Identification of Life History Parameters for Two Exploited Skate Species (*Amblyraja radiata* and *Malacoraja senta*) in the Gulf of Maine: Strategies for Fisheries Management

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Abstract: The primary objective of the proposed study was to foster a partnership between commercial fishermen and research scientists in order to quantify the life history parameters essential to the development of a fisheries management plan for the thorny skate, *Amblyraja radiata,* and smooth skate, *Malacoraja senta,* in the Gulf of Maine. For the thorny skate, we found that this species grows slowly (k=0.11 for males, and k=0.13 for females), is long lived (16+ years for both males and females), reaches sexual maturity at a late age and size (50% maturity occurs at a total length of 860 mm and near 11 years of age for males and a total length of 875mm and around 11 years of age in females) and is reproductively active all year round. While the data for the smooth skate is still being analyzed, our results suggest that like the thorny skate, this species reproduces continuously throughout the year.

Introduction: The Northeast skate complex consists of seven species indigenous to the Gulf of Maine. Remarkably, no direct biological data exists for six of these species. This lack of knowledge presents a unique opportunity to study several aspects of life history information that are crucial not only for the advancement of general elasmobranch biology, but more importantly, it is a necessity for the conservation of these fish. Skates, like other elasmobranchs, are categorized as a K selected species, and as such reach sexual maturity at a late age, have a low fecundity and are relatively longed lived. Furthermore, after oviposition, egg gestation rates can range from one to six years depending on skate size and ambient water temperature. These characteristics, coupled with the fishery practice of the selective removal of large individuals, make these animals highly susceptible to exploitation by commercial fisheries.

In the past, skates collected during ground fishing operations were limited in number and discarded as bycatch. However, the rapidly expanding markets for skate wing have made these fish commercially more viable in recent years, especially in the western north Atlantic. To complicate matters, there are no current regulations that govern the harvesting of skates in the U.S. (at the time this grant was submitted). The lack of biological data together with increased fishing pressure and the failure to implement a proper fishery management plan resulted in a drastic depletion of common skates, *Raja batis*, in the Irish Sea. Based on work completed at the 30th Northeast Regional Stock Assessment Workshop (SAW 30), the National Marine Fisheries Service (NMFS) identified three Northeast Atlantic species that are currently over fished and appear to be following the same destructive trend as *R. batis*. These include, the barndoor, *Dipturus laevis*, thorny, *Amblyraja radiata* and smooth, *Malacoraja senta*, skates. For all species, the 1997-1999 Northeast Fisheries Science Center (NEFSC) autumn survey biomass index were well below the sustainable fisheries act (SFA) biomass and threshold target levels. Moreover, there is no data on age or growth to determine fishing mortality rates or propose SFA fishing mortality reference points. A fourth species, the winter skate (*Leucoraja ocellata*), is currently not classified as overfished but reduction in biomass near the SFA threshold levels indicates that overfishing of this species may be occurring .

The over fished status of the barndoor, thorny and smooth skates necessitates the development of management measures to end over fishing 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 and socioeconomic information. Biological samples of *D. laevis* on southern Georges Bank and *L. ocellata* in the Gulf of Maine have been collected and are being examined for age, growth, population genetics and reproductive ecology. However, no biological data are available or currently being collected for *A. radiata* and *M. senta* in the Gulf of Maine. Due to the lack of biological information for these skate species, the primary objective of the proposed study is to collect this necessary life history data so that an effective fisheries monitoring, management, and conservation plan can be developed for *A. radiata* and *M. senta*.

Project objectives and scientific hypotheses: The primary objective of the proposed study was to quantify the life history parameters essential to the development of a fisheries management plan for *A. radiata* and *M. senta* in the Gulf of Maine. To achieve this overall objective, we intend to provide the first histological analysis of temporal changes in gonad maturation and the first analysis of temporal changes in reproductive steroid hormone concentrations. Together, we will correlate peaks in plasma hormone concentrations with histological changes in the gonads, and determine the breeding/seasonal cycle for these two species of skate. We will also provide the first determination of age and growth for *A. radiata* and *M. senta*, and when coupled with the reproductive information, we will be able to elucidate age and size at sexual maturity. Finally, the collective results of the study can be (have been) used to create effective fisheries monitoring, management, and conservation for *A. radiata,* in the Gulf of Maine. Once finalized, data for the smooth skate will also be used to properly manage this species.

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Paul Tsang, James Sulikowski, and Joe Jurek all played key roles in project design and implementation.

Methods:

Study Area and Fishing Protocol

Collection of skates took place from June 2001 to May 2003. The bulk of the areas chosen for the study were off the coast of southern Maine, New Hampshire and northern Massachusetts, and encompasses inshore waters and the southern part of Jeffreys Ledge. These waters support the vast majority of commercial fishing and were easily accessed during normal fishing operations.

Joe Jurek, a local fisherman, used his vessel, gear and expertise to capture *A. radiata* and *M. senta* in these areas. Incorporated into his normal fishing regime, Joe conducted short duration tows in areas that were known skate habitats. In a typical sea day, we were able to make three to four tows.

Numbers Collected and General Processing

A total of 931 thorny skates (404 males and 527 females) and 487 smooth skates (265 females and 222 males) were used for this study. Upon capture, skates were either euthanized (2g/l bath of MS222) immediately and processed at sea or were transported back to the University of New Hampshire's Coastal Marine Laboratory, where they were held until the time of sampling (usually within 24 hours of capture).

Analyses of Age and Growth

Fish age was determined by enumerating growth bands found in the vertebral centra. Here, a section of their vertebral column was removed, sectioned, processed, stained, viewed through a compound microscope and the number of growth bands counted. Nonconsecutive band counts were made independently by two readers for each specimen used in the study, without prior knowledge of the skate's length or previous counts. A Tukey's test was used to test for differences between ages. Age determination bias between readers was assessed through the use of an age-bias plot. This type of graph plots band counts of one reader versus the second reader in reference to an equivalence line. Specifically, reader 2 is represented as mean age and 95% confidence intervals corresponding to each of the age classes estimated by reader 1. Divergence from the equivalence line, where reader 1 = reader 2, would indicate a systematic difference between readers. Precision estimates of each reader were calculated using the coefficient of variation (CV).

Marginal increment analysis:

The annual periodicity of band pair formation was investigated using marginal increment analyses (MIA). Since the annuli in older adult specimens were compressed, marginal increments were calculated from randomly selected juvenile specimens. For MIA determination, the distance of the final opaque band and the penultimate opaque band, from the centrum edge, was measured using a compound microscope and optical micrometer. The marginal increment was calculated as the ratio of the distance between the final and penultimate bands. Average increments were plotted by month of capture to identify trends in band formation, and a Kruskal-Wallis one-way analysis of variance on ranks was used to test for differences in marginal increment by month.

Growth rates

A von Bertalanffy growth function (VBGF) was used fit to the data and then calculated by using FISHPARM, a computer program for parameter estimation of nonlinear models with Marquardt's algorithm for least-square estimation of non-linear parameters.

Reproduction studies

Collections:

After capture, the skates were euthanized (2g MS222/liter of water) and then livers, reproductive organs and tracts were removed, blot dried and weighed to the nearest milligram. Gonads were fixed in 10% formalin and stored in 50% isopropyl alcohol until examined. Male and female gonadosomatic index (GSI) and hepatosomatic index (HSI) were determined 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.

Gross sexual development

All gross morphological changes corresponding to sexual maturity were compared to the histological and steroid analyses, allowing for a complete description of reproductive status. Attainment of sexual maturity in males will be analyzed through an abrupt change in the relationship of clasper length to disk width. Attainment of sexual maturity in female skates will be examined through ovarian follicle (egg) development (by measuring and counting all eggs from 1mm in diameter to ovulatory size), nidamental gland (by size), and the relationship of egg diameter to total body length. Cloacal, uterine and nidamental gland swabs will be taken with a sterile scalpel, smeared on chrom-alum coated slides, dried and stained with 0.3% Wright's stain in methanol to visualize the presence of any sperm. All egg cases removed from gravid females will be maintained at the U.N.H. Coastal Marine Lab at ambient conditions in order to determine gestation length.

Histology

A single 2-3 mm thick segment was removed from the center of each testis and ovary and stored in 10% formalin until processed by the U.N.H. Veterinary Diagnostic Laboratory. Prepared slides of gonadal tissue were scrutinized microscopically for structural changes occurring at the cellular level over a consecutive 12-month period. Ovaries and testes were also examined to verify the development from immature oocytes to mature oocytes and from immature spermatocytes to mature spermatocysts, respectively.

Plasma steroid analysis

A 10 ml volume of blood was obtained via cardiac puncture from each skate utilized in the histological study described above. This blood was centrifuged at 1700 x g, and stored at -20ºC for later analysis. The concentrations of progesterone, testosterone and estradiol, representing the three major classes of steroid hormones found in blood, were determined by specific radioimmunoassay (RIA). Antibodies for each steroid have been characterized and were available for use. Radiolabeled hormones were purified by thin layer chromatography before RIA. Also, all blood samples were extracted with petroleum ether or ethyl ether prior to RIA. Radioactivity in samples was determined in a liquid scintillation counter. A resulting standard curve was used to interpolate blood concentrations of each hormone using a RIA computer program. Solvent extraction efficiencies, intra- and inter-assay coefficients of variance were also calculated.

Data Analysis

Steroid hormone concentrations were compared by an analysis of variance (ANOVA) followed by Tukey's to pinpoint differences between months. This will enable us to determine the relationship between peak hormone concentrations and reproductive activity. Additionally, the relationships between hormone concentrations and gross morphological characteristics (such as clasper length, follicle size, nidamental gland weight) can also be determined using correlation analysis.

Unexpected difficulties: The enumeration of growth zones deposited in vertebral centra has provided the most reasonable method of estimating age at length in most elasmobranchs, including the winter and thorny skates here in the Gulf of Maine. However, for many other of batoids, there have been unforeseen difficulties in using the same techniques as those employed successfully in other species. This has led to incomplete or absent information for most batoid species. Unfortunately, in our study of smooth skates, we have encountered similar difficulties and have exhausted all available techniques here at UNH. In order to work through this problem, we have modified an alternative, and novel, aging method that has been used to successfully age a few species of sharks but has yet to be attempted in skates or rays. Working in collaboration with Dr. Lisa Natanson, the vertebrae are prepped and are in the process of being histologically analyzed for the annual periodicity of growth band formation. As a result, another manuscript is forthcoming.

Data: Data on age, growth, maturity and reproductive cycles have been collected and analyzed for the thorny skate. The data for age, growth and reproduction have been published in Fishery Bulletin. The online links to those manuscripts can be found at:

Age and growth; http://fishbull.noaa.gov/1031/suli.pdf

Reproduction; http://fishbull.noaa.gov/1033/sulikowski.pdf

A third manuscript containing data for maturity in this species is currently under review in the Journal of Fish Biology. However, we have provided the maturity ogives in Figure 1 that describes age and size at maturity for this species. Like wise, the reproductive cycle of the smooth skate is under review in the Journal of Marine and Freshwater Research. We have provided the pertinent information in Figure 2 that illustrates smooth skates are reproductively active year round. Plasma steroid hormone concentrations are currently being analyzed and will be sent to an appropriate journal for peer review in 2006.

Results and conclusions: Our project met several goals of the Northeast Consortium. It developed partnerships between commercial fishermen and researcher scientists. It also fit the Northeast Consortium's suggested topic area of "Fish habitat studies" as our study improved the accuracy and reliability of the underlying biological information, or lack thereof, for *A. radiata* and *M. senta*. In doing so, our study has provided life history parameters for the thorny skate. This information has been utilized by the New England Fishery Management Council in implementing a proper fishery management plan and recovery strategy for this over-exploited species. Once the data for the smooth skate has been thoroughly analyzed, this information will also be used to update and improve the management strategies for this species of skate. By doing so, the objectives of this project will have been met.

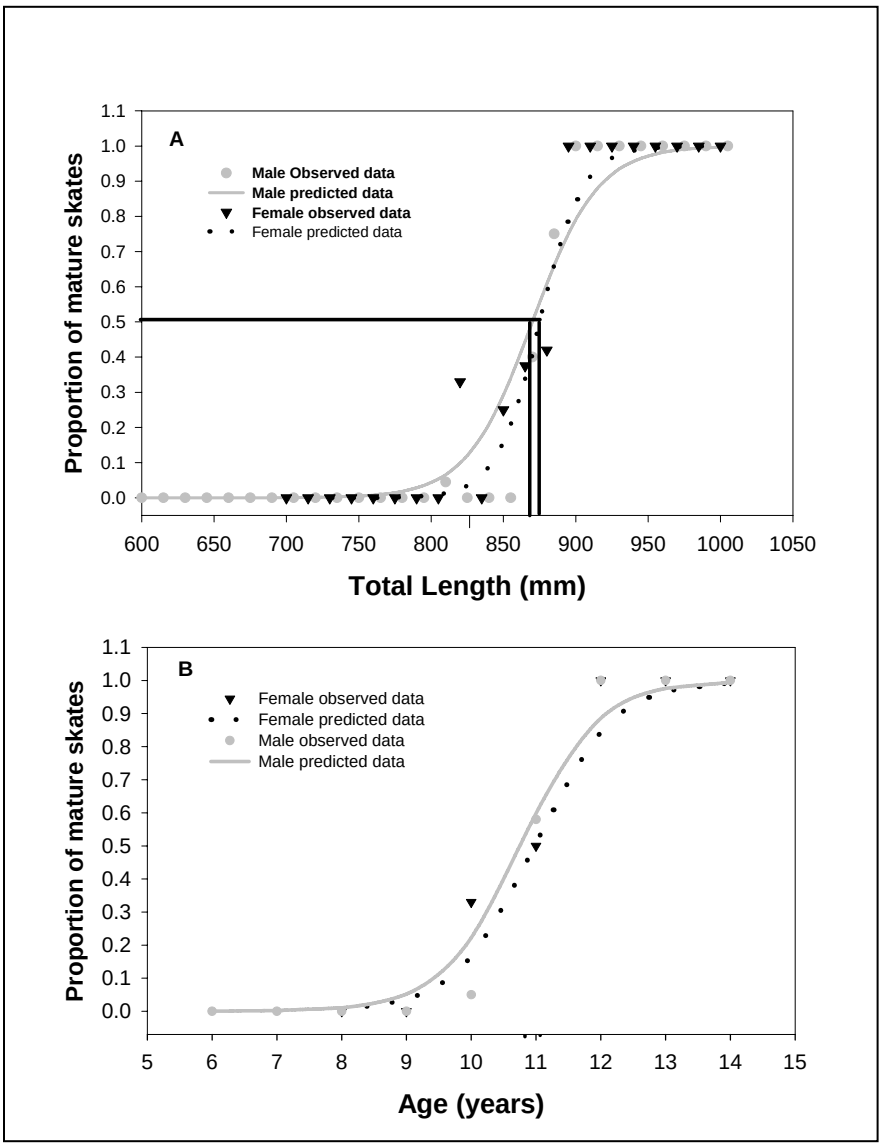

Figure 1. Maturity ogives based on morphological, histological and steroid hormone analyses for male and female thorny skates. The upper panel (A) represents the total length of male and female *R. radiata* given in 15 mm intervals, and the lower panel (B) represents the age of male and female *A. radiata* given in one year increments.

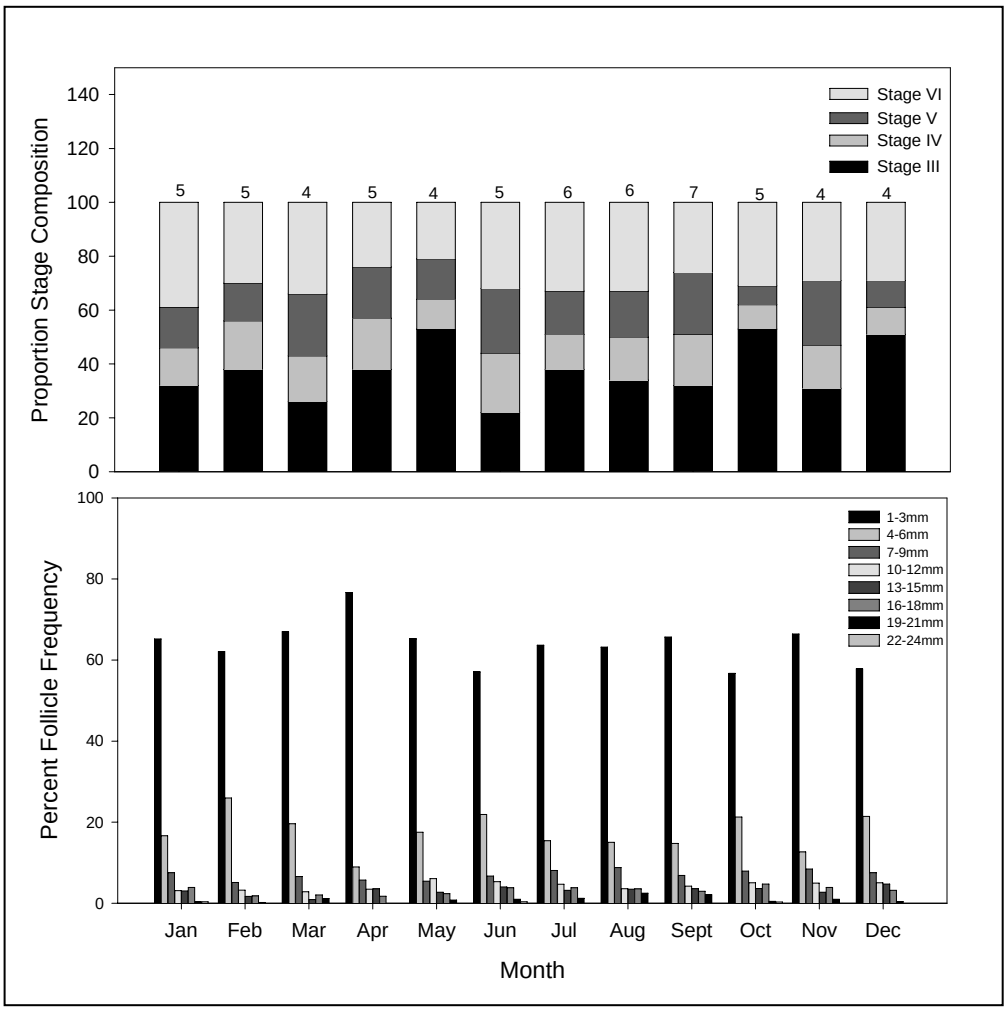

Figure 2. Comparisons between (upper panel) the percentage of each stage of spermatogenesis (Stages III-VI) and (lower panel) ovarian follicular dynamics, in smooth skates (*Malacoraja senta*) over the course of the sampling period. In the upper panel, the values represent the mean percent of each stage of spermatogenesis (III-VI) occupied along a transect line across one representative full lobe cross section of testis (n=60). In the lower panel, follicles were assigned to one of eight size classes and the number of follicles in each size class was expressed as the percent of the total number of follicles ≥ 1 mm in diameter (percent follicle frequency).

Partnerships: As described above, we developed partnerships with commercial fishing industry. In doing so, it will enabled fishermen to participate in research. In particular, Captain Joe Jurek was instrumental in collection of the skates used in this study.

Impacts and applications: As describe above, the information gathered from our biological study of these skates has had a direct impact on fishery management. Our data has been utilized by the New England Fishery Management Council in implementing a proper fishery management plan and recovery strategy for the thorny skate, and once data for the smooth skate are finalized, this information will also be used in the same manner.

Related projects: N/A

Presentations:
Sulikowski*, J.A., J. Kneebone, S. Elzey, P. Danley, W. H. Howell and P.C.W. Tsang. 2005. Age and size at sexual maturity for the thorny skate, *Amblyraja radiata*, in the western Gulf of Maine based on a three parameter estimate. Ann. Mtg. Am. Soc. Ichty. and Herp., Tampa, Fl.

Sulikowski*, J.A., J. Kneebone, S. Elzey, P. Danley, W. H. Howell and P.C.W. Tsang. 2004. The reproductive cycle of the thorny skate, *Amblyraja radiata*, in the Gulf of Maine. Ann. Mtg. Am. Soc. Ichty. and Herp., Norman, OK.

Sulikowski, J. A., S. Elzey, J. Kneebone* and P.C.W. Tsang. 2005. The reproductive cycle of the smooth skate, Malacoraja senta, in the western Gulf of Maine. Ann. Mtg. Am. Soc. Ichty. and Herp., Tampa, Fl.

Student participation:
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Published reports and papers:

Published
Sulikowski, J.A., J. Kneebone, S. Elzey, P. Danley, W. H. Howell and P.C.W. Tsang. 2005. The reproductive cycle of the thorny skate, *Amblyraja radiata*, in the Gulf of Maine. Fish Bull. 103(3):536-543.

Sulikowski, J.A., J. Kneebone, S. Elzey, P. Danley, W. H. Howell and P.C.W. Tsang. 2005. Age and growth estimates of the thorny skate, *Amblyraja radiata*, in the Gulf of Maine. Fish Bull. 3(1):161-168.

In review
Sulikowski, J.A., W.H. Howell and P.C.W. Tsang. Age and size at sexual maturity for the thorny skate, *Amblyraja radiata*, in the western Gulf of Maine based on morphological, histological and steroid hormone analyses. J. Fish Biol.

Sulikowski, J.A., S. Elzey, J. Kneebone, P. Danley, W. H. Howell and P.C.W. Tsang. The reproductive cycle of the smooth skate, *Malacoraja senta*, in the Gulf of Maine. J. Mar. Fresh. Res.

Images: See attached

Future research: Research is needed to ascertain the essential fish habitats for these species. Particularly what areas serve as nursery grounds? Also, both seasonal and daily movement patterns need to be evaluated in order to gain a better understanding of these skate species.