大型海産魚マンボウ *Mola mola* の生殖生態に関する研究

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Summary

A study on the reproductive biology of ocean sunfish ***Mola mola***

Toshiyuki Nakatsubo

1. Introduction

Ocean sunfish, *Mola mola*, which belongs to the family Molidae in the order Tetraodontiformes, is widely distributed in temperate and tropical waters worldwide. This fish is the world's heaviest bony fish, reaching 2000 kg in body weight (BW) and 3 m in total length (TL). The heaviest specimen ever captured was a female with a TL of 2.7 m weighing 2.3 metric tons that was caught in a set net off the coast of the Boso peninsula in Kamogawa, Chiba prefecture, Japan.

The appearance of the ocean sunfish is extremely unique among fishes as the posterior half of the body appears to be truncated; they have no caudal fin or caudal peduncle, and the posterior end of body consists of a pseudo-caudal fin (clavus), which is a fusion of the dorsal and anal fins. Further, they lack several organs common to most species of fish, such as a pelvic fin, swim bladder, and otolith.

Due to the unique features of these fish, many aquariums have long attempted to display them, but as it is very difficult to rear ocean sunfish in captivity, they were rarely reared or displayed in aquariums in the past. With the recent advances in rearing techniques and equipment, they can now be kept for long periods of time and they are one of the most popular marine creatures in aquarium.

Additionally, ocean sunfish are popular with scuba divers, and some fishery cooperative associations and diving shops offer opportunities for scuba divers to swim with them in net cage. However, the value of ocean sunfish as aquatic resources is low; consumption was limited to local consumption by fishermen, and the fish were generally tossed back into the sea because of the low market value. However, ocean sunfish is gaining popularity in aquarium, and is now gradually increasing in value as an aquatic resource due to its consumption as local specialties and delicacies.

Several characteristics of ocean sunfish have been described to date, but direct observation is required to confirm and further expand basic knowledge about this species. While ocean sunfish is known as the most prolific fish in the world, this is merely based on the fecundity of an immature specimen. It has been reported that larval fish have a caudal fin and that they undergo metamorphosis during growth, assuming a confetti-like shape before attaining the adult form; however, this knowledge is not based on actual observations, but merely estimations based on morphological observation of hatchlings from feeding studies of tunas. Chronological observation of the metamorphosis of ocean sunfish starting from the juvenile stage and using live specimens will likely provide valuable data on fish development and evolution. Additionally, the display of live ocean sunfish in various stages of development in aquariums can then serve as educational materials to inform the public about marine organisms. However, information about reproductive biology, such as spawning period and maturity age, is largely unknown.

In the present study, the reproductive biology of ocean sunfish was investigated to gather basic information about reproduction. Using methods to determine sex, estimate age and maturation, specimens captured in Kanto coastal waters were examined for sexual differences in growth and maturation as well as spawning period.

2. Determination of sex

To establish a technique for determining sex, karyotype analysis was carried out, morphological differences were analyzed, and gonads were anatomically and histologically analyzed. Using three captive specimens kept in an aquarium, karyotype analysis was conducted by tissue culturing and in vitro colchicine treatment methods, and the results showed that the ocean sunfish has 46 chromosomes（2n）, all of which are acrocentric（46A）, but no sexual dimorphism was confirmed. Additionally, a total of 21 morphological parameters were measured on 330 specimens captured in Kanto coastal waters（178 females and 152 males; TL: 36-252 cm）, and significant differences were observed between males and females in the distributions of body length（$p=0.00023$）, dorsal fin base length ($p=0.010$), anal fin base length ($p=0.012$), and clavus base length ($p=0.001$), but the maximum difference was only 1.38% of the total length (mean, clavus base length). Hence, macroscopically determining sex based on morphological parameters was shown to be difficult. In the present study, sex was determined using anatomical examination and histological analysis（paraffinized sections） of gonads.

3. Age estimation

To develop a method for estimating the age of mature ocean sunfish that are capable of spawning, captive specimens in an aquarium were repeatedly measured to produce a growth curve. The consecutive TL measurements of eight specimens taken over the rearing period of 315 - 1556 days showed that growth for all eight specimens was linear. Next, one specimen that did not grow normally was excluded, and the growth data of the other seven specimens was subjected to the von Bertalanffy growth curve, producing the following formula:

$$TLt = 318.4 \times \{1-\exp[-0.149 \times (t-0.031)]\} \quad (R^2=0.886)$$

(TLt: total length (cm) at age t; t: age (days); R^2: correlation coefficient)

Using this derived growth curve, a specimen with a TL of 3 m was estimated to be about 20 years of age.

4. Estimation on maturation

In general, fish maturation is estimated by the gonadosomatic index (GSI) based on gonad weight (GW) and body weight (BW) as follows: GSI = (GW/BW) × 100. However, in the case of ocean sunfish, which are large, it is often difficult to accurately measure BW, so the gonad index (GI) based on TL was considered in the present study as follows: GI = (GW/TL^3) × 10000. Using 180 wild specimens and 148 captive specimens, the relationship between BW and TL was investigated and a strong correlation was confirmed (R^2 = 0.959-0.987). Next, GSI and GI were confirmed to be strongly correlated, and based on this finding, GI was used to estimate maturation using the following equations:

Reared specimen: GI = 5.15 × GSI (R^2 = 0.969)

Wild specimen: GI = 6.06 × GSI (R^2 = 0.834)

(GI: gonad index; GSI: gonadosomatic index; R^2: correlation coefficient)

5. Sexual difference in growth and maturation

Using 180 wild specimens and 148 captive specimens, sexual differences in growth and maturation were investigated. First, the following relationships between TL and BW were determined in the wild and captive specimens for ocean sunfish with TL $<$ 200 cm:

Captive specimen: $BW = 3.00 \times 10^{-5} \times TL^{3.11}$ (R^2=0.959)

Wild specimen: $BW = 7.64 \times 10^{-5} \times TL^{2.90}$ (R^2=0.987)

(BW: body weight; TL: total length; R^2: correlation coefficient)

The growth of wild females with TL $\geq$ 200 cm differed. Analysis of covariance was performed using the log transformed TL and BW data, and no significance difference was found between wild and captive specimens with TL $<$200 cm (p=0.553). However, the growth of ocean sunfish with TL $\geq$ 200 cm was significantly different from that of specimens with TL $<$200 cm ($p<0.0001$). The relationship between TL and BW in ocean sunfish is summarized as follows:

TL <200 cm: $BW = 9.34 \times 10^{-5} \times TL^{2.85}$ (R^2=0.982)

TL ≥200 cm: $BW = 3.43 \times 10^{-13} \times TL^{6.50}$ (R^2=0.979)

(TL: total length; BW: body weight; R^2: correlation coefficient)

Analysis of sexual differences in TL using 460 specimens showed that females are larger than males (t-test, $p<0.0001$), and that the ratio of females increased with growth (chi-square test, $p<0.0001$), with all specimens with TL $\geq$ 250 cm being females in this study. In the Japanese pufferfish, *Takifugu rubripes* and *T. chinensis*, which also belong to the order Tetraodontiformes, the females grow better than the males, and in *T. niphobles*, the females are also larger than the males. Thus, it is thought that in ocean sunfish, the females also grow larger than the males. In addition, because it takes at least seven years for ocean sunfish to grow larger than 200 cm based on the growth curve estimated in Section 3, it would follow that there are sexual differences in the size and age at reproduction.

6. Estimation of seasonal maturation

A total of 183 wild specimens captured year-round from 1992 to 2006 using such equipment as set nets along the both the pacific ocean and Tokyo bay sides of Boso peninsula in Chiba prefecture, in addition to the Miura peninsula in Kanagawa prefecture were examined.

The GI was monitored seasonally and the highest GI value was found to occur in August for both sexes of wild specimens. Subsequently, histological analysis using paraffinized sections of large specimens was conducted to evaluate maturation. In the ovarian tissue of large specimens, oocytes were classified in four distinct maturational stages: perinucleolus stage, yolk vesicle stage, yolk globule stage, and migratory nucleus stage. Ovaries in the migratory nucleus phase, the most mature stage, were found in August. In these ovaries, many oocytes in other maturational stages were also found, suggesting that the mode of maturation is asynchronous oocyte development type. Additionally, in testes, mature sperm were detected from June to August and active sperm were collected from a male specimen in August. No large specimens were caught in September (females: TL ≥185 cm and males: TL ≥165 cm), but the ovaries of females captured at the end of October were more atretic and many atretic oocytes were confirmed. Furthermore, in the testis of male specimens captured in December, vacuolization was notable in the lobular lumen.

These observations suggest that the spawning period for ocean sunfish is relatively long, extending from late August to late September, and that they are multiple spawners.

7. Conclusions

Ocean sunfish, *Mola mola*, is widely known for its unique shape, but its biology, particularly its reproductive biology, has generally remained unknown. Therefore, to clarify the reproductive biology of ocean sunfish, we first investigated methods for determining sex as well as estimating age and maturation. In terms of determining sex, chromosomal analysis did not reveal any sexual dimorphism and morphological parameters failed to show marked differences. In the present study, sex was determined using macroscopic and histological observations of gonads. In estimating age, absolute growth was investigated using captive specimens in aquariums to produce a growth curve. The growth curve has made it possible, for the first time, to estimate the age and growth of ocean sunfish, consequently to estimate the age of maturation. Furthermore, use of the GI based on TL was shown to be a convenient and accurate tool for assessing maturation, which had previously been difficult to assess due to the size of ocean sunfish.

Based on the findings of this study, sexual differences in growth and maturation of ocean sunfish were assessed and maturation was estimated for ocean sunfish captured in Kanto coastal waters. In this study, all wild ocean sunfish larger than 250 cm in size were females; female ocean sunfish were larger than male ocean sunfish; and the ratio of female ocean sunfish increased with growth. Additionally, the changes in GI and histological analysis of gonads revealed that both the ovary and testis were most mature in August, and that both regressed by the end of October and December, respectively. In ovaries with oocytes in the migratory nucleus phase, oocytes in other stages of maturation were also observed, suggesting that ocean sunfish are multiple spawners with a relatively long spawning period extending from late August to late September.