

Activity of Reproductive Management Committee for Sand Tiger Sharks

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ABSTRACT: Reproductive Management Committee for Sand Tiger Sharks (RMCS) is working with six aquariums to exchange rearing record since 2015. In RMCS, under different water temperature and photoperiod condition, male breeding behavior is observed in different season, spring (seasonal change) and autumn (constant). MARINEWORLD started to analyze changes in blood levels of steroid hormones (17- β -Estradiol, Progesteron, Teststeron) since 2015. Many investigations are still undergoing to reveal a mechanism of reproduction of *C. taurus* and to make a success in breeding of this shark in the future. Sand tiger sharks are distributed around Ogasawara islands in Japan, but biological and ecological data of them is very limited. We started data collection program (photo identification survey) in cooperation with local diving services to complement the lack of data.

INTRODUCTION

Sand tiger sharks, *Carcharias Taurus*, are distributed in warm temperate coastal waters worldwide. They are known to undergo seasonal migration that corresponds to their reproductive cycle (Gilmore et al.1983; Gilmore 1993; Branstetter and Musick 1994; Pollard et al. 1996; Lucifora et al. 2002; Dicken et al. 2006a). *C. Taurus* is listed as Vulnerable on the International Union for Conservation of Nature's Red List of Threatened Species (Pollard et al.2018) and the population in Japan is listed as Endangered (Ministry of the Environment 2017).

The first *C. taurus* were transported to Japanese aquarium from Australia in 1995, and afterwards other *C. taurus* were transported from Republic of South Africa. Since then, they have fascinated visitors and they are very popular species in aquarium. However, the breeding of this species is very challenging. Successful reproduction of live pups has been recorded at only four institutions in the world: Underwater World SEA LIFE Mooloolaba (Australia), Manly Sea Life Sanctuary (formerly known as Oceanworld Manly) (Australia), Sea World South Africa Durban, Ushaka Marine World (South Africa) and the Scientific Center of Kuwait (Middle East) (Henningsen et al. 2017) One premature still-born was observed at Ibaraki Prefecture Oarai aquarium on 16 December 2015, but success breeding has not been recorded in Japan.

To exchange more reproductive information about *C. taurus*, Reproductive committee for Sand Tiger Sharks (RMCS) in Japan was established with Six aquariums, MARINEWORLD unionnakamichi (MW), Keikyū Aburatsubo marine park (KAM), Ibaraki Prefecture Oarai aquarium (IPO), Science museum Tokai University (TUM) and Yokohama

Hakkeijima Sea paradise (YHS), and Shinagawa aquarium (SA) joined from 2018. We introduce the basic data of RMCS, our current study, and future plan in this paper.

C. taurus is known to migrate long distance. In the NW Atlantic, SW Atlantic and South Africa pregnant *C. taurus* are thought to aggregate in warmer water during the gestation period (Bass et al. 1975, Branstetter & Musick 1994, Lucifora et al. 2002) which may enhance embryo development (Bass et al. 1975). Near-term pregnant female sharks in South Africa waters migrate to cooler water in July and August prior to parturition which may occur in August or September (Bass et al. 1975) or between November and February (Dicken et al. 2006a, 2007). In Australian east coast, photo identification surveys identified 181 sharks. Mature female *C. taurus* migrate northward in their mating season, arrive at Wolf Rock, and remain at this northern site for much of their pregnancy. Most pregnant sharks leave Wolf Rock for their migration to the south, which is assumed to be their pupping site (Bansemmer & Bennett 2009).

In Japan, the wild *C. taurus* is distributed in Ogasawara islands (27°6'0"N, 142°12'0"E) and it is an important resource for tourism. However, biological data, including information of reproduction and seasonal migration, has not been recorded in precise. We have launched monitoring program of the population in Japan. It will be helpful to study ecology of the sharks in Japan.

BASIC PHYSICAL CHARACTERISTICS AND CONDITIONS OF RMCS

Sex ratio of population.

There are totally 21 sharks kept in aquariums join RMCS. Five aquariums keep both male and

Table 1. Basic characteristics of RMCS (2015-2017)

Facility	Tank(m ³)	Monthly Average Water Temperature (°C)	Photoperiod	Population (M:F)	Copulation	Release egg capsules	Remarks
KAM	600	Seasonal 20.1-26.3	Semi-seasonal	1 : 1	-	○	South Africa
TUM	530	Seasonal 21.0-25.5	L8-10 D14-12	1 : 1	-	-	South Africa
IPO	500	Seasonal 21.0-25.5	L10-14 D14-10	3 : 4	○	○	South Africa
YHS	1,302	Constant 19.4-20.3	Constant L11-12	1 : 2	○	○	South Africa
MW	1,400	Constant 21.4-22.0	Constant L8-12	3 : 2	○	○	Australia/ South Africa
SA	180	Constant 21.9-22.8	Constant L8	0 : 2	-	○	Australia

female, and the other aquarium keeps only female. Copulation was observed in three aquariums, and premature still-born was recorded at IPO (16 Dec. 2015). This is the only parturition record of *C. taurus* in Japan.

Water temperature condition

All aquariums use natural seawater. Three aquariums make seasonal change in water temperature, and other three aquariums water temperature is almost constant through a year (Table.1).

Lighting

All aquariums use a combination of metal halide and LED for lighting, and one aquarium has a window and natural light comes in. Photoperiod was the same as opening hours until 2014 in all aquariums, and there was no seasonal change. But from 2015, Two aquariums (IPO,TUN) started to change photoperiod, imitating natural day time (Table.1).

REPRODUCTIVE BEHAVIOR

Male - Effect of water temperature and photoperiod

In the wild population, breeding season of *C. taurus* starts from spring to early summer. Under condition of seasonal water temperature change, male breeding behavior is observed in spring, which fits to the natural rhythm. However, under condition of constant water temperature, male breeding behavior is observed in autumn (Fig.1).

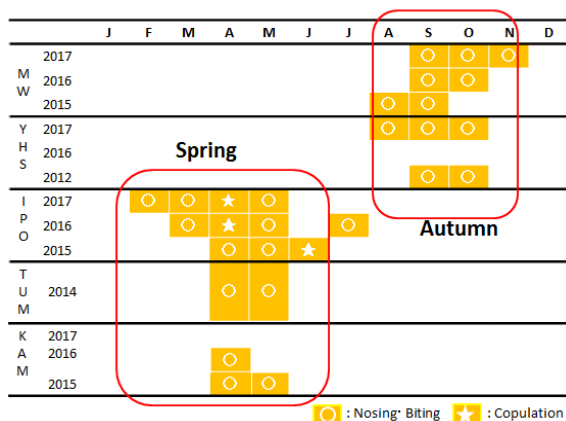


Fig. 1. The timing of male sexual action in RMCS (2015-2017)

At IPO, *C. taurus* were kept under constant water temperature until 2009 but they started to imitate seasonal water temperature change and photoperiod from 2010. Before the change, the breeding behavior was observed in winter. However, since 2010, male breeding season shifted from winter to spring (Fig.2). According to this fact, seasonal water temperature change and/or lighting time change might have some effect on male breeding behavior. The exact key is still unknown. so study must be continued.

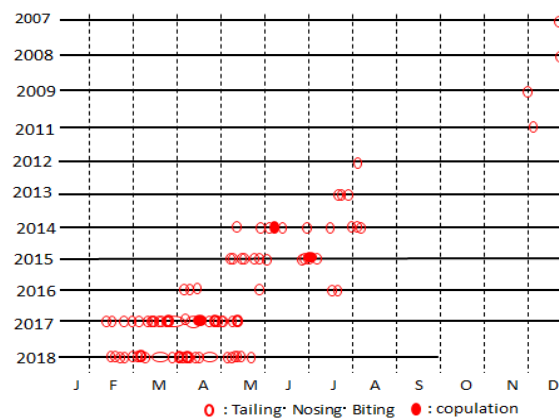


Fig. 2. Changes of season of male sexual action at IPO (2007-2018).

Female

Females at five aquariums have released egg capsules, which indicates they are all matured. From 2015 to 2017, female at MW and IPO released infertile egg capsules. At MW, the cycle of releasing egg capsules is every two years, constantly. The previous study revealed that levels of reproductively-related steroid levels across the annual and biennial cycle for male and a female respectively (Henningsen et al. 2008), which matches the result at MW. However, at IPO, the cycle is not constant. And at MW, both females No.8 and 10 released egg capsules on the same season, from July to August. But at IPO, females (No.1, 3, 5, 7) released egg capsules in a different season (Fig.3). This difference may be caused by variation of the timing of ovulation and/or egg staying period in their uterus among individuals, but it is still unclear. More study must be needed to explain this point.

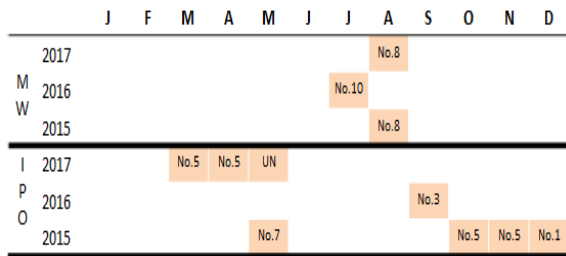


Fig. 3. Female egg release (2015-2017) Each number represents identification numbers. UN: undermined

Reproductive steroid hormone levels

Blood collection has been performed in almost every month on female No.10 and No.8 since March 2015 in free swimming situation, without any restriction (Fig.4). Plasma is collected from the blood and stored at -30°C until analyzing. Steroid hormones, 17- β -estradiol (E2), progesterone (P4), testosterone (T) were measured by EIA method at Kyushu University. Blood sampling was done on female No.10 for 39 months, and on No.8 for 15 months.



Fig. 4. Free swimming blood sampling.

E2 peak appears on October 2015, and again on September to November 2017 at No.10. As E2 gets highest level just before ovulation in mammals, we assumed ovulation occurred on these periods. In fact, on 22 January 2018, after the second E2 peak, we confirmed egg capsules stayed in the uterus by ultrasound examination in cooperation with Okinawa Churaumi Aquarium (Fig.6).

On the other hand, at No.8, clear peak of E2 and P4 did not appear from June 2017 to August 2018, which indicates no ovulation during this period (Fig.5). We will continue blood sampling and measuring reproductive steroid hormones level and other parameters (water temperature, photoperiod, etc.).

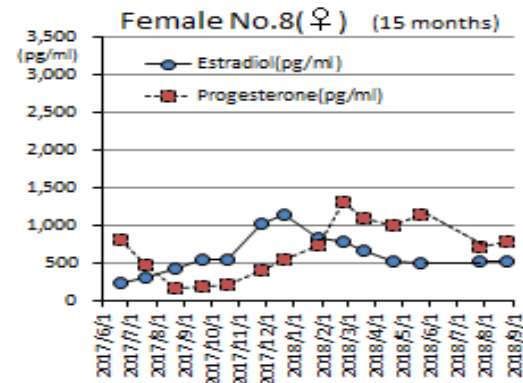
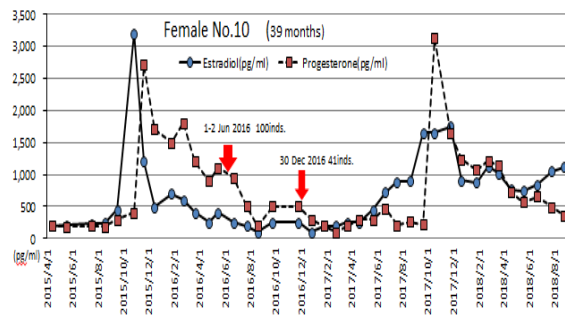


Fig.5. Changes of female reproductive steroid hormone levels. Arrows are the timing of egg releasing.

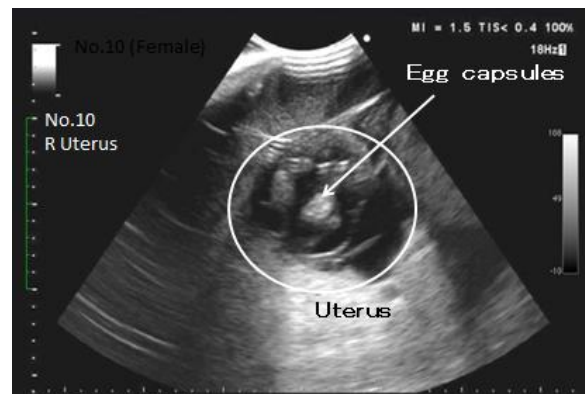


Fig.6. Ultrasound examination (No.10).

Relationship between food consumption and breeding status in female.

In MW, moving annual total of daily food consumption of females shows clear two years cycle. After releasing egg capsules, food consumption increases, and after the peak, food consumption decreases, and release of egg capsules occurs again (Fig.7).

Fig.8 shows relationship between food consumption, reproductive steroid hormone levels and the timing of releasing egg capsules. The peak of food consumption and reproductive steroid hormone levels matched. In the previous study, it was revealed that liver mass and liver lipids of female *C. taurus* changes seasonally, corresponding to reproductive status (Davidson et al.,2011). This might result from appetite change like this.

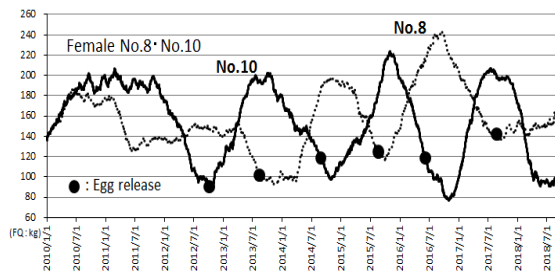


Fig.7. Relationship between food intake and the timing of egg releasing. Dot line: No.8, Liner line: No. 10 (2010-2018)

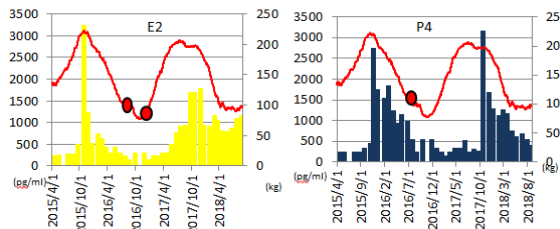


Fig.8. Food consumption and steroid hormones (Female No.10).

In Situ, Resarch

Ogasawara islands are 1,035 km far to the south from Tokyo. The islands were registered to World Natural Heritage in 2011 and many tourists visit there. *C. taurus* in Ogasawara islands is a famous shark among divers. On 1977, Tokyo prefecture researched distribution of sharks around the islands. Though *C. taurus* is known that they migrate more than 500 km, this research reported *C. Taurus* was not distributed in Hachijyo island, 760 km north from Ogasawara islands. Therefore, our hypothesis is “*C. taurus* in Ogasawara islands settles there and will not migrate so much”.

Monitoring program –Photo identification

From February 2018, RMCS started monitoring program, photo identification program on *C. taurus* in Ogasawara islands in cooperation with local diving services. We made a poster about this program to ask divers to take photographs or videos of the sharks and send them to us via email. Until now, we identified 16 individuals from right flank, and 15 individuals from left flank, from data we received. All identification data is saved at Kanagawa Prefectural Museum of Natural History.

Fresh mating scars in female

The first fresh mating scars were observed on 17 Jun 2018 (Fig.9). From this fact, mating season might be on June in Ogasawara islands. By re-identification, female migration was recorded from Ototo-jima to Futami port in Chichi-jima, 9.7km distance. We will continue this program and test our hypothesis.



Fig.9. First observation of fresh mating scars.

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