by four or more years.

K values, an estimation of how quickly an animal grows to L_∞, were similar for both sexes of winter skate. These growth rates are commensurate with other skate species of similar size, but slower than skate species of smaller size (Table 1.3). The oldest ages obtained for the winter skate were 19 and 18 years for males and females, respectively. These data are in agreement with the assumption that larger batoids, such as *L. ocellata* and *R. pullopunctata* (Walmsley-Hart *et al.*, 1999) are longer lived and grow slower than smaller species, such as *R. erinacea*, which has been aged to 8 years with a k value of 0.352 (Johnson, 1979; Waring, 1984).

Summary

Accurate stock assessment data for skates is difficult to collect in the northeast U.S. because species are rarely differentiated in landings information (New England Fishery Management Council, 2001). Because of this, fluctuations in stock size will be difficult to detect and successful implementation of fisheries management plans will remain problematic. Our study provides some basic age/growth parameters for the winter skate and it supports the hypothesis that *L. ocellata*, like other elasmobranchs, require conservative management because they grow slowly and thus are susceptible to over-exploitation (Brander, 1981; Kusher *et al.*, 1992; Zeiner and Wolf, 1993; Frisk *et al.*, 2001).

Table 1.3. Comparison of von Bertalanffy growth parameters for several skate species (L_∞ (mm) =Disk width; L_∞ (mm)=Total Length).

Scientific Name	Sex	L_∞ (mm)	k	t_0(years)	Max age (yr)	Source
Raja rhina	♀♂	1047 (TL)	0.17	-0.16	13	Zeiner and Wolf, 1993
Raja microocellata	♀♂	1370 (TL)	0.086	-3.009	9	Ryland and Ajayi, 1984
Raja montagui	♀♂	978 (TL)	0.152	-1.719	7	Ryland and Ajayi, 1984
Raja erinacea	♀♂	527 (TL)	0.352	-0.449	8	Waring, 1984
Raja wallacei	♀♂	422 (DW)	0.26	-0.17	15	Walmsley-Hart *et al.*, 1999
Raja clavata	♀♂	1050 (TL)	0.215	0.045	10	Brander and Palmer 1985
Raja pullopunctata	♂	771 (DW)	0.05	-2.20	18	Walmsley-Hart *et al.*, 1999
Raja pullopunctata	♀	1327 (DW)	0.08	-1.95	14	Walmsley-Hart *et al.*, 1999
Leucoraja ocellata	♀♂	1314 (TL)	0.064	-1.531	19	This study

CHAPTER II

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.

<u>Introduction</u>

The winter skate, *Leucoraja ocellata*, is a large species belonging to the

family Rajidae (Collette and Klein-MacPhee 2002; Robins and Ray 1986). It 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.

(Collette and Klein-MacPhee 2002). Traditionally, skates caught by ground

fishing operations were discarded (Martin and Zorzi 1993; Junquera and Paz

1998; Sosebee 1998). However, new and expanding commercial markets for

skates have made retention of these fish more lucrative in recent years. (Sosebee

1998; Dulvey *et al.*, 2000; New England Fishery Management Council 2001).

This is particularly true of *L. ocellata* collected in New England waters, as adults

are processed for their wing meat (New England Fishery Management Council

2001).

Despite a wide geographic range, important biological such as age at

maturity, reproductive cycles and annual fecundity, are lacking for the winter

skate, (Casey and Myers 1998; Simon and Frank 1998). Recently we have

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collected age and growth data from specimens inhabiting the western Gulf of Maine (Sulikowski *et al.*, 2003). In this study, males were aged to 19 years with a k value of 0.074, while females were aged to 18 years with a corresponding k value of 0.059. According to characteristics outlined by Winemiller and Rose (1992) and the comparative analyses of Frisk *et al.* (2001), this species, like other elasmobranchs, displays characteristics of equilibrium strategists because they grow slowly and are relatively long-lived. These characteristics, coupled with fisheries that selectively remove large individuals (especially those over 100 cm total length), make the winter skate highly susceptible to overfishing (Hoenig and Gruber 1990; Dulvey *et al.*, 2000; Frisk *et al.*, 2001; Frisk *et al.*, 2002). Recent assessment studies in the northeast U.S. (Northeast Fisheries Science Center 1999), suggest 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).

The recent intensification in commercial fishing of *L. ocellata* has made the collection of this species' life history information essential to the management of their populations in the western Gulf of Maine (Cailliet and Tanaka 1990; Ryland and Ajayi 1984; 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 animal becomes sexually mature. Information regarding sexual maturity in elasmobranchs is largely descriptive. Some studies either address changes in morphology, such as clasper length in males and ovary weight in females (Walmsley-Hart *et al.*, 1999; Mollet *et al.*,

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2000; Francis *et al.*, 2001; Conrath and Musick, 2002), while others use gonadosomatic index (GSI) to help assess reproductive activity (Parsons, 1983; Ryland and Ajayi, 1984; Snelson *et al.*, 1988; Loefer and 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 and Grier, 1992; Maruska *et al.*, 1996). Furthermore, few studies have linked histological changes in gonadal tissues or with circulating steroid hormones when these fish become reproductively capable. We present the first determination of size at sexual maturity for a cartilaginous fish by correlating morphological and histological characteristics to steroid hormone concentrations. This information was then coupled with interpreting annular counts on vertebral centra to elucidate age at sexual maturity (Sulikowski *et al.*, 2003).

Materials and methods

Sampling techniques

One hundred eighty four winter skates, *L. ocellata*, were captured by otter trawl at locations that ranged from 1.6 to 32 km off the coast of New Hampshire. Approximate depths at these locations ranged between 9 and 107 m. Immediately after capture 5-10 ml of blood was collected by cardiac puncture using chilled, heparinized syringes with a 21 gauge needle. Samples were centrifuged at 1300g for five minutes, and the separated plasma placed in ice for 4-8 and stored at -20°C. Skates were maintained alive on board the vessel until transport to the University of New Hampshire's (UNH) Coastal Marine

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Laboratory (CML). Individual fish were euthanized (in a bath of 0.05 MS222 g L^{-1}) before removing reproductive and vertebral tissues. Total length (TL in mm) was measured as the straight line distance from the tip of the rostrum to the end of the tail, and disc width (DW in mm) as the straight line distance between the tips of the widest portion of pectoral fins. Total wet weight (kg) was also recorded. For males, clasper length was measured as the straight line distance from the posterior point of the cloaca to the end of the clasper.

Assessment of sexual maturity

Testes were removed from the skates at the UNH CML, blot dried and weighed to the nearest gram. A single 2-3 mm thick segment was removed from the central portion of a single lobe in the medial area of each testis (Maruska *et al.*, 1996), placed in a tissue cassette and fixed in 10% buffered formalin until processed by the UNH Veterinary Diagnostic Laboratory. Here, each sample was dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Prepared slides of testicular tissue were examined using a compound microscope for changes in gametogenic development, as outlined by Maruska *et al.* (1996) to assist in determining sexual maturity. Specifically, the mean proportion of the testes occupied by mature spermatocysts was measured along a straight line distance across one representative full lobe cross section of the testes. Histologically, mature spermatocysts were identified by the organization of spermatozoa into tightly shaped packets that were arranged spirally along the periphery of the spermatocysts (Figure 2.1). Collectively, maturity in males was

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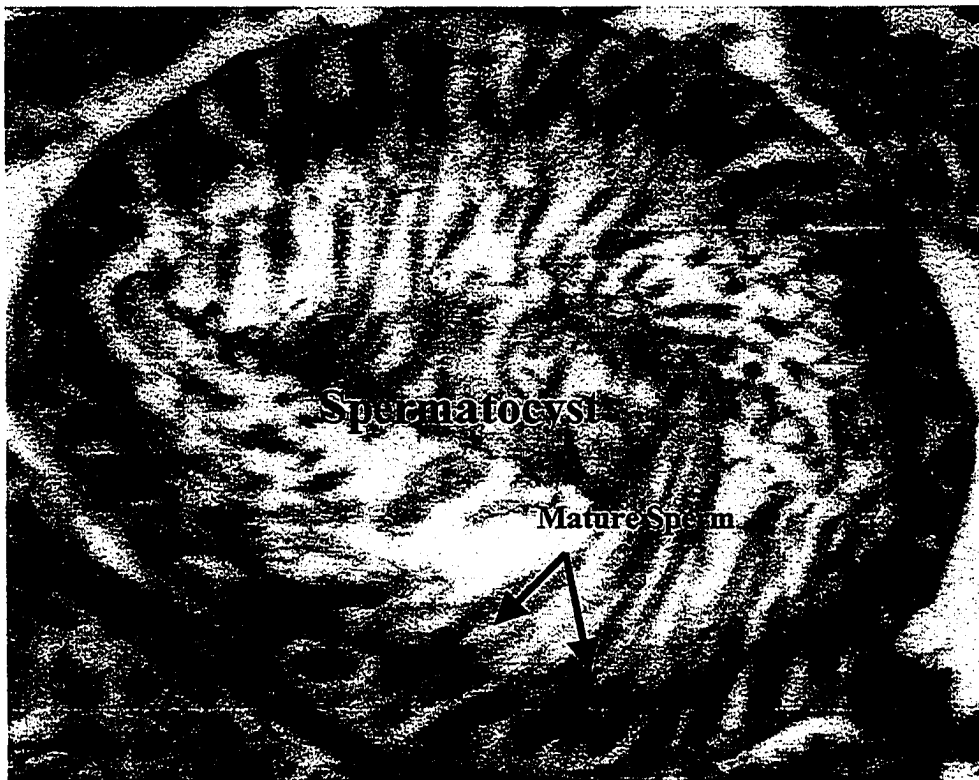
Figure 2.1. Histological section of an *L. ocellata* testis at 20x magnification. Spermatocysts are characterized by mature sperm that are organized into cone shaped packets arranged spirally along the outside of the spermatocysts.

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based on a testosterone value of 30,000 pg/ml or larger, a total length to clasper ratio of 0.23 or larger and a proportion of mature spermatocysts of 26% or larger

The female reproductive tract was removed and the ovaries, shell glands and uterus separated, blot dried and weighed 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 (Tsang and Callard 1987; Martin 1982; Snelson et al. 1988). Changes in mean ovary weights, mean shell gland weights and the presence of vitellogenic follicles in different age groups were used in determining sexual maturity. Moreover, females whose reproductive tract contained follicles 26 mm in diameter or larger, a shell gland weight of 25 gm or larger and estradiol concentrations of 1000pg/ul or greater were considered sexually mature.

Analysis of steroid hormones

Stock solutions of radiolabeled steroids (Amersham Biosciences, Piscataway, NJ) were purified by thin layer chromatography (TLC). The following radiolabeled steroids were used in this study: $[1,2,6,7-^3H]$ testosterone (TRK 92; specific activity= 101Ci/mmol), and $[2,4,6,7,16,17-^3H]$ estradiol (TRK 587; specific activity= 152Ci/mmol). Silica gel plates (HLF, scored 20 x 20 cm, 250 microns) were obtained from Analtech, Inc (Newark, DE). All organic solvents (ACS certified) were obtained from Fisher Scientific (Pittsburg, PA). For E_2, the solvent system was ether:hexane, 3:1 part and for T, the solvent system was benzene:acetone, 4:1 part. Adjacent lanes were spotted with respective nonradioactive standards (20 μg/10 μl absolute ethanol). Each plate

was run twice before viewing under short wave ultraviolet light to localize the steroid standards by fluorescence. The silica gel areas containing radiolabeled steroid, which co-migrated with the respective standard, were scraped from the plate into 13 x 100 mm borosilicate glass tubes containing 0.4 ml water and extracted (3x) with 2.0 ml methanol. A 100 ul aliquot was removed from the combined methanol fractions to determine recovery before evaporation under nitrogen. The dried extract was reconstituted in absolute ethanol to yield a stock concentration of 5 uCi/ml for T and 8 uCi/ml for E_2. They were stored at $-20^{\circ}C$.

Aliquots of 0.6 ml plasma were pipetted into 16 x 100 mm borosilicate tubes and approximately 1000 counts per minute (cpm) of an appropriate radiolabeled steroid was added to account for procedural losses. Each sample was extracted with 10 volumes of diethyl ether (anesthesia grade) by vortexing for 1 minute. The aqueous layer was snap frozen in an acetone/dry ice bath before decanting the organic layer into 16 x 100 mm borosilicate glass tubes. The ether was then evaporated to dryness in a $37^{\circ}C$ water bath under a stream of nitrogen (Airgas East, Dover, NH). The aqueous phase was re-extracted as described above and the extracts combined. Buffer blanks, charcoal stripped plasma and plasma spiked with steroids (to test for linearity and parallelism; R^2 for T= 0.98, R^2 for E_2= 0.97) were processed in an identical manner. The dried extract was reconstituted in a volume of phosphate buffered saline with 0.1% gelatin (PBSG; 0.06M $Na_2HPO_4*7H_2O$, 0.04M $NaH_2PO_4*H_2O$, 0.15M NaCl, 0.1% sodium azide) equivalent to the plasma aliquot and stored at $-20^{\circ}C$. The overall recoveries for E_2 and T were 76% and 74% respectively.

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Plasma concentrations of testosterone (T) in males and estradiol-17β (E_2)

in females were determined by radioimmunoassay using procedures modified

from Tsang and Callard (1987). All nonradioactive steroids were obtained from

Steraloids, Inc (Wilton, NH). Two aliquots of the reconstituted extract in

duplicate were placed in 12 x 75 mm borosilicate tubes and brought to 0.2 ml with

PBSG. All tubes received 0.1 ml of the appropriate labeled steroid (containing

approximately 10,000 to 15,000 cpm) and 0.1 ml of diluted antiserum. The final

concentration of the antibodies were 1:64,000 for T (#250 anti-testosterone-11-

BSA; from Gordon Niswender, Colorado State, Fort Collins, CO) and 1:200,000

for E_2 (#244 anti-estradiol -6-BSA; from Gordon Niswender, Colorado State, Fort

Collins CO). The characteristics of the E_2 and T antibodies have been described

by Korenman et al., (1974) and Gay and Kerlan (1978) respectively. All tubes

were incubated at 4°C overnight. Free steroids were separated from bound using

1.0 ml of a suspension containing 0.2% washed Norit A charcoal with 0.02%

Dextran T-70 (Amersham Pharmacia Biotech, Piscataway, NJ) in PBSG. After

mixing, the tubes were incubated for 10 minutes at 4 °C, followed by

centrifugation at 2500 *rpm* for 10 minutes at 4°C. The supernatant was decanted

into 7 ml minivials (USA Scientific Inc., Ocala, Fl) containing 3.5 ml Ready

Safe™ scintillation cocktail (Beckman Coulter, Somerset, NJ). Radioactivity was

determined in a Beckman LS6000IC (Fullerton, CA) liquid scintillation counter.

A logit bound vs. log dose standard curve was used to interpolate hormone

concentrations. The intra-assay coefficients of variance were 5.5% for E_2 and

8.6% for T, and the inter-assay coefficients of variance were 12.8% for E_2 and 10.4% for T.

Preparation and age analysis of vertebrae

Vertebrae, taken from above the abdominal cavity, were removed from 88 females and 96 males, labeled, and stored at -20 °C. The methods utilized for processing vertebrae and age analyses were identical to those employed by Sulikowski *et al.* (2003). Briefly, 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.

Three nonconsecutive annulus counts were made for the 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 two years, that particular specimen was eliminated from further analyses. Count reproducibility was estimated using the Index of Average Percent Error (IAPE) described by Beamish and Fournier (1981). An upper limit for the IAPE was arbitrarily set at 15% for each vertebra. The average of the mean counts for all three centra defined the age estimate for each specimen (Casey et al. 1985; Wintner and Cliff 1996).

Statistics

Results are presented as mean ± 1 SEM. Differences in morphological and histological parameters and hormone concentrations between age groups were determined using an analysis of variance followed by a tukey's post-hoc test.

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Statistical significance was accepted at P < 0.05. Maturity ogives were fitted to length (at 20 mm intervals) and age at maturity data by sex using probit analyses.

Results

Justification for sizes used

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

Reproductive parameters

A total of 184 specimens were used for this study. Males ($n = 96$) ranged from 551-940 mm TL and 1.2–6.0 kg body weight . Females ($n = 88$) ranged from 529-940 mm TL and 0.88–8.0 kg body weight. To avoid any bias that might be associated with reproductive seasonality, skates collected during the months July and August (in 1999, 2000 and 2001) were used for this study. Data collected by Sulikowski *et al.* (manuscript in preparation) suggest that these months represent high reproductive activity in this species. We were unable to perform radioimmunoassays on plasma on male skates belonging to the year 8 age-class.

Also, for age-classes 7 and 9, plasma from two skates for each age-class was assayed for testosterone.

The reproductive system of male winter skates followed the morphological classification of rajid batoids outlined by Pratt (1988). Overall, a steady and increasing trend in the total length to clasper length (TL/CL) ratio, testes weight and circulating testosterone concentrations was observed until age 10 (Table 2.1). Beginning at age 10, dramatic increases in several reproductive parameters were observed until age 13, when values leveled off and remained relatively constant in individuals between 14 and 19 years of age. The TL/CL ratio increased (P<0.05) from age 10 to age 11 (Table 2.1, Figure 2.2). Likewise, a significant increase (P<0.05) in testes weight occurred from age 10 to age 11(Table 2.1, Figure 2.2), while the proportion of mature spermatocysts increased (P<0.05) between age 11 and 12 years (Table 2.1, Figure 2.3) . A second increase (P<0.05) in the TL/CL ratio and the proportion of mature spermatocysts occurred in skates from age 12 to age 13 years. Although testosterone concentrations increased dramatically from ages 8 to 13 years (Table 2.1, Figure 2.3), no statistical differences were detected between any two consecutive age groups. However, testosterone concentration was correlated to the TL/CL ratio (r^2= 0.62), percent mature spermatocysts (r^2= 0.55), and testes weight (r^2= 0.63).

Based upon the abrupt increases in TL/CL ratio, testes weight and testosterone concentration and the inception of mature spermatocysts within the

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Table 2.1. Morphological measurements and reproductive parameters for the male winter skate, *Leucoraja ocellata*. Values given as mean ± SEM. * For each column, arrowed brackets and asterisks represent significant differences (P< 0.05; *ANOVA*) between skates in consecutive age groups. [A] Consists of two samples.

Age	Sample Size	Total length (mm)	Weight (kg)	Total length to Clasper ratio	% mature spermatocytes	Average testes weight (g)	Testosterone (pg/ml)
7	6	593 ± 15	1.6 ± 0.1	0.09 ± 0.01	0 ± 0	2.0 ± 0.3	10 ± 2[A]
8	6	639 ± 2	1.9 ± 0.1	0.12 ± 0.01	0 ± 0	2.2 ± 0.3	dnm
9	5	660 ± 7	2.0 ± 0.2	0.13 ± 0.01	0 ± 0	2.6 ± 0.3	2602 ± 315[A]
10	6	693 ± 4	2.3 ± 0.0	0.17 ± 0.01*	0 ± 0	3.4 ± 0.3 *	10621 ± 2261
11	7	735 ± 6	2.6 ± 0.2	0.23 ± 0.01*	13 ± 4*	10.9 ± 1.9 *	19366 ± 4069
12	22	775 ± 3	3.8 ± 0.1	0.23 ± 0.01*	29 ± 4 *	15.8 ± 1.3	32144 ± 4451
13	12	820 ± 2	4.1 ± 0.2	0.27 ± 0.01*	42 ± 4 *	19.0 ± 1.0	46626 ± 7174
14	4	838 ± 2	4.9 ± 0.1	0.27 ± 0.01	44 ± 3	21.0 ± 1.2	49974 ± 13970
15	8	849 ± 1	4.5 ± 0.1	0.27 ± 0.01	44 ± 4	21.0 ± 1.0	50785 ± 6304
16	8	861 ± 3	4.9 ± 0.2	0.26 ± 0.01	44 ± 2	22.6 ± 1.0	47267 ± 6969
17	10	902 ± 5	5.7 ± 0.2	0.26 ± 0.01	41 ± 2	25.8 ± 1.2	46049 ± 6476
19	2	934 ± 7	5.8 ± 0.2	0.26 ± 0.01	43 ± 7	24.1 ± 3.3	47338 ± 562
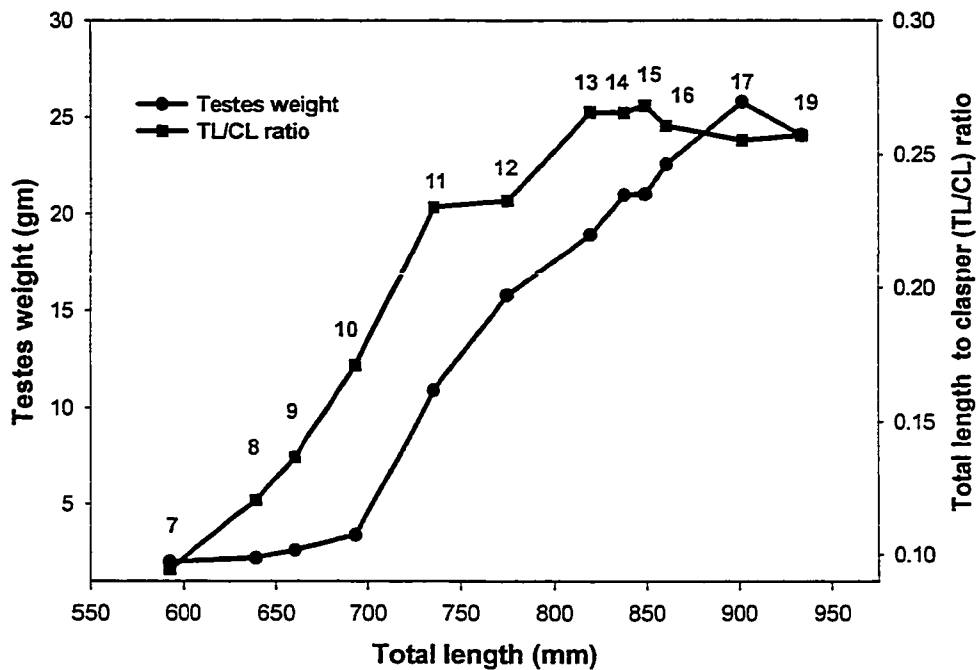
Figure 2.2. Changes in the total length to clasper length ratio and testes weight as male winter skates, *L. ocellata,* progress through sexual maturity. Average age is given above each representative size class. Values are expressed as mean ± SEM. For Error bars, sample sizes and statistical significance please refer to Table 2.1.

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Figure 2.3. Changes in 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. For Error bars, sample sizes and statistical significance please refer to Table 2.1.

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testes, the smallest sexually mature male within our sampling population measured 711 mm TL and was aged to 11 years. Maturity ogives predict that 50% maturity occurs at a total length of 730 mm and at age 11 years (Figures 2.4A and 2.4B). Indeed, in our sampling population, increases in TL/CL ratio, circulating testosterone concentrations, testis weight, and proportion of mature spermatocyts within the testes are in overall agreement with the ogives. The data in Table 2.1 and Figures 2.2-2.3, suggest that 50% maturity occurs between total lengths of 735 and 775 mm and between the ages of 11 and 12 years.

The reproductive system of female winter skates followed the morphological classification of rajid batoids outlined by Pratt (1988). In general, a steady and increasing trend in ovary weight, shell gland weight, average size of the largest follicle and E_2 concentration was observed until age 14, when values leveled off and remained relatively constant in individuals between 15 and 18 years of age (Table 2.2, Figures 2.5 and 2.6). Interestingly, the first and only significant (P<0.05) change in ovary weight, shell gland weight, and average size of the largest follicle between consecutive age groups occurred between the 8 and 9 year age-classes. Similar to testosterone concentrations, no statistical differences were detected for estradiol between any two consecutive age-groups. However, E_2 concentration was correlated to ovary weight (r^2= 0.68), shell gland weight (r^2= 0.65), and average size of the largest follicle (r^2= 0.64).

Furthermore, when follicles were enumerated, those that ranged in size from 1 to 3 mm in diameter were predominant in ovaries of skates 8 to 14 years of
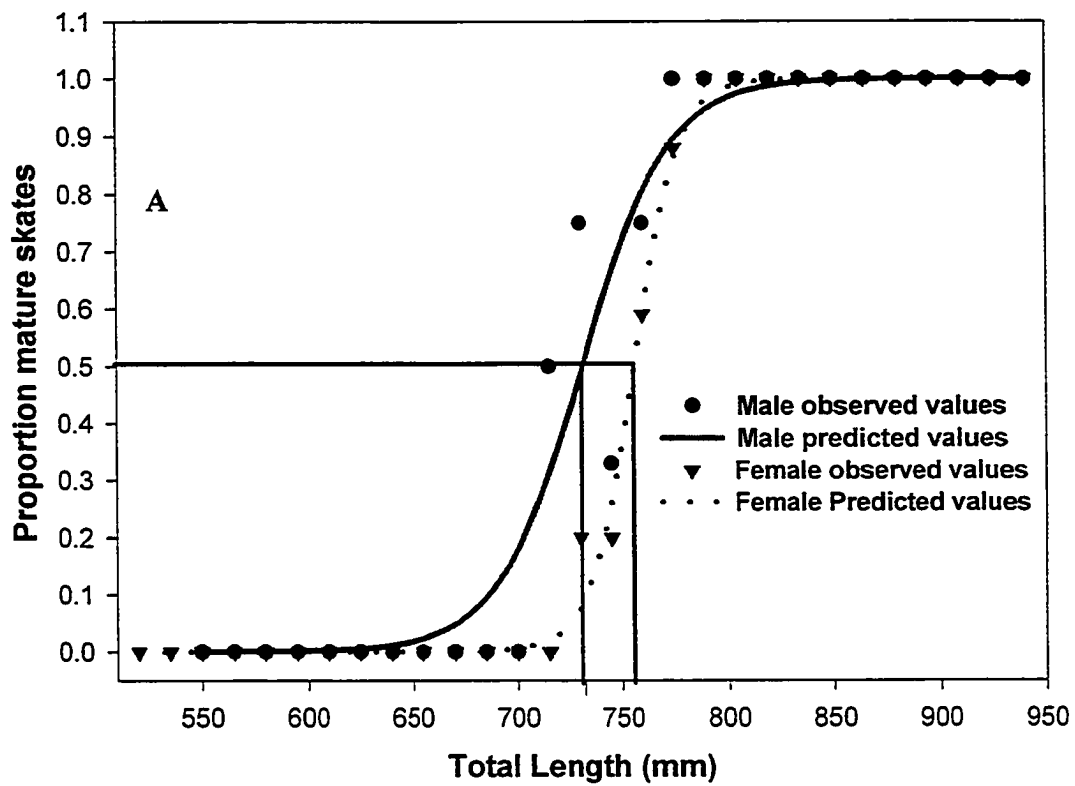
Figure 2.4A. Maturity ogives for total length of male and female *L. ocellata*.

Maturity ogives predict that 50% maturity occurs at a total length of 730 mm for

males and a total length of 760 mm for females.

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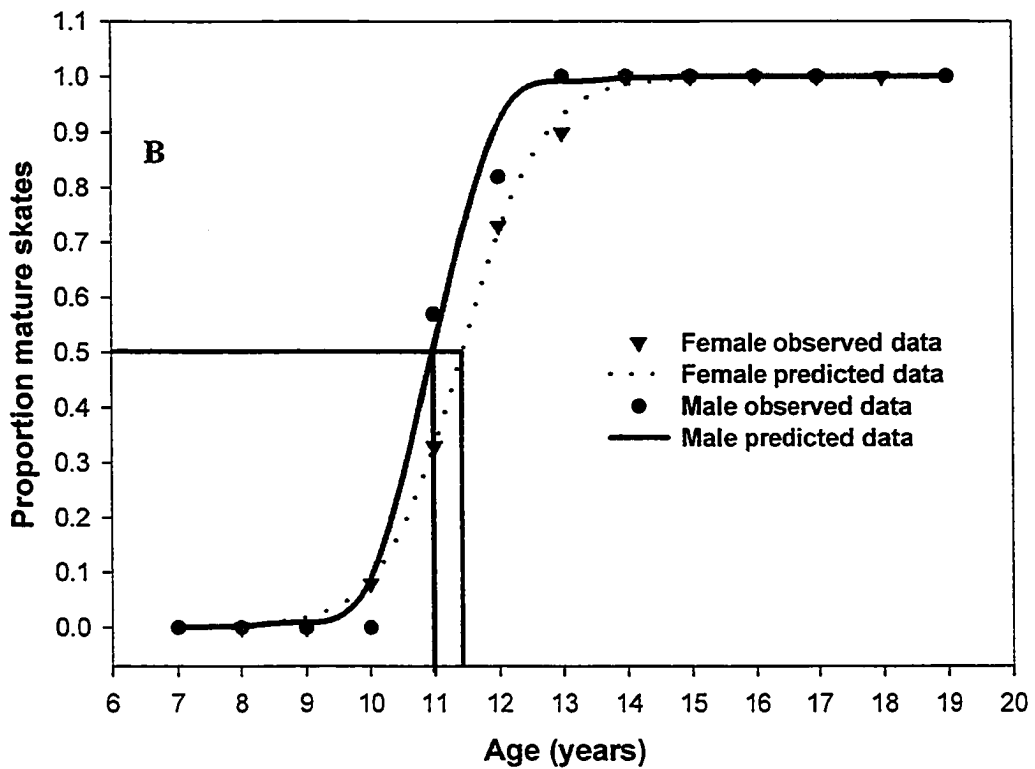
Figure 2.4B. Maturity ogives for age of male and female *L. ocellata* given in 20

mm intervals. Maturity ogives predict that 50% maturity occurs at 11 years of

age for males and between 11 and 12 years of age for females

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Table 2.2. Morphological measurements and reproductive parameters for the female winter skate, *Leucoraja ocellata*. Values given as mean ± SEM. * For each column, arrowed brackets and asterisks represent significant differences (P< 0.05; *ANOVA*) between skates in consecutive age groups.

Age	N	Total Length	Weight (kg)	Ovary weight (g)	Shell gland weight (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	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 ± 4	7.7 ± 0.3	52 ± 5	27 ± 7	21 ± 1	2073 ± 194

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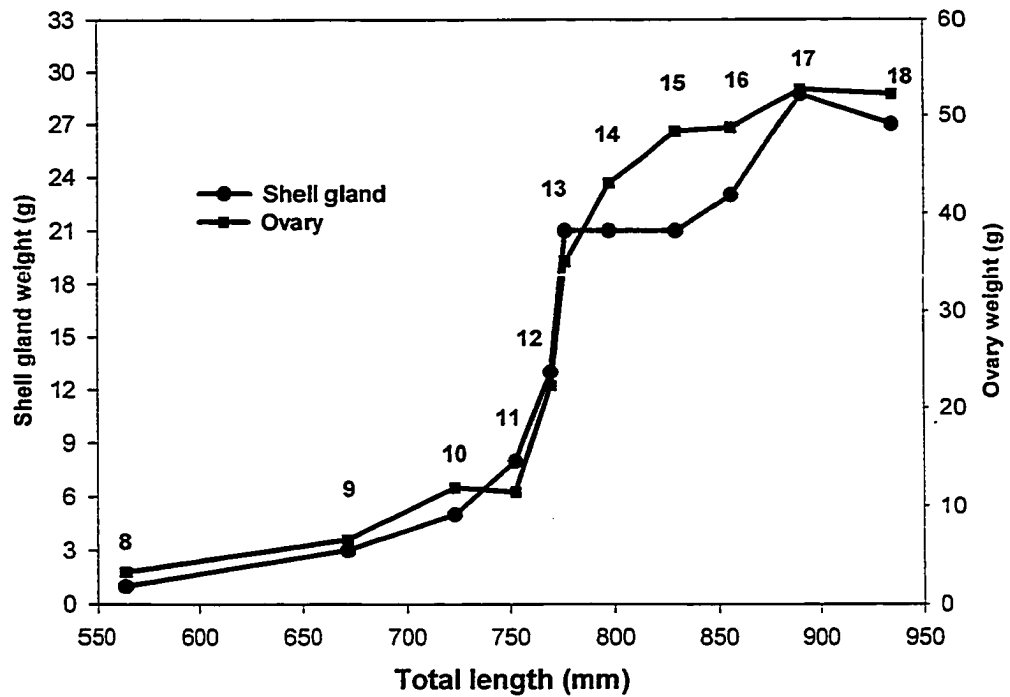
Figure 2.5. Changes in ovary weight and shell gland weight as female winter skates, *L. ocellata*, progress through sexual maturity. Average age is given above each representative size class. Values are expressed as mean ± SEM. For Error bars, sample sizes and statistical significance please refer to Table 2.2.
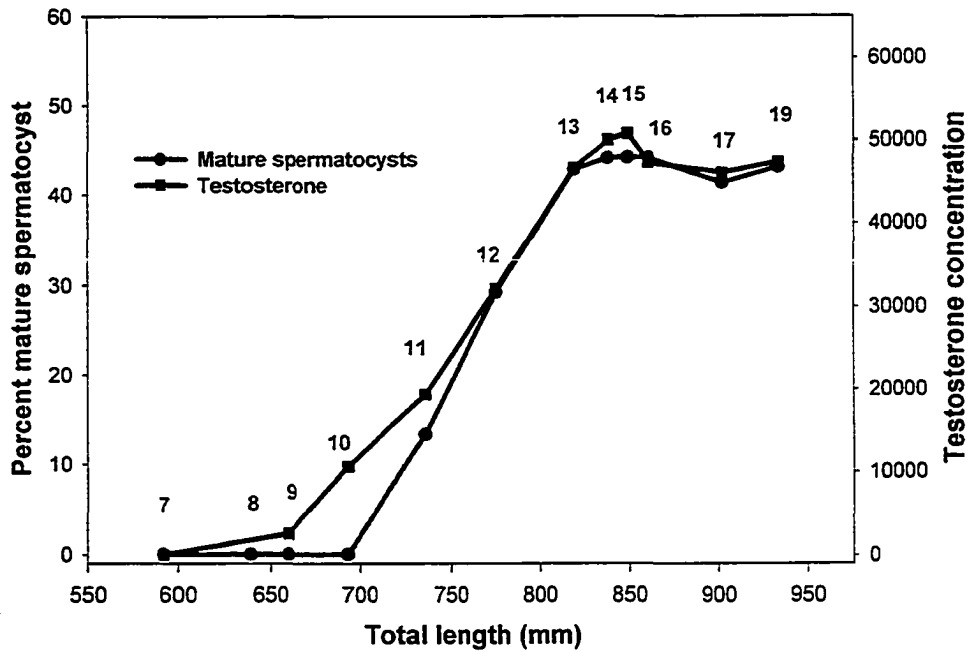
39
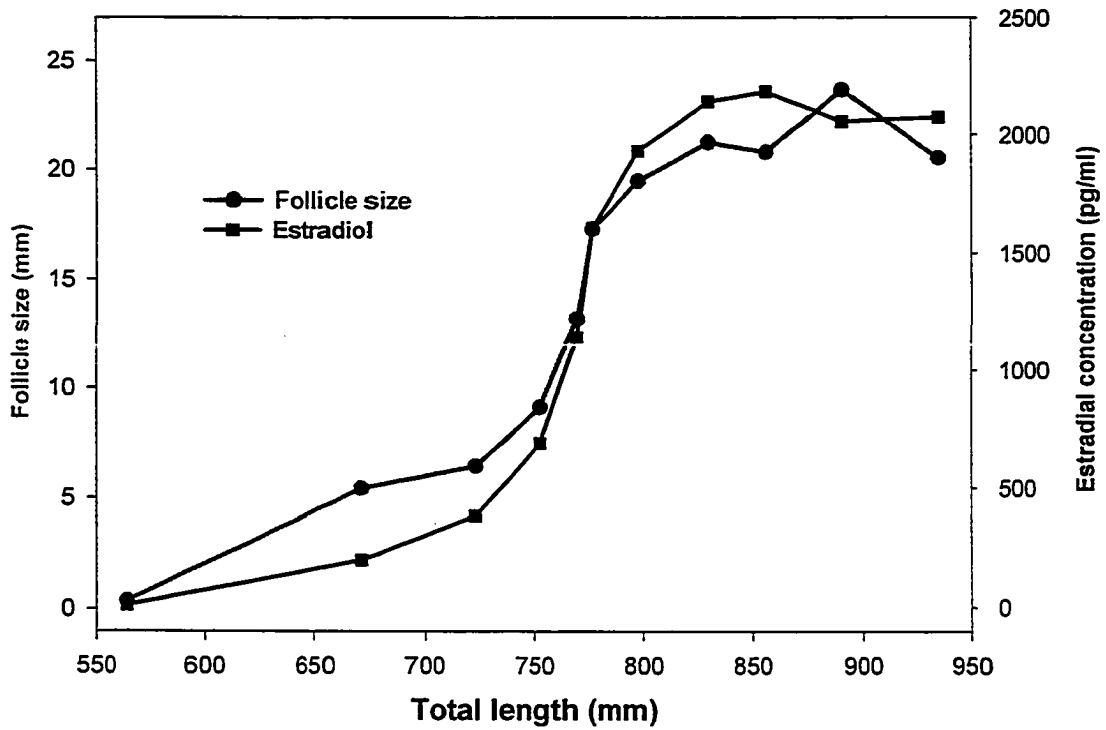
Figure 2.6. Changes in follicle diameter and estradiol concentrations as female winter skates, *L. ocellata,* progress through sexual maturity. Average age is given above each representative size class. Values are expressed as mean ± SEM. For Error bars, sample sizes and statistical significance please refer to Table 2.2.

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age (Figures 2.7A-G). In 8 to 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. Based on the increases in the measured reproductive parameters, particularly the pattern of follicle growth and E_2 levels (Figure 2.6), the smallest mature female measured 735 mm TL and was aged to 11 years. Maturity ogives for females predict that 50% maturity occurs at a total length of 760 mm and between 11 and 12 years of age (Figures 2.4A and 2.4B). In our sampling population, the increases in reproductive tract size, circulating E_2 concentrations and follicle dynamics are in agreement with the ogives. The data in Table 2.2, and Figures 6 and 7, suggest that 50% maturity occurs between 769 and 776 mm total length and between the ages of 12 and 13 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 two years, resulting in 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.
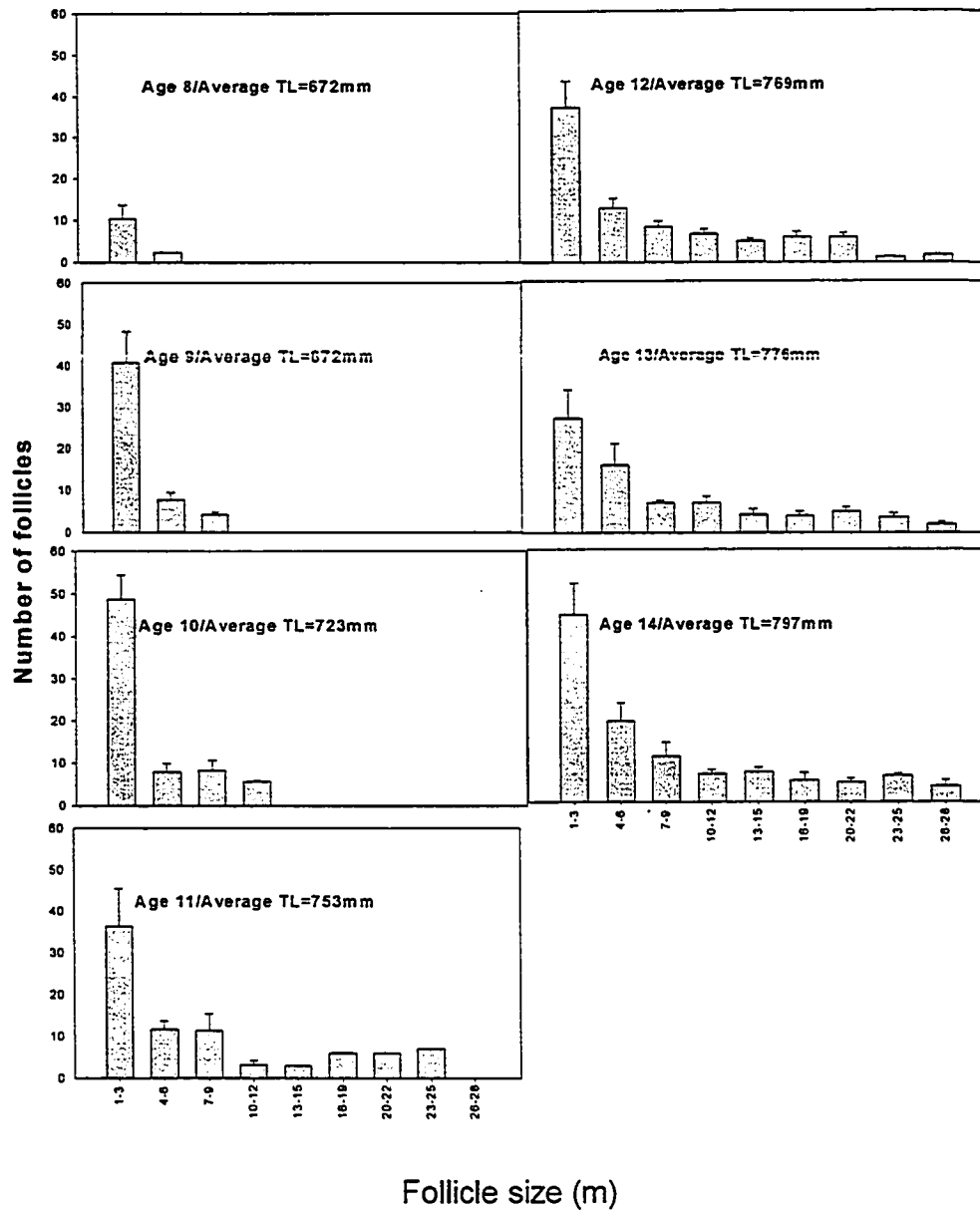
Follicle size (m)

Figures 2.7A-G.   Follicle dynamics in winter skates. Patterns of follicular development (average follicle number verses average follicle size, in mm) as *L. ocellata* mature from age 8 and total length of 672 mm (sexually immature) to age 14 and a total length of 797 mm (sexually mature).   Average follicle number is expressed as mean ± SEM.

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Discussion

Previous studies have profiled steroid hormone concentrations to monitor reproductive cyclicity (*e.g.* Tsang and Callard 1987; Rasmussen and Murru 1992; Manire *et al.,* 1995; Snelson et al. 1997; Tricus *et al.,* 2000) or they have described temporal patterns of gonadal activity in adult elasmobranchs (Dobson 1974; Parsons and Grier 1992; Maruska *et al.,* 1996; Walmsley-Hart 1999; Conrath and Musick 2002). Studies that have evaluated age and size at maturity, have done so largely with descriptive measures, addressing changes in GSI or morphology, such as clasper length in males and ovary weight in females (*i.e.* Walmsley-Hart *et al.,* 1999; Mollet *et al.,* 2000; Francis *et al.,* 2001; Conrath and Musick, 2002). The present study is the first to combine morphological changes such clasper length, pattern of follicle development, histological changes in percent mature spermatocysts, and steroid hormone concentrations to assess development in an elasmobranch as it matures. Furthermore, we used these parameters, in combination with age analyses, to determine the age and size at which an elasmobranch, the winter skate, reaches sexual maturity.

A variety of approaches have been used to estimate age (Frisk *et al,.* 2002) and size (Simon and Frank 1998; Sosebee 2002) at maturity for winter skates. Frisk *et al.* (2002) estimated age at 50% maturity for winter skates at 9 years of age, using empirical life history approaches based on a TL of 113 cm and a life span of 20 years. Simon and Frank, (1998) estimated 50% maturity in females to occur near 750 mm in total length based on follicle characteristics. Sosebee (2002) employed methods developed for assessment of crustacean

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maturity (Somerton 1980) to estimate a TL range at 50% maturity for males between 530 mm to 580 mm and a 50% maturity for females between 660 mm to 730 mm total length. Sosebee's (2002) estimates were 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 the findings of the present study, Frisk *et al.* (2002) may have underestimated the age at 50% maturity by as much as 3 years, while Sosebee (2002) appears to have underestimated total length at 50% maturity by as much as 200 mm in males and 116 mm in females. Indeed, based on the present study's data, the smallest mature male (TL=711 mm , age= 11 years) and mature female (TL=735mm, age=11 years) are older and larger than predicted by empirical analyses of Frisk (2002) and the morphological techniques of Sosebee (2002). Furthermore, maturity ogives for male and female winter skate (Figures 2.4A and 2.4B) support our findings. Moreover, Simon and Frank's (1998) estimate of 750 mm for females using follicle characteristics is very close to the present studies estimation. Collectively, the findings of the present study suggest that analyzing a combination of reproductive parameters, offers a more accurate estimation of age and size at sexual maturity when compared to those calculated from empirical (Frisk, 2002) or morphological measurements (Sosebee, 2000) other than follicle size (Simon and Frank, 1998).

Several studies suggest that an abrupt increase in clasper length marks the onset of sexual maturity in elasmobranchs (*e.g.* Ryland and Ajayi, 1984; Martin, 1982; Zeiner and Wolf 1993; Mollet *et al.,* 2000; Francis *et al.,* 2001; Conrath and

Musick, 2001; Loefer and Sedberry, 2003). Such an abrupt increase also occurs in winter skates (Table 2.1). In *L.ocellata* males, the TL/CL ratio displayed a 34% (P<0.05) increase as the fish matured from age 10 to 11 years (Table 2.1). Changes at the onset of sexual maturity were also observed for the other reproductive parameters in winter skates (Table 2.1). For males maturing from age 10 to 11 years, testes weight increased by 221% , mature spermatocysts by 120% and testosterone concentration by 82%. However, the increase in testes weight was the only measured parameter of the three that changed significantly (P<0.05) during this time. Yoccoz (1991) discussed judging biological significance based on statistical tests, and suggested it is not always associated with statistical significance. This may be the case in our study of sexual maturity in the male winter skate as the abrupt increases in TL/CL ratio, testes weight, testosterone concentration and the inception of mature spermatocysts within the testes appear to signal a biological shift towards maturity in this species (Table 2.1). Moreover, all changes in these measured reproductive parameters , as the skates progressively matured from age 8 to age 14 yeas, were strongly correlated ($r^2 > 0.55$), further suggesting a biological significance to the observed alterations. Previously, Rasmussen and Murru (1992) determined that testosterone concentrations in an immature sandbar, *Carcharhias plumbeus*, and an immature bull shark, *C. leucas*, were considerably lower (P>0.05) than their adult counterparts. As both sharks reached sexual maturity, testosterone concentrations increased to values similar to those found in the sampled adults (Rasmussen and Murru, 1992). In addition, our findings of an association between spermatocyst

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development and testosterone concentration (Figure 2.3) in the winter skate is consistent with *in vitro* experiments that have demonstrated the production of T by Sertoli cells of mature spermatocysts (Callard and Cuevas, 1992). Also, Tricas *et al.*, (2000) reported a maximum peak in T corresponded to maximum spermatocyst density in the Atlantic stingray, *Dasyatis Sabina*.

For females, several studies suggest that an abrupt increase in ovary weight and follicle size marks the onset of sexual maturity (*e.g.* Martin, 1982; Zeiner and Wolf, 1993; Mollet *et al.*, 2000; Francis *et al.*, 2001; Conrath and Musick, 2001; Loefer and Sedberry, 2003). Such changes also occurs in winter skates as ovary weight increased by 95% and shell gland weight by 60% , when the fish matured from 11to 12 years of age (P = 0.1; Table 2.2, Figure 2.5). Other changes for females maturing from age 11 to 12 were also observed in follicle size and estradiol concentration, which increased by 44% and 66%, respectively (Table 2.2, Figure 2.6). Although these changes were not statistically significant (P>0.05), the large observed increases in ovary weight, shell gland weight, follicle size and E_2 concentration may represent a biological significance (Yoccoz, 1991), signaling a shift towards sexual maturity in female winter skates beginning at age 11 years. Similar to our observations in males, all changes in these measured reproductive parameters, as the skates progressively matured from age 7 to 13, were strongly correlated ($r^2 > 0.65$). This is consistent with the results of previous studies on elasmobranchs. For example, Rasmussen and Murru (1992) found E_2 concentrations in two immature grey nurse sharks, *C. taurus*, to be considerably lower (P>0.05) than the sampled adults of this species. Moreover,

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as these sharks progressively matured, E_2 concentrations increased to levels that were observed for female specimens that were known to be sexually mature. This trend was also observed female winter skates (Table 2.2, Figure 2.6). In the oviparous little skate, *L. erinacea*, (Koob *et al.*, 1986), spotted catshark, *Scyliorhinus canicula* (Sumpter and Dodd, 1979), aplacental viviparous dogfish, *Squalus acanthias* (Tsang and 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, this phenomenon was observed in the winter skate as increases in follicle size and E_2 concentrations, as the skates matured, were correlated (r^2=0.64). Moreover, similar to Koob *et al.* (1986) and Tsang and Callard (1987) a strong correlation between shell gland weight, and plasma E_2 concentrations (r^2=0.65) existed in *L. ocellata*. 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 (Figures 2.5, 2.6 and 2.7A-G), the results collectively suggest that these observed changes at sexual maturity may be biologically relevant.

Summary

In summary, the results of the present study indicate that the coordinate examination of morphological and histological parameters, along with steroid hormone concentrations, are a more accurate approach to determining age and size at sexual maturity for the winter skate when compared to other studies that utilized empirical or morphological measurements alone. Moreover, the results

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suggest that hormone analyses alone may be a sufficient technique to elucidate size at maturity. When used in combination with K values and age estimates (male = 0.074 and 19 years, female = 0.059 and 18 years, respectively; Sulikowski *et al.*, 2003), the 50% maturity ogives for total length and age (males 730 mm and 11 years, females 760 mm and between 11 and 12 years, respectively) indicate that the winter skate is a late maturing and long lived species. Like many other elasmobranchs, these characteristics make their populations highly susceptible to exploitation by commercial fisheries (Brander 1981; Kusher *et al.*, 1992; Zeiner and Wolf 1993; Frisk *et al.*, 2001, 2002).

CHAPTER III

SEASONAL CHANGES IN STEROID HORMONE CONCENTRATIONS AND

GONAD DEVELOPMENT IN THE WINTER SKATE *LEUCORAJA OCELLATA*

Introduction

Elasmobranch fish display a wide range of complex reproductive strategies that

includes such attributes as internal fertilization, sperm storage, low fecundity, and

morphological and physiological specializations for either oviparous or viviparous

reproduction (Wourms and Demski, 1993; Hamlett and Koob, 1999). It is assumed that

these strategies are associated with one of the three basic types of reproductive cycles:

1) reproduction throughout the year, 2) a partially defined annual cycle with one or two

peaks, and 3) a well defined annual or biennial cycle (Wourms, 1977; Hamlett and

Koob, 1999). Unfortunately, how the major events that occur during the many phases

of reproduction, such as gamete production, gestation, parturition and oviposition, are

controlled by or associated with cycles in sex steroid hormone production remains

largely unknown for the majority of elasmobranchs. Few studies have characterized the

cyclic changes in gonad development and profiled patterns of sex steroid hormones that

are associated with each of these reproductive strategies (Rasmussen and Murru, 1992;

Manire *et al.*, 1995; Rasmussen *et al.*, 1992; Snelson *et al.*, 1997; Tricus *et al.*, 2000).

Moreover, the available information from the limited number of species that have been

studied, suggests that there are both similarities and differences in hormonal control

among the elasmobranch fish. For example, in the oviparous little skate, *Luecoraja erinacea* (Koob *et al.*, 1986) and aplacental viviparous dogfish, *Squalus acanthias* (Tsang and Callard, 1987), elevated concentrations of estradiol are associated with the follicular phase of egg development. Manire *et al.* (1995) found a peak in progesterone after ovulation, while in the little skate, *L. erinacea*, progesterone was elevated at ovulation. Elevated concentrations of progesterone in the spiny dogfish, *S. acanthias*, may inhibit vitellogenesis induced by estradiol (Callard *et al.*, 1992) and in *L. erinacea* may regulate ovulation, encapsulation, and oviposition (Koob *et al.*, 1986).

According to Winemiller and Rose (1992) and Frisk *et al.*, (2001), elasmobranchs display characteristics of equilibrium strategists. As such, they grow slowly, are relatively long-lived, and have low fecundities. More precise knowledge of their life history, such as reproductive parameters, is essential to guide wise management decisions for these demographically fragile fish that are becoming increasingly exploited (Branstetter, 1993, Stevens *et al.*, 2000; New England Fishery Management Council, 2001). Despite paucity of information, in the few studies conducted so far, the measurement of serum hormone concentrations have yielded useful scientific and practical information concerning elasmobranch reproduction (Rasmussen and Murru, 1992; Parsons and Grier 1992; Snelson *et al.*, 1997; Rasmussen *et al.*, 1999; Tricas 2000). Moreover, when annual endocrinological changes are used in combination with other aspects of reproduction, characterization of gonad development, a more accurate interpretation of the reproductive cycle in elasmobranch is attained (Rasmussen *et al.*, 1996; 1999; Tricas, 2000).

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The purpose of this study was to characterize the circulating concentrations of steroid hormones in relation to changes in gonadal tissue development in the winter skate, *Leucoraja ocellata*. This species was chosen for several reasons. First, *L. ocellata* is a oviparous skate from the family Rajidae that is endemic to the inshore waters of the western Atlantic (Robins and Ray, 1986; Collette and Klein-MacPhee, 2002). Despite a wide range, the only primary literature available on the biology of this species is a description of age and growth characteristics (Sulikowski *et al.*, 2003). Second, studies that have determined the hormonal status of batoids are especially limited. Changes in annual reproductive cycles have only been described for three species, the oviparous clearnose skate, *Raja eglanteria* (Rasmussen *et al.*, 1999), the viviparous torpedo ray, *Torpedo marmorata* (Fasano *et al.*, 1992), and the viviparous Atlantic stingray, *Dasyatis Sabina* (Snelson *et al.*, 1997; Tricus *et al.*, 2000). Finally, assessment studies in the northeast U.S. (Northeast Fisheries Science Center, 1999) suggest over-exploitation of the winter skate may be occurring. To our knowledge, the present study is the first to correlate seasonal changes in plasma hormone titers with specific stages of gonadal development from a feral Rajid population.

Materials and methods

Sampling techniques

Winter skates were captured by otter trawl at locations that ranged from 1.6 to 32 km off the coast of New Hampshire. Approximate depths at these locations ranged from 9 to 107 m. Collection of a target sample size of four adult male and four adult female skates was attempted around the 15th of each month. Immediately after capture, a 5-10 ml aliquot of blood was collected by cardiac puncture using chilled, heparinized

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syringes with a 21 gauge needle. Samples were centrifuged at 1300g for five minutes, and the separated plasma placed on ice for 4-8 and stored at -20°C. Skates were maintained alive on board the vessel until transport to the University of New Hampshire's Coastal Marine Laboratory (CML). Individual fish were euthanized (in a bath of 0.05 MS222 g L^{-1}) before removing reproductive tissues. Total length (TL in mm) was measured as the straight line distance from the tip of the rostrum to the end of the tail, and disc width (DW in mm) as the straight line distance between the tips of the widest portion of pectoral fins. Total wet weight (kg) was also recorded. For males, clasper length was measured as the straight line distance from the posterior point of the cloaca to the end of the clasper. The gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as (gonad weight/total body weight) x 100 and (liver weight/total body weight) x 100, respectively. The epigonal organ was included in both male and female GSI measurements due to its close association with the reproductive tissue (Maruska *et al.*, 1996).

Gross morphology of the female reproductive tract

The female reproductive tract was removed at the UNH CML, and the ovaries, shell glands and uteri separated, blot dried and weighed to the nearest gram. Ovarian follicle dynamics was analyzed by measuring each follicle (calipers) and counting all eggs that were $\geq$ 1 mm in diameter (Tsang and Callard, 1987; Martin, 1982; Snelson *et al.*, 1988). Mean ovary weights, mean shell gland weights and the presence of vitellogenic follicles were used to determine their temporal pattern during the cycle. Females whose reproductive tract contained follicles 26 mm in diameter or larger, a

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shell gland weight of 25 gm or larger and estradiol concentrations of 1000 pg/ml or greater were considered reproductively capable of encapsulation and oviposition.

Histology of the testis

Testes were removed from the skates, blot dried and weighed to the nearest gram. A single 2-3 mm thick segment was removed from the central portion of a single lobe in the medial area of each testis (Maruska *et al.*, 1996), placed in a tissue cassette and fixed in 10% buffered formalin until processed by the UNH Veterinary Diagnostic Laboratory. Here, each sample was dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Prepared slides of testicular tissue were examined using a compound microscope and classified into stages of spermatogenic development based on criteria outlined by Maruska *et al.* (1996), Hamlett (1999) and Tricas *et al.* (2000). Of the distinct stages described in other species, hormone concentrations appear to be associated with stages III through VI (Tricas *et al.*, 2000). For this reason, we concentrated on describing the spermatogenic changes that occurred in stages III through VI in the winter skate (Figure 3.1). Briefly, in Stage III, spermatocysts contain early spermatocytes and growing Sertoli cells. Stage IV exhibits spermatids within the spermatocysts. The characteristics of Stage V include immature spermatozoa which associate with Sertoli cells but are unorganized within the spermatocysts, and Stage VI is characterized by mature sperm that are organized into packets around the inner periphery of the spermatocysts (for a full review of each stage, please see Maruska *et al.*, 1996). The mean proportion of testes occupied by each stage was measured along a straight line distance across one representative full lobe cross section of the testes (Maruska *et al.*, 1996; Conrath and Musick, 2002).

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Stage III   Stage IV
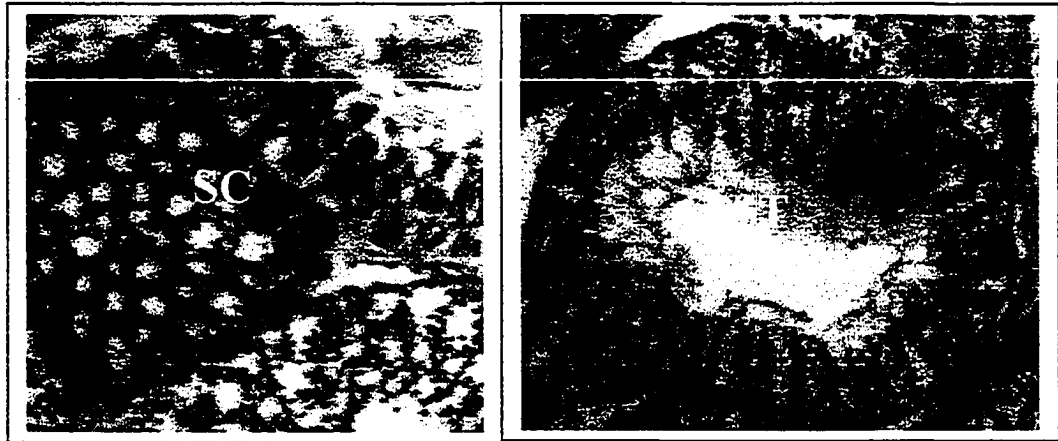
Stage V   Stage VI
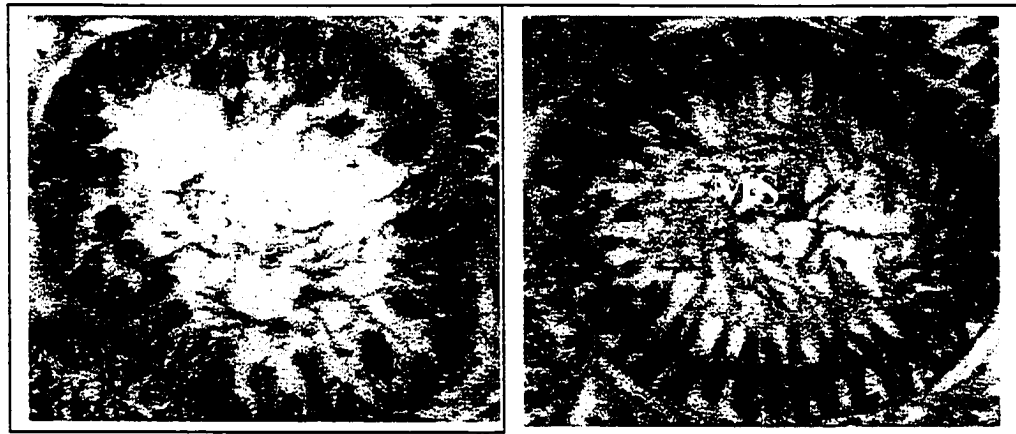
5 *u*m _____

Figure 3.1. Histological section of an *L. ocellata* testis at 10x magnification representing the sperm stages III to VI. Spermatocytes=SC, spermatids= ST, immature sperm =IM, mature sperm= MS.

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<u>Analysis of steroid hormones</u>

Stock solutions of radiolabeled testosterone (T) and estradiol (E$_2$) (Amersham Biosciences, Piscataway, NJ) but not progesterone (P$_4$) (Perkin Elmer Life Sciences, Boston, MA) were purified by thin layer chromatography (TLC). The following radiolabeled steroids were used in this study: [1,2,6,7-^{3}H] testosterone (TRK 92; specific activity= 101Ci/mmol), [2,4,6,7,16,17-^{3}H] estradiol (TRK 587; specific activity = 152Ci/mmol), and [1,2-^{3}H] progesterone (NET20825UC; specific activity = 53Ci/mmol).

Silica gel plates (HLF, scored 20 x 20 cm, 250 microns) were obtained from Analtech, Inc (Newark, DE). All organic solvents (ACS certified) were obtained from Fisher Scientific (Pittsburg, PA). For E$_2$ the solvent system was ether:hexane, 3:1 part and for T, the solvent system was benzene:acetone, 4:1 part. Adjacent lanes were spotted with respective nonradioactive standards (20 µg/10 µl absolute ethanol). Each plate was run twice before viewing under short wave ultraviolet light to localize the steroid standards by fluorescence. The silica gel areas containing radiolabeled steroid, which co-migrated with the respective standard, were scraped from the plate into 13 x 100 mm borosilicate glass tubes containing 0.4 ml water and extracted (3x) with 2.0 ml methanol. A 100 ul aliquot was removed from the combined methanol fractions to determine recovery before evaporation under nitrogen. The dried extract was reconstituted in absolute ethanol to yield a stock concentration of 5 uCi/ml for T and 8 uCi/ml for E$_2$. The stock concentration for P$_4$ was 5 uCi/ml. They were stored at - 20°C.

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Aliquots of 0.6 ml plasma were pipetted into 16 x 100 mm borosilicate tubes and approximately 1000 counts per minute (cpm) of an appropriate radiolabeled steroid was added to account for procedural losses. Each sample was extracted with 10 volumes of diethyl ether (anesthesia grade) by vortexing for 1 minute. The aqueous layer was snap frozen in an acetone/dry ice bath before decanting the organic layer into 16 x 100 mm borosilicate glass tubes. The ether was then evaporated to dryness in a $37^{\circ}C$ water bath under a stream of nitrogen (Airgas East, Dover, NH). The aqueous phase was re-extracted as described above and the extracts combined. Buffer blanks, charcoal stripped plasma and plasma spiked with steroids were processed in an identical manner. To test for linearity and parallelism the R^2 for standard curve/samples were as follows; R^2 for T= 0.98/.097, R^2 for E_2= 0.97/0.98, R^2 for P_4= 0.96/0.94). The dried extract was reconstituted in a volume of phosphate buffered saline with 0.1% gelatin (PBSG; 0.06M $Na_2HPO_4*7H_2O$, 0.04M $NaH_2PO_4*H_2O$, 0.15M NaCl, 0.1% sodium azide) equivalent to the plasma aliquot and stored at $-20^{\circ}C$. The overall mean recoveries for P_4, E_2 and T were 68%, 76% and 74% respectively. Each sample was correct for recovery.

Plasma concentrations of testosterone, estradiol-17β, and progesterone were determined by radioimmunoassay modified from procedures previously outlined by Tsang and Callard (1987) and Goldberg et al. (1996). All nonradioactive steroids were obtained from Steraloids, Inc (Wilton, NH). Two aliquots of the reconstituted extract in duplicate were placed in 12 x 75 mm borosilicate tubes and brought to 0.2 ml with PBSG. All tubes received 0.1 ml of the appropriate labeled steroid (containing

approximately 10,000 to 15,000 cpm) and 0.1 ml of diluted antiserum. The final concentration of the antibodies were 1:64,000 for T (#250 anti-testosterone-11-BSA; from Gordon Niswender, Colorado State, Fort Collins, CO), 1:200,000 for E_2 (#244 anti-etradiol-6-BSA; from Gordon Niswender, Colorado State, Fort Collins CO), and 1:10,000 for P_4 9#337 anti-progesterone-11-BSA; from Gordon Niswender, Colorado State, Fort Collins CO). The characteristics of the E_2, T, and P_4 antibodies have been described by Korenman et al., (1974), Gay and Kerlan (1978) and Gibori et al. (1977), respectively. All tubes were incubated at 4°C overnight. Free steroids were separated from bound using 1.0 or 0.75 ml of a suspension containing 0.2% washed Norit A charcoal with 0.02% Dextran T-70 (Amersham Pharmacia Biotech, Piscataway, NJ) in PBSG. After mixing, the tubes were incubated for 10 minutes at 4 °C, followed by centrifugation at 2500 *rpm* for 10 minutes at 4°C. The supernatant was decanted into 7 ml minivials (USA Scientific Inc., Ocala, Fl) containing 3.5 ml Ready Safe™ scintillation cocktail (Beckman Coulter, Somerset, NJ). Radioactivity was determined in a Beckman LS6000IC (Fullerton, CA) liquid scintillation counter. A logit bound vs. log dose standard curve was used to interpolate hormone concentrations. The intrassay coefficients of variance were 5.5% for E_2, 8.6% for T and 5.1% for P_4. The interassay coefficients of variance were 12.8% for E_2, 10.4% for T, and 12.1% for P_4.

Statistics

The results are presented as mean ± 1 SEM, and significant differences were evaluated by Kruskal Wallis analysis of variance followed by a tukey's post-hoc test. Statistical significance was accepted at P < 0.05.

One hundred thirty two winter skates were collected over the 18 month sampling period. Mature female skates (n=66) ranged in size from 735-940 mm (mean = 823 ± 3.4) and from 3.0-7.3 kg (mean= 5.1 ± 0.1) in total body mass. Mature male skates (n=66) were slightly larger in size, ranging from 750-960 mm TL (mean = 840 ± 6), but weighed slightly less, ranging from 2.5-6.0 kg (4.6 ± 0.1).

Reproductive parameters

In general, numerical values for ovary weight (Figure 3.2A), shell gland (Figure 3.2B), and size of the largest follicle (Figure 3.2C), displayed a steady increasing trend in females collected from January to July and August before declining through December (Table 3.1). Statistical analyses revealed that ovary weight and the size of the largest follicle in July and August animals were significantly different (P<0.05) than in January animals. Similarly, the shell gland weight in July skates was heavier (P<0.05) than in January skates. The GSI followed a comparable pattern, with September animals having a greater index (P<0.05) than those in January. No significant differences were observed in HSI. Notably, it is interesting that reproductively capable females with ovarian follicles greater that 25 mm in diameter (presumptive preovulatory follicle; Figure 3.3) were captured during most months of the year (Figure 3.4). However, more females with these presumptive ovulatory follicles were captured from June through September, which coincides with the cyclic trends in ovary weight, shell gland weight and estradiol concentrations (Figures 3.2A-C, 3.5A).
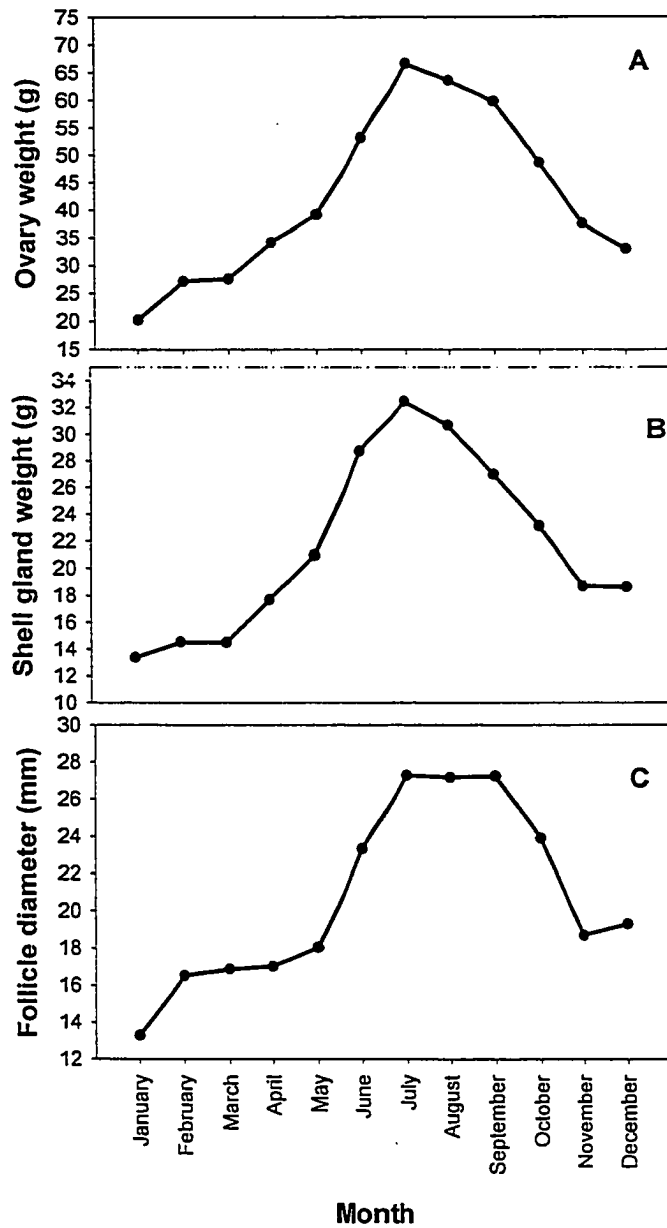
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Figure 3.2A-C. Seasonal changes in the ovary weight (A), shell gland weight (B) and

follicle size (C) during each month for *L. ocellata*. All values are expressed as mean ±

SEM. For standard error, please refer to Table 3.1.

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Table 3.1. Measured reproductive parameters and serum hormone concentrations for female winter skates, *Leucoraja ocellata*. Values are mean ± SEM. Significant differences were evaluated by Kruskal Wallis analysis of variance followed by a Tukey's post-hoc test ($P < 0.05$). Asterisks signify significant difference between all groups. Months with designated different letters were found to be significantly different from each other.

Month	N	Average ovary (gm)	Average shell gland (gm)	Largest follicle size (mm)	Estradiol (pg/ml)	Testosterone (pg/ml)	Progesterone (pg/ml)	GSI	HIS
J	5	20 ± 3^{A}	13 ± 2^{B}	13 ± 1^{A}	775 ± 316^{CF}	2107 ± 854	76 ± 7	0.41^{B}	9.27
F	4	27 ± 4	15 ± 6	17 ± 4	1014 ± 216^{DF}	2924 ± 731	72 ± 14	0.47	8.60
M	9	28 ± 9	15 ± 4	17 ± 3	1099 ± 298^{EF}	4610 ± 1375	188 ± 64	0.64	5.30
A	7	34 ± 7	18 ± 4	17 ± 2	1638 ± 159	3222 ± 911	210 ± 28	0.62	7.95
M	6	39 ± 6	21 ± 4	18 ± 2	2408 ± 561	3101 ± 703	98 ± 37	0.66	8.59
J	6	53 ± 6	29 ± 2	23 ± 2	3425 ± 878^{A}	3428 ± 467	180 ± 36	0.89	7.41
J	5	67 ± 4^{BD}	33 ± 3^{A}	27 ± 1^{BD}	$5143 \pm 521^{*}$	1295 ± 210	$109 \pm 28^{*}$	1.18	7.63
A	6	64 ± 7^{CD}	31 ± 3	27 ± 1^{CD}	2787 ± 427	2073 ± 591	124 ± 38	1.15	9.03
S	3	60 ± 9	27 ± 1	27 ± 1	2628 ± 283	3973 ± 584	436 ± 146	1.23^{A}	6.47
O	4	49 ± 7	23 ± 1	24 ± 2	1736 ± 572	3981 ± 1651	1114 ± 407	1.00	6.90
N	5	38 ± 11	19 ± 4	19 ± 4	1646 ± 298	3086 ± 735	412 ± 127	0.91	5.66
D	5	33 ± 8	19 ± 3	19 ± 3	704 ± 90^{BF}	2334 ± 499	248 ± 154	0.76	6.71

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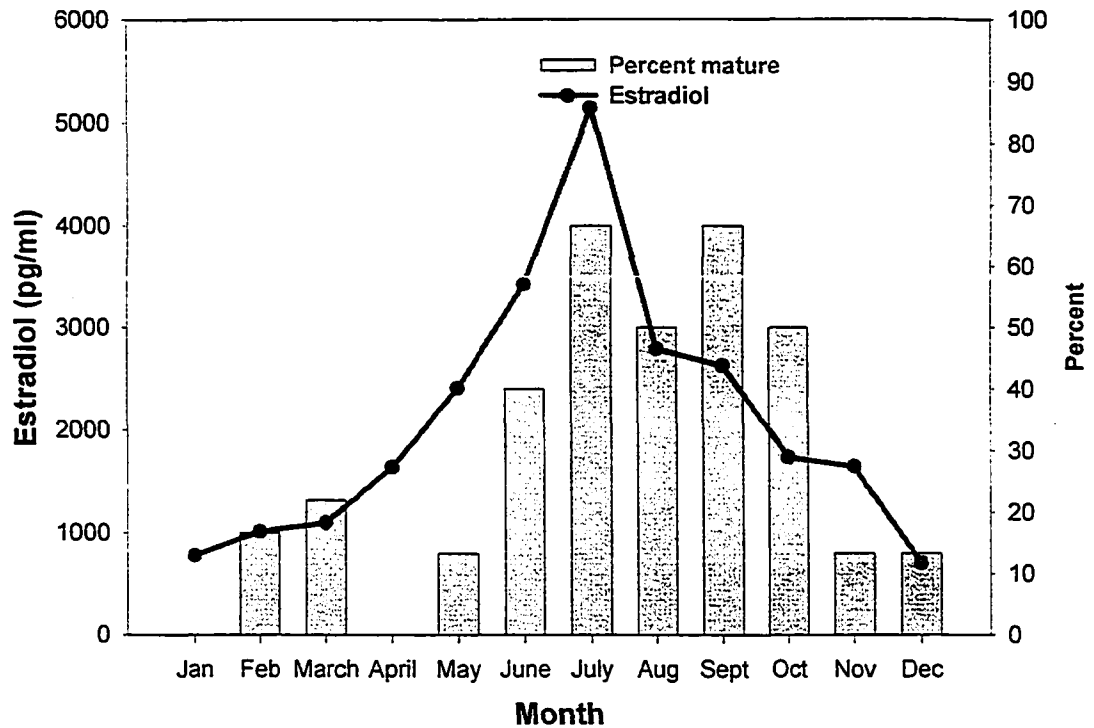
Figure 3.3. Proportion of reproductively capable female winter skates, *Leucoraja ocellata*, from each month within the sampled population. Reproductive capability was based on the presence of pre-ovulatory follicles (26 mm in diameter or larger), shell gland weight (25 gm or larger) and estradiol concentration (1000pg/ml or greater). More reproductively capable females were captured from June through September, which coincides with the cyclic trends in ovary weight, shell gland weight and estradiol concentrations (shown on above).

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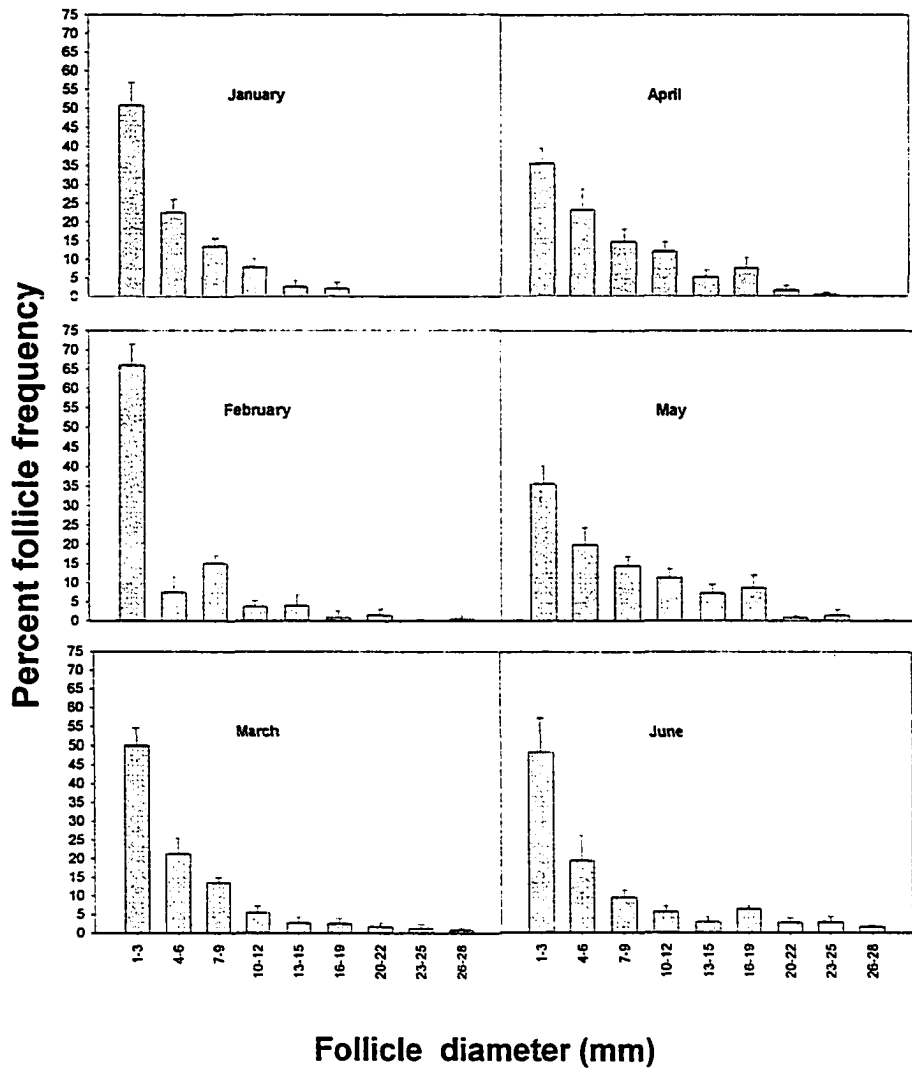
Figure 3.4A. Seasonal changes in the follicle dynamics of the winter skate. Patterns of

follicular development (percent frequency verses average follicle size, in mm) from

January to June for *L. ocellata.* Average follicle number is expressed as mean ± SEM.

Follicle diameter (mm)
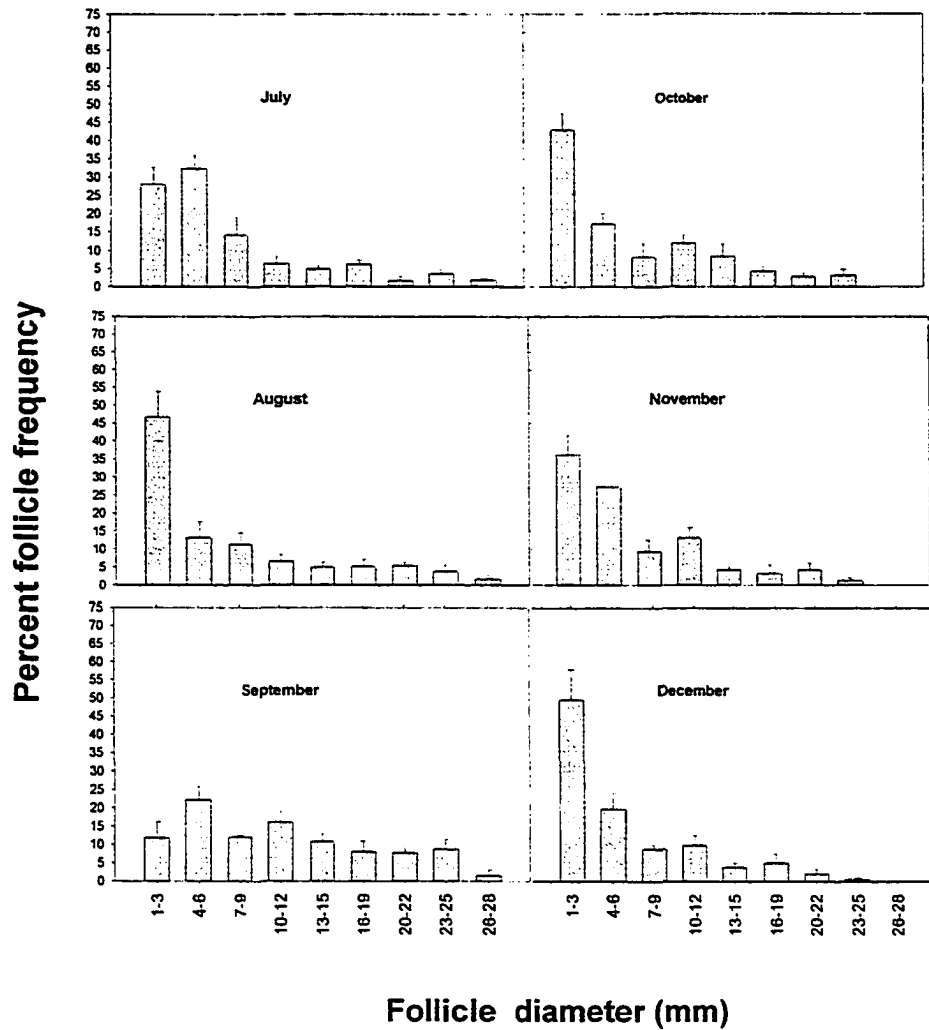
Figure 3.4B. Seasonal changes in the follicle dynamics of the winter skate. Patterns of follicular development (percent frequency verses average follicle size, in mm) from July to December for *L. ocellata*. Average follicle number is expressed as mean ± SEM.

Steroid hormone concentrations-Females

Estradiol concentrations displayed a distinct pattern from January to December (Table 3.1, Figure 3.5A). Based on our observations of numerical values, there was a steady increasing trend in E_2 concentrations in skates collected from January to July followed by a progressive decreasing tend from July to December. Statistical analyses revealed that E_2 concentrations peaked in June and July (P>0.05 for these two months), with the July value being greater (P<0.05) than the other 10 months of the study. In addition, E_2 concentrations were correlated to ovary weight (r = 0.57), shell weight (r = 0.53) and size of the largest follicle size (r = 0.57). Furthermore, although a similar trend existed between E_2 concentration and GSI, they were weakly correlated (r = 0.24), while no clear association (r = 0.14) was observed for E_2 concentration and HSI over the course of the study (Table 3.1).

Testosterone concentrations in the plasma of female winter skates were maintained at relatively high concentrations over the course of the study (Table 3.1, Figure 3.5B). Although July animals exhibited the lowest numerical T concentration, which was coincident with the peak in E_2, no significant statistical differences were detected between monthly samples for this androgen. Unlike E_2 concentrations, T concentrations were weakly correlated to ovary weight (r = 0.11) and size of the largest follicle (r=0.16), and negatively correlated to shell weight (r = -0.02), GSI (r = -0.30) and HSI (r = -0.18) over the course of the sampling period.

Progesterone concentrations, overall, were numerically lower than E_2 and T. In October, P4 concentrations peaked and were greater (P<0.05) than samples collected for all other months of the study (Table 3.1 Figure 3.5C). No correlations were found
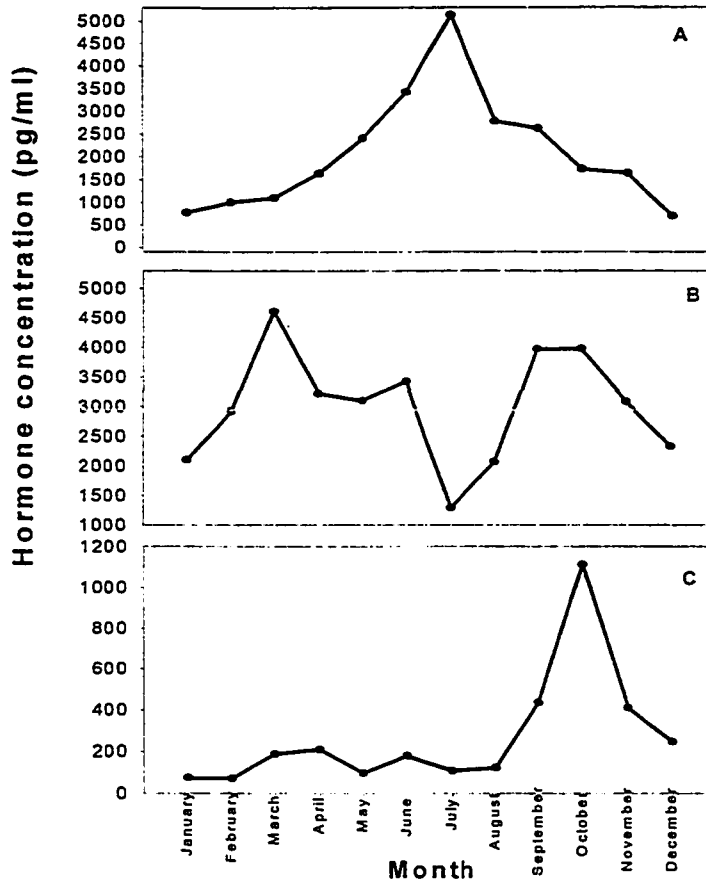
Figure 3.5. Plasma steroid hormone concentrations in female winter skates, *Leucoraja ocellata*. Female estradiol (A), testosterone (B) and progesterone (C) each display a unique seasonal pattern. However, during September through December, E_2 concentration displays a marked decrease while T and P_4 display a coincident surge which peaks in October, followed by a decline in November and December. The coincident increases in T and P_4 concentrations during the months of September, October and November suggest a possible interaction of these hormones in relation to the events leading to and involving encapsulation and ovipostion. Standard error of the mean values are given in Table 3.1. Significant differences were evaluated by Kruskal Wallis analysis of variance followed by a tukey's post-hoc test ($P < 0.05$).

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between P_4 and any of the other measured reproductive parameters.

Plasma steroid hormone concentration-males

Testosterone production was robust in male skates throughout the year (Table 3.2, Figure 3.6A). From January to June, concentrations declined, reaching a nadir in June ($P<0.05$). Then, T concentrations numerically increased in July before a decreasing trend was observed through November (Table 3.2, Figure 3.6A). Furthermore, there was little to no relationship between GSI; ($r^2 = 0.02$) or HIS ($r^2 = -0.12$) and T concentration.

Compared to testosterone, plasma E_2 concentrations were relatively low throughout the year (Table 3.2, Figure 3.6B). A similar trend was observed between male T and E_2 concentrations. Starting in February, values numerically declined where E_2 concentrations increased from May to June. However, this increase was not observed until June and July for T values. Thereafter, both declined numerically until September. This association was reflected by a correlation ($r = 0.38$) between these two hormones. No relationship existed between GSI or HSI and estradiol concentration.

Steroid Production and testis development

Developing spermatocysts in the winter skate testis has been characterized as a series of histologically identifiable gametogenic stages. In the present study the histological stages III through VI (SIII-SVI) were examined in relation to T and E_2 concentrations for 48 males collected during the sampling period, except for the months of May and September (Figure 3.1). Stage III is characterized by development of spermatocysts and associated Sertoli cell growth and migration. From January to December, the percent testis occupied by SIII was inversely related to T and E_2

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Table 3.2. Measured reproductive parameters and serum hormone concentrations for male winter skates, *Leucoraja ocellata*. Values are mean ± SEM. Significant differences were evaluated by Kruskal Wallis analysis of variance followed by a tukey's post-hoc test (P < 0.05). Months with designated different letters were found to be significantly different from each other.

Month	N	Testosterone (pg/ml)	Estradiol (pg/ml)	Gonadosomatic index	Hepatosomatic index
January	7	60670 ± 6642 [B]	78 ± 7	0.44 ± 0.02	4.7 ± 1
February	9	54133 ± 6326	106 ± 21	0.53 ± 0.06	5.1 ± 1
March	12	48941 ± 4374	97 ± 14	0.48 ± 0.02	4.5 ± 0.4
April	5	44129 ± 12237	72 ± 20	0.49 ± 0.04	4.9 ± 0.3
May	4	44129 ± 12237	30 ± 10	0.53 ± 0.04	4.2 ± 0.3
June	4	22242 ± 2938 [A]	106 ± 26	0.43 ± 0.05	6.3 ± 0.7
July	3	51861 ± 4332	106 ± 34	0.44 ± 0.01	5.9 ± 0.3
August	4	47321 ± 3062	77 ± 12	0.43 ± 0.02	4.4 ± 0.1
September	3	33788 ± 17407	76 ± 8	0.47 ± 0.03	4.4 ± 0.1
October	4	31567 ± 2633	117 ± 21	0.50 ± 0.03	5.9 ± 0.0
November	5	31658 ± 3290	50 ± 11	0.42 ± 0.01	5.5 ± 0.7
December	4	54732 ± 14045	41 ± 11	0.51 ± 0.02	5.3 ± 0.3
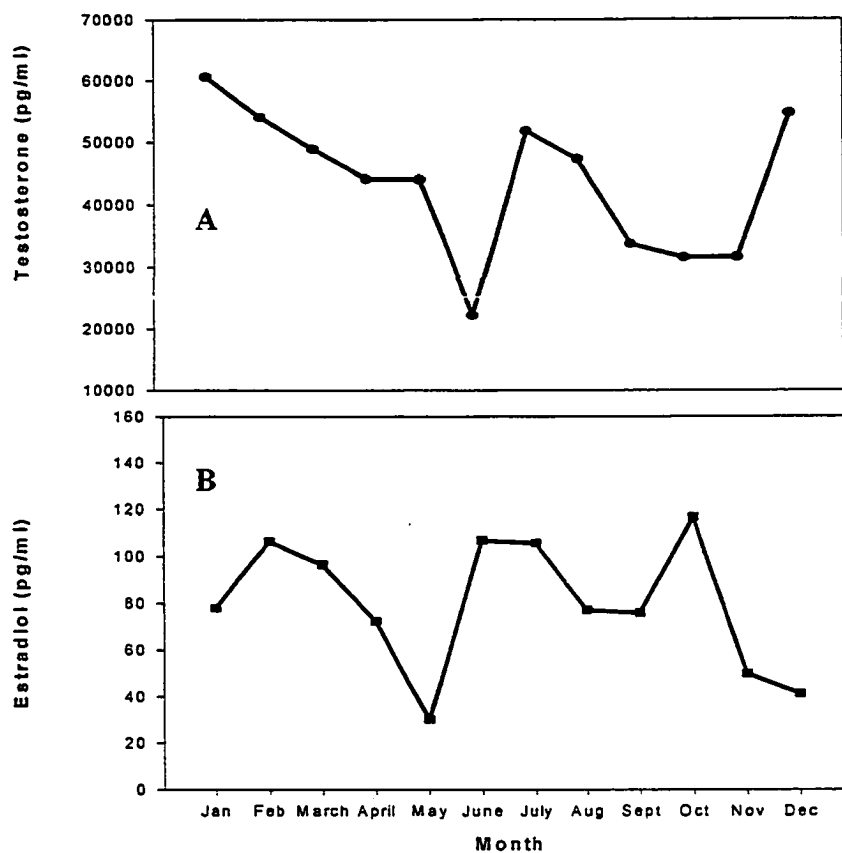
Figure 3.6. Plasma steroid hormone concentrations in male winter skates, *Leucoraja ocellata*. Testosterone concentrations (A) in male skates displayed a seasonal cycling that consisted of two peaks; one in December and January, and the other in July during the 12 sampling period. Similar to testosterone, male estradiol concentrations (B) also demonstrate a periodic cycling with peaks in February, July and December. Standard error of the mean values are given in Table 3.2. Significant differences were evaluated by Kruskal Wallis analysis of variance followed by a tukey's post-hoc test ($P < 0.05$).

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concentrations. Lower concentrations of T (as observed in June, October and November samples; Figure 3.7A) and higher concentrations of E_2 (as observed in June, October and November samples; Figure 3.8A) were associated with the greater percent of SIII. Overall, there was no observable relationships between patterns of T and E_2 concentrations and spermatogenic stages IV (characterized by secondary spermatocytes becoming spermatids, Figures 3.7B and 3.8B) and V (characterized by immature spermatozoa Figure 3.7C and 3.8C). However, there was a coordinate trend Between the formation of mature spermatocysts (SIV) and T concentrations (Figure 3.7D). Conversely, while the pattern of E_2 concentration also paralleled the proportion of stage VI spermatocysts from January to September, an inverse relationship was observed from October to December (Figure 3.8D).

Discussion

Steroid hormones and the reproductive cycle in females

The results of the present study indicate that female winter skates displayed a partially defined annual reproductive cycle. This conclusion was based on concurrent peaks in ovary weight, shell gland weight, size of the largest follicle, follicle dynamics and E_2 concentrations. These indicators alone would suggest a well defined annual reproductive cycle for this species, but the fact that reproductively capable females were found during most months of the year implied that reproduction can occur anytime (Figure 3.3). As a result a clear annual cycle has not been defined. This type of reproductive cycle is similar to the oviparous little skate, a cogener species, which displays a partially defined annual cycle consisting of two peaks (Johnson, 1979). Although the population as a whole is reproductively active all year, a higher

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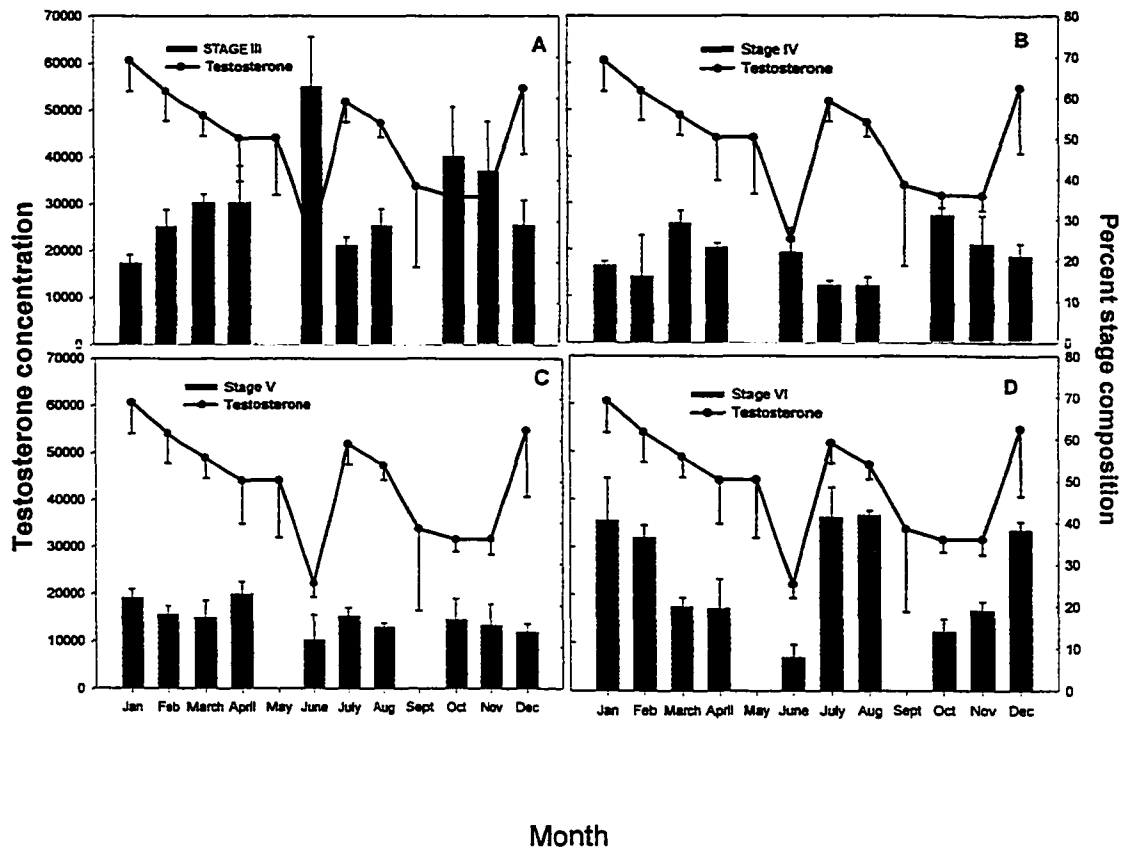
Figures 3.7A-D. Stages of testes development and changes in testosterone levels in male winter skates, *Leucoraja ocellata*. The percentages of gametogenesis (SIII–SVI) were determined by measuring a transect line across one representative full lobe cross section of the testes. The mean proportion each stage occupied along that straight line was then calculated. Steroid concentrations for testosterone are expressed as pg/ml. Testosterone and mature spermatocytes (SVI) display a coincident peak in activity during January, July and December, while testosterone displayed an inverse relationship with primary and secondary spermatocytes (SIII) over the course of the study.

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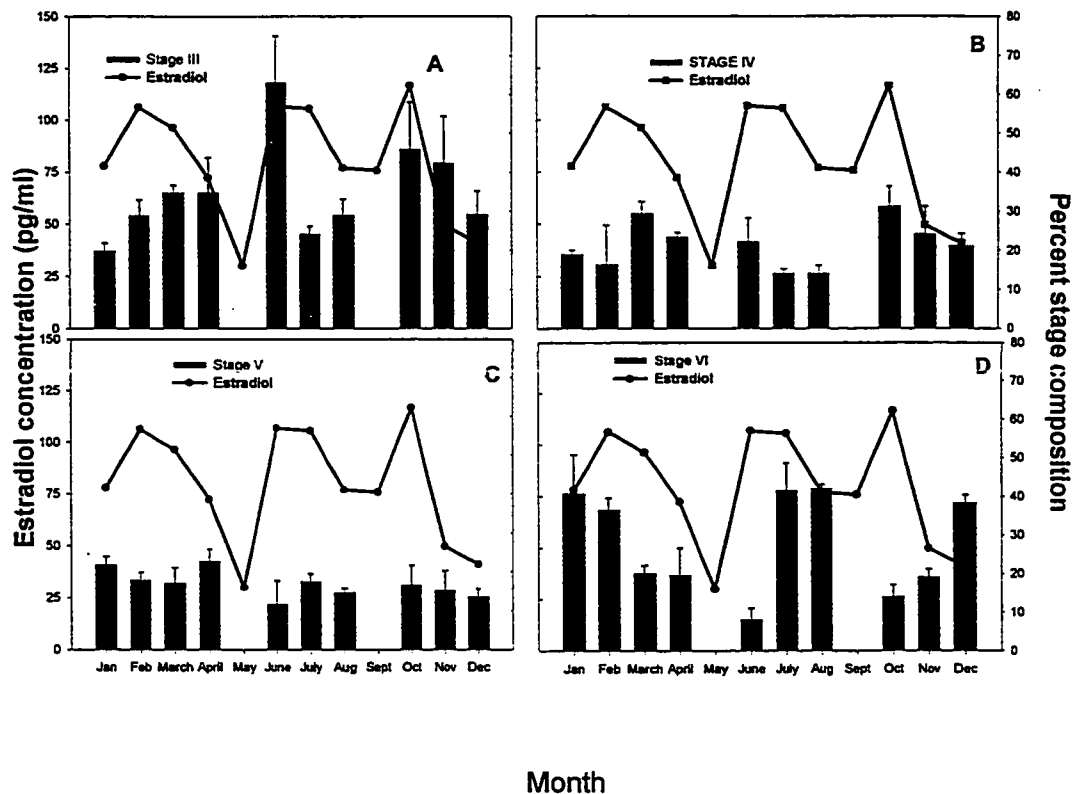
Figures 3.8A-D. Stages of testes development and changes in estradiol levels in male winter skates, *Leucoraja ocellata*. The percentages of gametogenesis (SIII–SVI) were determined by measuring a transect line across one representative full lobe cross section of the testes. The mean proportion each stage occupied along that straight line was then calculated. Steroid concentrations for estradiol are expressed as pg/ml. Male E_2 concentrations displayed a periodic cycling with two peaks in February and November and a prolonged peak in June and July. The elevation of E_2 during June and October, closely follows testis surge in SIII spermatocytes and a decline in T concentration (Figure 3.7A).

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proportion of females are reproductively active during the observed peaks (Richards *et al.* 1963).

In female winter skates, there is a strong association between E_2 concentration and ovary weight, shell gland weight and size of the largest follicle. A similar relationship was observed by Koob *et al.* (1986) and Tsang and Callard (1987) in the little skate, and the spiny dogfish, respectively. This pattern of association between elevated E_2 concentrations and egg development during the follicular phase has also been documented in several other elasmobranch species (Sumpter and Dodd 1979; Rasmussen and Gruber, 1993; Manire *et al.* 1995; Snelson *et al.* 1997; Tricas *et al.*, 2000), and would suggest that the ovarian follicle is a source of E_2 production. Indeed, follicular cells from large and small follicles of *L. erinacea* produce E_2 (Tsang and Callard, 1982). In addition, in the spiny dogfish the granulosa and thecal cells of the follicle synthesize both T and E_2 (Tsang and Callard, 1992). Thus while it appears that ovarian follicles may be a possible site of E_2 biosynthesis in the winter skate, this awaits further confirmation by future in vitro studies.

Previously, Koob *et al.* (1986) reported that E_2 and T are the predominant steroids during the follicular phase of the little skate. Similarly, high concentrations of E_2 and T were present in the plasma of the winter skate throughout the year. Because no statistical differences were detected between any month during the year and no clear correlations existed between reproductive parameters and T concentrations, the role of this hormone in the winter skate is open to speculation. Elevated testosterone concentrations were observed during the months of September, October and November which coincided with peak egg case formation and oviposition (Figure 3.5B). This is in

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contrast to the little skate (Koob *et al.*, 1986), where testosterone concentrations were low during egg case production, but similar to the clearnose skate (Rasmussen *et al.*, 1999) where increases in testosterone concentrations coincided with the observations of egg cases in both the uterus and cloaca. Studies have correlated testosterone titers with follicle development in the little skate (Koob *et al.*, 1986) and the spiny dogfish (Tsang and Callard, 1992), implicating follicles as the principle source of this hormone. An earlier study by Tsang and Callard (1982) suggest that T is a substrate for E_2 biosynthesis by follicular cells. Addition of increasing concentrations of T substrate to cells obtained from both small (5-10mm) and large (>10) follicles resulted in corresponding increases in E_2 production. To this end, in July winter skates, ovary weight, shell gland weight and size of the largest follicle peaked. This occurred when E_2 concentrations were highest and when T concentrations were at a numerical nadir. Future studies are necessary to determine if aromatase activity is increased at this time.

Among the three steroids, plasma P_4 concentrations were overall the lowest. A significant peak occurred in October, coincident with our observation that egg case production appeared to begin in September, peak in October then decline during November. Simon and Frank (1998) report similar findings for winter skates captured off the coast of Sable Island, Canada. Previously, Koob *et al.* (1986) reported a strong relationship between elevated P_4 concentrations and egg retention and oviposition in the little skate. In the clearnose skate, the greatest frequency of P_4 elevations occurred immediately after oviposition (Rasmussen *et al.*, 1999). Furthermore, a transient pulse of P_4 was associated with parturition in the Atlantic stingray (Snelson *et al.*, 1997; Tricas *et al.*, 2000). For the winter skate, the average P_4 concentrations for September,

October and November were 638 ± 140 pg/ml while the average P_4 concentrations for the other nine months were 139 ± 15 pg/ml. Based on results gathered on other species and our findings, P_4 in winter skates collected in September, October and November may have a role in encapsulation and oviposition.

Male steroid hormones and spermatocyst development

The results of the present study show that T concentrations were associated with spermatogenesis in the winter skate. A similar observation in the Atlantic stingray was reported by Tricas *et al.* (2000). In *L. ocellata*, higher concentrations of T paralleled the increase in percent composition of spermatocyst and their association with the presumed steroidogenic Sertoli cells (Figure 3.7D). On the other hand, T concentrations and the percent composition of SIII in the testes were inversely related. The cellular sources of T biosynthesis within the winter skate testis remain to be determined. However, our finding of a close association between mature spermatocyst development and T production in the winter skate is consistent with invitro experiments that demonstrate T production by the Sertoli cells of *S. acanthias* spermatocysts (Du Bois *et al.*, 1989; Cuevas and Callard, 1989). It is also important to consider the possible action of other androgens not measured in this study, such as 11-ketotestosterone, which is the most active form in teleosts (Borg, 1994), and dihydrotestosterone (DHT). Manire *et al.* (1999) recently reported 11-ketotestosterone in the bonnet head shark, while DHT in male *D. sabina* has been shown to closely follow the production of T.

The lack of correlation between GSI or HSI and T concentrations in the winter skate was not surprising since studies do not support the assumption that relative gonad size and reproductive readiness are not positively correlated (Teshima 1981; parsons

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and Grier 1992; Maruska *et al.* 1996). For instance, neither peak sperm production (Maruska *et al.* 1996) nor the pattern of androgen hormones (Snelson *et al.*, 1997) were correlated to GSI in *D. Sabina*.

The results of the present study show that E_2 concentrations were associated with SIII spermatocytes. Tricas *et al.* (2000) described a distinct periodic increase in serum E_2 that closely followed testis growth and the first surge in T concentration in the Atlantic stingray. Interestingly, the SIII and E_2 increases in the winter skate did not coincide with surges in T concentration. In the testis of spiny dogfish, Callard *et al.* (1985) demonstrated that aromatase activity is highest in the primary and secondary spermatocytes. Based on Callard *et al.'s* study (1985), the occurrence of high E_2 concentrations coinciding with peak spermatocyte activity (SIII) is not unexpected in the winter skate. As the Atlantic stingrays reproductive season progressed Tricas *et al.* (2000) noted a continued decline in E_2 during the second surge in *D. sabina* T concentration. Tricas *et al.* (2000) suggested that the decline in E_2 indicates aromatase activity may diminish as spermatocytes mature, or possibly the production of an aromatase inhibitor may be effecting the concentration of E_2. This may be the case in the winter skate as E_2 concentrations declined or were at reduced levels during SVI proliferation. If, or, how aromatase activity plays a role in spermatocyst formation requires further investigation.

Although a few trends existed, no clear pattern between E_2 concentrations and GSI was exhibited during the course of the study. For example, the peaks in January and Octobers for GSI and E_2 concentrations coincided, while May and December surges in GSI were offset by declines in E_2 concentrations. In contrast, serum levels of

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E_2 displayed a pattern which correlated with testicular regression and recrudescence in the Atlantic stingray (Snelson *et al*. 1997). For this species, E_2 levels increased in late summer and reached maximum levels in the fall when GSI levels were largest. This pattern, along with the data from Tricas *et al*. (2000) suggests that E_2 may be involved in the initial phases of gonadal maturation and spermatogenesis in the Atlantic stingray.

<u>Summary</u>

In summary, our data suggests annual cycling of steroids is closely associated with specific phases of gametogenesis in male winter skates. In female winter skates, a strong association between E_2 concentration and ovary weight, shell gland weight and size of the largest follicle existed over the course of their reproductive cycle. Further, it appears that males are capable of producing mature spermatocysts on an annual basis. When combined with the information regarding reproductively capable females within the sampled population, our data implies reproduction can occur through-out the year, but appears to peak in July for this species.

SYNTHESIS AND FUTURE DIRECTIONS

The results of this study have provided important information regarding the life history of the winter skate, *Leucoraja ocellata*, captured within the western Gulf of Maine. Estimates of von Bertalanffy growth parameters suggest that females attain a slightly larger asymptotic TL (L_∞=1374mm) than males (L_∞=1218mm) and grow slower (k=0.059 and 0.074, respectively). The oldest ages obtained for the winter skate were 19 years for males and 18 years for females, which corresponded to total lengths of 932mm and 940mm, respectively. Maturity ogives for males, based on data gathered for TL/CL ratio, circulating testosterone concentrations, and proportion of mature spermatocytes within the testes, predict that 50% maturity occurs at a total length of 730 mm and at 11 years. For females, maturity ogives, based on data gathered for ovary weight, shell gland weight, follicle size and circulating estradiol concentrations predict that 50% maturity occurs at a total length of 760 mm and between 11 and 12 years of age. Reproduction can occur throughout the year in this species, but appears to peak in July. Egg case formation appears to begin in September, peak in October and then decline in November. Overall, when these results are combined, they indicate that the winter skate is a late maturing, slow growing, long-lived species, with an apparent succinct reproductive cycle. Like many other elasmobranchs, these characteristics make *L. ocellata* population highly susceptible to exploitation by commercial fisheries.

As in other vertebrates, the physiological mechanisms central to this specie's reproduction are likely regulated by endocrine factors. In the few elasmobranchs that have been similarly examined, the biosynthesis and secretion of steroid hormones are

correlated to specific events during the course of the reproductive cycle. I attempted to associate morphological changes in gonadal tissues with circulating steroid hormones by using radioimmunoassay to determine plasma estradiol concentrations in females and testosterone concentrations in males at different stages of sexual maturity during the winter skate's life cycle. In all cases, abrupt increases in several reproductive parameters, such as spermatogenesis, follicle size, testosterone and estradiol concentration, appeared to mark the onset of sexual maturity in both male and female winter skates. Moreover, these morphological and histological changes were correlated to estradiol concentrations in females and testosterone concentrations in males. Thus, the overall results indicate that the coordinated examination of morphological and histological parameters, along with steroid hormone concentrations, is a more accurate approach to determining age and size at sexual maturity for the winter skate when compared to other studies that have utilized empirical or morphological measurements alone. However, based on the fact that elevated titers of the reproductive steroids are correlated with advancing sexual maturity, the results suggest that hormone analyses alone may be a sufficient technique to elucidate size at maturity, offering an alternative to more invasive methods.

Furthermore, the morphological changes in gonadal tissues and patterns of circulating steroid hormones also provided insight regarding mechanisms that regulate key events that occur over the course of the winter skate's reproductive cycle. For example, the annual cycling of steroids was associated with specific phases of gametogenesis in males and reproductive tract dynamics in females. In males, higher concentrations of testosterone paralleled the increase in percent composition of spermatocysts (SVI), while testosterone concentrations and the percent composition of

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spermatocytes (SIII) were inversely related. Interestingly, an opposite trend was observed for estradiol concentrations; higher concentrations of estradiol paralleled the increase in SIII while estradiol concentrations and SVI were inversely related. In female winter skates, a strong association between E_2 concentration and ovary weight, shell gland weight and size of the largest follicle existed over the course of the reproductive cycle. Moreover, it appears that the hormones progesterone and testosterone may have a role in encapsulation of the egg and oviposition.

Although the results of this study have led to a better understanding of several life history characteristics of the winter skate, more investigations are needed in order to answer questions that address the role of steroid hormones and their underlying mechanisms during the reproductive cycle of the winter skate. Specifically, future investigations are needed to:

1) determine if the ovarian follicle is a source of E_2 production;

2) determine if testosterone is a substrate for E_2 biosynthesis;

3) determine if peak E_2 concentration and the corresponding nadir in T concentration is due to an increase in aromatase activity;

4) determine the role of T and P_4 in encapsulation of the egg and oviposition;

5) determine the concentrations of other androgens not measured in this study, such as 11-ketotestosterone and dihydrotestosterone (DHT);

6) determine the cellular sources of T biosynthesis within the winter skate testis;

7) determine if or how aromatase activity plays a role in spermatocytes.

Given that I have indicated throughout my dissertation how vulnerable the winter skate is to overfishing, I feel compelled to offer some suggestions as to how this species might be effectively managed. Since fishing could be particularly destructive to *L.*

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ocellata, I feel two measures could be implemented immediately to reduce exploitation of this resource. First, because landed skate wings are seldom identified to species, available fishery data that has been collected are incomplete. This means that no time series of landings are available for any of the species within the Gulf of Maine. This represents a significant impediment to developing skate specific management measures, evaluating the potential effectiveness of these measures, and monitoring the success of a fisheries management plan. Thus one of the most important problems to overcome is the proper identification and separation of landed species. Skate identification guides already created could be distributed to local government agencies, scientists, skate wing processors, and commercial fisherman. This would facilitate proper identification and help coordinate data collection on landings not only for the winter skate but other species as well.

Another aspect of fishing that could prove detrimental to winter skate populations is harvest size. Skate wing processors prefer *L. ocellata* wings that are at least 1-1 ¼ pounds skin on. This equates to a winter skate approximately 700 mm in TL. Based on size at sexual maturity, these individuals are not yet capable of reproducing and adding new recruits to the population. In order to alleviate this fishing pressure on the species but still allow for a fishery to exist, regulations that include a legal minimum length and a legal maximum length could be implemented. Based on my results, I would set the legal minimum size at 800 mm TL and the legal maximum size at 900 mm TL. This would allow for skates below and above the legal limits to have the chance to reproduce while still allowing for a marketable wing size to be fished.

Several other important parameters need to be collected in order for a winter skate fisheries management plan to be successful within the Gulf of Maine. These include but are not limited to:

1) determine composition and size structure of landings;

2) determine catch locations, sizes, weights, and sexes of specimens;

3) design and implement a winter skate-tagging program to determine the extent and characteristics of skate nursery areas and the degree of exchange of skates into and out of the Gulf of Maine;

4) determine the true level of discards and the discard mortality rates for different fishing gears;

5) investigate trophic interactions between winter skate species and other groundfish.

These types of strategies have proved useful in the management of other elasmobranchs such as Tope shark, *Galeorhinus galeus*, which is managed by the Australian Fisheries Management Authority. However, without this information for the *L. ocellata*, uncertainty will constrain the ability to develop, implement and monitor the success of a winter skate fisheries management plan within the Gulf of Maine.

LIST OF REFERENCES

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

Bigelow, H.B., and Schroeder, W.C. 1953. Fishes of the Gulf of Maine. Fish. Bull. 53:63-65.

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

Brander, K. 1981. Disappearance of common skate *Raia batis* from Irish Sea. Nature. 290 (5801):48-49.

Branstetter, S., and Musick, J.A. 1984. Age and growth estimates for the sand tiger in the northwestern Atlantic ocean. Trans. Am. Fish. Soc. 123:242-254.

Branstetter, S., Ed. (1993). Conservation biology of elasmobranchs. NOAA Technical Report NMFS 115.

Brown, C. B., and Gruber, S. H. 1988. Age assessment of the lemon shark, *Negaprion brevirostris*, using tetracycline validated vertebral centra. Copeia. 3: 747-753.

Cailliet, G. M., Martin, L. K., Kusher, D., Wolf, P., and Weldon, B.A.1983. Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. NOAA Tech. Report, NMFS. 8:157-165.

Cailliet, G. M., Radtke, R. L. and Weldon, B. A.. 1986. Elasmobranch age determination and verification: a review *in* Indo-Pacific fish biology: Proceedings of the second international conference on Indo-Pacific fishes (T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds), p. 345-360. Icthyol. Soc. Jpn., Tokyo.

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 (H. L. Pratt Jr, S. H. Gruber and T. Tanuichi eds), NOAA Technical Report, NMFS 90:157-65.

Cailliet, G. M., and Tanaka, S. 1990. Recommendations for research needed to better understand the age and growth of elasmobranchs. *In* Elasmobranchs as living resources: advances in the biology, ecology, systematics and the status of the fisheries. *Edited by* H. L. Pratt Jr, S. H. Gruber and T. Tanuichi. NOAA Technical Report, NMFS 90:505-508.

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Callard, G., Pudney, J.A., Mak, P., and Canick, J.A. 1985. Stage-dependent changes in steriodogenic enzymes and estrogen receptors during spermatogenesis in the testis of the dogfish *Squalus acanthias*. Endocrinology. 117:13281335.

Callard, G., and Cuevas, M.E. 1992. In vitro steroid secretion by staged spermatocysts (Sertoli/germ cell units) of dogfish (*Squalus acanthias*) testis. Gen Comp. End. 88(1): 151-165.

Casey, J. G., Pratt, H.L., and Stillwell, C.E. 1985. Age and growth of the sandbar shark (*Carcharhinus plumbeus*) from the western North Atlantic. Can. J. Fish. Aquat. Sci. 42(5): 963-975.

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

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

Conrath, C.L., and Musick, J.A. 2002. Reproductive biology of the smooth dogfish, *Mustelus canis*, in the northwest Atlantic ocean. Environ. Bio. Fish. 64:367-377.

Cuevas, M.E. and Callard, G.V. 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.

Dobson, S. 1974. Endocrine control of reproduction in the male *Scyliorhinus canicula*. Ph.D. Dissertation, University of Wales.

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

Dulvey, N.K., Metcalfe, J.D., Glanville, J., Pawson, M.G., and Reynolds, J.D., 2000. Fishery stability, local extinctions, and shifts in community structure in skates. Cons. Biol. 14 (1): 283-293.

Fasano, S., M. D'antonio, R. Pierantoni, and G. Chieffi. 1992. Plasma and follicular tissue steroid levels in the elasmobranch fish *Torpedo-marmorata*. Gen Comp. End. 85 (2): 327-333

Francis, M., Maolagain, C.O., and Stevens, D. 2001. Age, growth, and sexual maturity of two New Zealand endemic skates, *Dipturus nasutus* and *D. innominatus*. N. Z. J. Mar. Freshwat. Res. 35(4) 831-842.

Frisk, M.G. 2000. Estimation and analysis of biological parameters in elasmobranch fishes and the population dynamics of the little skate *Raja erinacea*, winter skate *R. ocellata* and barndoor skate *R. laevis*. Masters thesis, University of Maryland, Center for Environmental Science.

Frisk, M. G., Miller, T.J., and Fogarty, M.J. 2001. Estimation and analysis of biological parameters in elasmobranch fishes: a comparative life history study. Can. J. Fish. Aquat. Sci. 58(5): 969-981.

Frisk, M. G., Miller, T.J., and Fogarty, M.J. 2002. The population dynamics of little skate *Leucoraja erinacea*, winter skate *Leucoraja ocellata*, and bandoor skate *Dipturus laevis*: predicting exploitation limits using matrix analyses. ICES J. Mar. Sci. 59(3): 576-586.

Gay, V.L., and Kerlan, J.T. 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.

Gibori. G., Antczak., E. and Rothchild, I. 1997. The role of estrogen in regulation of luteal progesterone secretion in the rat after day 12 of pregnancy. Endocrin. 100:1483-1495

Goldberg, M.J. Moses, M.A., and Tsang, P.C.W. 1996. Identification of matrix metalloproteinases and metalloproteinase inhibitors in bovine corpora lutea and their variation during the estrous cycle. J. Anim. Sci. 74849-857.

Hamlett, W.C., and Koob, T.J. 1999. Female reproductive system. In Sharks, skates and rays; the biology of elasmobranch fish. W.C. Hamlett, (eds). Pages 398-443. Johns Hopkins University Press.

Hoenig, J.M., and Gruber, S.H. 1990. Life history patterns in the elasmobranchs: Implications for fisheries management. *In* Elasmobranchs as living resources: advances in the biology, ecology, systematics and the status of the fisheries. *Edited by* H. L. Pratt Jr, S. H. Gruber and T. Tanuichi. NOAA Technical Report, NMFS. 90:1-16.

Holden, M. J. and Vince, M. R. 1973. Age validation studies on the centra of *Raja clavata* using tetratcycline. J. Cons. Int. Explor. Mer. 35:13-17.

Holden, M. J. 1977. Elasmobranchs *in* Fish population dynamics (J. A. Gulland, ed), p. 187-214. J. Wiley and Sons, London England.

Johnson, G.F. 1979. The biology of the little skate, *Raja erinacea*, in Block Island Sound, Rhode Island. Masters Thesis, University of Rhode Island. Kingston, RI.

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

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

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

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

Loefer, J.K., and Sedberry, G.R. 2003. Life history of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*)(Richardson,1836) off the southeastern United States. Fish. Bull. 101 (1):75-88.

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

Marquardt, D. W. 1963. An algorithm for the least squares estimation of nonlinear parameters. J. Soc. Ind. Appl. Math. 2:431-441.

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

Martin, L. K. 1982. Growth and reproduction of the bat ray, *Myliobatis californica*, Gill, in California. Masters Thesis, San Jose State Univ. and Moss Landing Mar. Lab. Calif.

Martin L. K. and Cailliet, G. M. 1988. Age and growth determination of the bat ray, *Myliobatis californica*, in central California. 3:762-763.

Martin, L., and Zorzi, G.D. 1993. Status and review of the California skate fishery. Conservation Biology of Elasmobranchs. NOAA Tech. Report, NMFS. 115: 39-52.

85

Mollet, H.F., Cliff, G., Pratt, H.L. Jr, Stevens, J.D. 2000. Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. Fish. Bull. 98(2): 299-318.

Natanson, L.J. 1993. Effect of temperature on band deposition in the little skate, *Raja erinacea*. Copeia (1):199-206.

Natanson, L.J., Casey, G.C., and Kohler, N.E. 1995. Age and growth estimates for the dusky shark, *Carcharhinus obscurus*, in the western North Atlantic Ocean. Fish. Bull. vol. 93(1):116-126.

New England Fishery Management Council. January 2001. PP-2 of the 2000 Stock Assessment and Fishery Evaluation (SAFE) Report for the Northeast Skate Complex. 50 Water Street, Mill 2 Newburyport, MA 01950.

Northeast Fisheries Science Center. 1999. 30th Northeast Regional Stock Assessment Workshop, 166 Water Street Woods Hole, MA 02543-1026

Otwell,W.S and Lanier, T.C. 1979. Utilization of North Carolina skates and Rays. North Carolina Department of Natural Resources and Community Development, Morehead City (USA). Publ. by: MCDMF; Morehead City, NC (USA), Compl. Rep. N.C. Div. Mar. Fish. February, 49 p.

Prager, M. H., Saila, S. B.,and Recksiek, C. W. 1987. Fishparm: A microcomputer program for parameter estimation of nonlinear models in fishery science. Old Dominion University, Norfolk Virginia, Tech. Rep. (87-10): 1-37.

Parsons, G.R. 1983. The reproductive biology of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Richardson). Fish Bull. (81)1: 61-74.

Parsons, G.R., and Grier, H.J. 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.

Pratt, H.L. 1979. Reproduction in the blue shark, *Prionace glauca*. Fish Bull. 77:445-470.

Rasmussen, L.E.L. and Murru, F.L. 1992. Long term studies of serum concentrations of reproductively related steroid hormones in individual captive carcharhinids. Aust. J. Mar. Freshwater Res. 43:273-81

Rasmussen, L.E.L., Hess, D.L., and Gruber, S.H. 1992. Serum steroid hormones during reproduction in elasmobranchs. In: Reproductive biology of south American vertebrates. W.C. Hamlett (ed.), pp 19-42. Springr-Verlag, New York.

Rasmussen, L.E.L., and Gruber, S.H. 1993. Serum concentrations of reproductively related circulating steroid hormones in the free ranging lemon shark, *Negaprion brevirostris*. Environ. Biol. Fishes 38:167-174

Rasmussen, L.E.L., Hess, D.L. and Luer, C.A. 1999. Alterations in serum steroid concentrations in the clearnose skate, *Raja eglanteria*: Correlations with season and Reproductive status. J. Exp. Zool. 284:575-585.

Richards, S. W., Merriman, D., and Calhoun, L. H. 1963. Studies inthe marine resources of southern New England. IX. The biology of the little skate *Raja erinacea* Mitchill. Bull Bingham Oceanogr. Coll. 18(3):311-407.

Robins, C.R. and Ray, G.C. 1986. A field guide to Atlantic coast fishes of North America. Houghton Mifflin Company, Boston, U.S.A. 354 p.

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

Simon, J.E., and Frank, K.T. 1996. Assessment of the Division 4VsW Skate Fishery. DFO Atl. Fish. Res. Doc. 96/105.

Simon, J.E., and Frank, K.T. 1998. Assessment of the winter skate fishery in Division 4VsW. DFO Atl. Fish. Res. Doc. 98/145.

Simpfendorfer, C.A. 1993. Age and growth of the Australian sharpnose shark, *Rhizoprionodon taylori* , from north Queens land, Australia. Environ. Biol. Fish. 36: (3), pp. 233-241.

Simpfendorfer, C.A.2000. Age and growth of the whiskery shark, *Furgaleus macki*, from south-western Australia. Environ. Bio. Fish. 58:335-343.

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

Snelson, F. F., Jr., Rasmussen, L.E.L., Johnson, M.R., and Hess, D.L. 1997. Serum concentrations of steroid hormones during reproduction in the Atlantic stingray, *Dasyatis sabina*. Gen. Comp. End. 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. 1998. Skates. Status of fishery resources off the northeastern United States. NOAA Tech. Mem. NMFS-NE. 115:114-115.

Sosebee, K. 2002. Maturity of skates in northeast United States waters. Northwest Atlantic Fisheries Organ., Dartmouth, NS. Sci. Counc. Res. Doc. NAFO. no. 02/134. 17 pp.

Sulikowski, J.A., Morin, M. D., Suk, S. H. and Howell, W. H. 2003. Age and growth of the winter skate, *Leucoraja ocellata*, in the Gulf of Maine. Accepted Fish. Bull. 101(2):405-413.

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

Teshima, K. 1981. Studies on the reproduction of the Japanese smooth dogfishes, Mustelus manazo and Mustelus griseus. J. Shimonoseki Univ. Fish. 29:113-199.

Tricas, T.C., Maruska, K.P., Rasmussen, L.E.L. 2000. Annual cycles of steroid hormone production, gonad development, and reproductive behavior in the Atlantic stingray. Gen Comp. End. 118 (2): 209-225.

Tsang, P. and I.P. Callard. 1982. Steroid production by isolated skate ovarian follicular cells. Bull. Mount Desert Island Biol. Lab. 22:96-97.

Tsang, P. and I.P. Callard. 1983. In vitro steroid production by ovarian granulosa cells of *Squalus acanthias*. Bull. Mount Desert Island Biol. Lab. 23:78-79.

Tsang, P., and Callard, I.P. 1987. Morphological and endocrine correlates of the reproductive cycle of the aplacental viviparous dogfish, *Squalus acanthias*. Gen. Comp. Endocrinol. 66:182-89.

Tsang, P.C.W., and Callard, I.P. 1992. Regulation of ovarian steriogenesis in vitro in the viviparous shark, *Squalus acanthias*. J. Exp. Zool. 261:97-104.

von Bertalanffy, L. 1938. A quantitative theory of organic growth (inquires of growth laws II). Human Biology 10:181-183.

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

Waring, G. T. 1984. Age, growth and mortality of the little skate off the northeast coast of the United States. Trans. Amer. Fish. Soc. 113: 314-321.

Winemiller, K.O., and Rose, K.A. 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., and Cliff, G. 1996. Age and growth determination of the blacktip shark, *Carcharhinus limbatus*, from the east coast of South Africa. Fish. Bull. 94: (1) 135-144.

Wourms, J. P. 1977. Reproduction and development in chondrichthyan fishes. Am. Zool. 17: 379-410.

Wourms, J. P., and Demski, L.S. 1993. The reproduction and development of sharks, skates, rays and ratfishes: introduction, history, overview and future prospects. Env. Bio. Fish. 38:7-21.

Yoccoz, N.G. 1991. Use, overuse, and misuse of significance tests in evolutionary biology and ecology. Bulletin of the Ecological Society of America 71(2):106-111.

Zeiner, S.J., and Wolf, P.G. 1993. Growth characteristics and estimates of age at maturity of two species of skates (*Raja binoculata* and *Raja rhina*) from Monterey Bay, California. Conservation Biology of Elasmobranchs, NOAA Technical Report NMFS, vol. 115, pp. 87-90.

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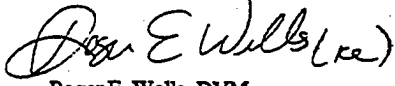Durham, New Hampshire 03824-3585
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OFF-CAMPUS ADDRESS (If applicable)			**IACUC #**	990402
			REVIEW LEVEL	1

PROJECT TITLE Native NH Skate Biology, Physiology, and Ecology: The Value of the Skate as a Marine Resource

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All cage, pen or other animal identification records must include your IACUC Protocol # as listed above.

The Institutional Animal Care and Use Committee has reviewed and approved the protocol submitted for this study under Category 1 on Page 3 of the "Application for Review of Animal Use or Instruction Protocol" - the program involves either no pain or potentially involves momentary, slight pain, discomfort or stress.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please note: Use of animals in research and instruction is approved contingent upon participation in the UNH Health Services Occupational Medical Clinic for persons handling animals. *Participation is mandatory* for all principal investigators and their affiliated personnel, employees of the University and students alike. A health survey is attached to this approval. *Please take a moment to make copies of the document and distribute them to all personnel under your supervision.* Completed questionnaires should be returned to Health Services and the Office of Environmental Health and Safety, as indicated on the forms. Project-related health services are provided at no cost to the employee or student. The occupational health physician will review questionnaires. Should she have questions or require further information, she will contact individuals directly.

Roger E. Wells, DVM
Chair, Institutional Animal Care and Use Committee

cc: File

       Hunt Howell, Zoology