The Elasmobranch Husbandry Manual:

Captive Care of Sharks, Rays and their Relatives

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Chapter 34
Notes on Reproduction of the Zebra Shark, *Stegostoma fasciatum*, in a Captive Environment

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Abstract: A pair of zebra sharks (*Stegostoma fasciatum*) successfully reproduced in the Shark Reef exhibit, Henry Doorly Zoo. Mating behavior was similar to that described for the nurse shark (*Ginglymostoma cirratum*) (Klimley, 1980; Carrier et al., 1994) and chain dogfish (*Scyliorhinus retifer*) (Castro et al., 1988). Between September 1998 and September 2000, 80 fertile eggs were laid. Mean incubation time was 152.5±26.5 days, newborns had a mean TL of 30.2±0.8 cm, and mean weight of 92.2±14.0 grams. Increased water temperature resulted in decreased incubation time (R^2=0.887, n=6).

The zebra shark (*Stegostoma fasciatum*) is a tropical, inshore shark of the Indo-West Pacific (Compagno, 1984). It is a hardy shark and a popular species for public exhibition. Zebra sharks are oviparous, laying eggs in large, dark brown or purplish-black cases (17cm long x 8 cm wide x 5 cm thick). These cases have fine, lateral tufts of hair-like tendrils which serve to anchor the cases to the substrate (Compagno, 1984).

The Shark Reef exhibit at the Henry Doorly Zoo (Omaha, Nebraska, USA) is an irregular, oval shaped tank having a depth range of 4.6-5.8 m and a volume of 3,400 m^3 (1,700 m^3 of which is on exhibit). The tank incorporates a public walk-through tunnel across one third of the exhibit floor. It is decorated with many artificial corals, covering one wall, a number of simulated coral bomboras, and patch reefs. The exhibit is maintained at a temperature range of 24.5-25.5 °C. Artificial seawater is used. The lighting regime is computer controlled and a 12:12 light:dark photoperiod is maintained. Sunrise and sunset are simulated every day and ambient light is allowed to fall on the exhibit at night. The exhibit contains six sandbar sharks (*Carcharhinus plumbeus*), five southern stingrays (*Dasyatis americana*), and a variety of Caribbean reef fishes.

Two adult zebra sharks, caught off northeastern Australia, were added to the Shark Reef exhibit in November of 1996: a female of 210 cm total length (TL), weighing 46.0 kg; and a male of 199 cm TL, weighing 33.0 kg. The male had a hardened-clasper length of 203 mm (measured from the point of flexion to the tip). Shortly after the zebra sharks were added to the exhibit they showed signs of breeding and data related to reproduction were recorded. This empirical information has been presented below.

COPULATION

Pre-copulatory behavior was observed shortly after the zebra sharks were introduced into the exhibit and recurred throughout the year. The male shark was observed biting the pectoral fins and tail of the female for periods of several hours. The zebra sharks would generally behave in one of two ways: either swimming through the water column, with the male biting the tail of the female; or lying motionless on the bottom of the exhibit with the male holding the female by the pectoral fin or tail. These behaviors were often intense and on one occasion it was considered necessary to separate the sharks. Pre-copulatory behavior was occasionally followed by copulation. Mating behavior was similar to that described for the nurse shark (*Ginglymostoma cirratum*) (Klimley, 1980; Carrier et al., 1994) and chain dogfish (*Scyliorhinus retifer*) (Castro et al., 1988). During mating the female became listless. As the male bit her pectoral fin she would sink to the exhibit floor, sometimes falling on her back. The male would then wrap his body around the female and insert one clasper into her cloaca. Copulation would last two to five minutes and the male would then release the female. Following copulation the sharks would frequently swim to separate areas.

EGG-LAYING

Mating was observed on several occasions and on the 23rd of September 1998, the first egg was laid. The female continued to lay eggs until early February, 1999. Egg-laying was repeated during the 1999-2000 season and the 2000-2001 season. Egg-laying behavior was similar to that described for the chain dogfish (Castro et al., 1988). As egg-laying approached, tendrils began to appear from the cloaca of the female and she would start to slowly circle vertical structures. The

Table 34.1. Data recorded during the incubation and hatching of zebra sharks (*Stegostoma fasciatum*) at the Henry Doorly Zoo, showing basic water parameters, incubation parameters, hatch success rates, and neonate morphometrics. Data has been sorted by increasing water temperature.

System name	System volume	Mean water temperature (°C)	Mean pH	Mean [NH_3] ($mg.l^{-1}$)	Number of fertile eggs	Eggs hatched	Hatch success rate
WT (2000)	$5.7m^3$	23.0 ± 0.4	8.00 ± 0.10	0.00 ± 0.01	7	0	0%
SR (1999)	$3{,}400m^3$	24.4 ± 0.8	8.30 ± 0.07	0.01 ± 0.01	35	3	9%
SR (2000)	$3{,}400m^3$	24.5 ± 0.8	8.25 ± 0.07	0.01 ± 0.01	9	5	56%
QS (1999)	$1.9m^3$	25.6 ± 0.2	8.19 ± 0.12	0.01 ± 0.01	4	3	75%
FR2 (2001)	$1.9m^3$	26.0 ± 0.7	8.49 ± 0.09	0.01 ± 0.06	6	3	50%
FR1 (2001)	$1.9m^3$	26.2 ± 0.6	8.32 ± 0.15	0.00 ± 0.00	10	2	20%
QS (2000)	$1.9m^3$	26.6 ± 0.4	8.19 ± 0.07	0.01 ± 0.01	9	6	67%

System name	Number of eggs with embryo	First embryo visible (days)	Mean yolk diameter at 15 weeks (mm)	Mean incubation time (days)	Mean neonate TL (cm)	Mean neonate weight (g)	Neonate gender (m.f.u)
WT (2000)	6	43	35.0 ± 5.0	-	-	-	-
SR (1999)	11	40	38.0 ± 5.5	195 ± 2.6	31.1 ± 0.9	73.5 ± 7.8	3.0.0
SR (2000)	6	38	31.0 ± 0.8	167 ± 2.9	28.8 ± 1.9	91.9 ± 4.4	3.2.0
QS (1999)	4	35	33.3 ± 1.3	159 ± 3.8	29.6 ± 0.6	77.0 ± 2.8	1.2.0
FR2 (2001)	3	26	25.0 ± 2.0	137 ± 3.6	30.5 ± 1.3	101.4 ± 3.5	0.3.0
FR1 (2001)	4	23	26.0 ± 2.8	134 ± 2.8	30.2 ± 3.1	106.3 ± 7.8	0.2.0
QS (2000)	7	30	18.2 ± 4.5	123 ± 2.2	30.8 ± 1.9	103.0 ± 7.7	3.3.0

tendrils would become entangled within the vertical structure and the egg would be pulled from the oviduct. The female would then stop circling and resume normal swimming behavior. Eggs were frequently found attached to artificial corals. The time interval between the first and second egg ranged from a few minutes to a few days. Up to six eggs were laid per day. In 1998-1999, 46 eggs (39 fertile) were laid in a 112-day period. In 1999-2000, 26 eggs (25 fertile) were laid in 49 days, while 18 eggs (16 fertile) were laid in 24 days during 2000-2001.

INCUBATION

Shortly after deposition, eggs were moved from the Shark Reef exhibit into smaller incubation systems, having different temperature regimes (Table 34.1). Eggs were never exposed to air. Each egg was placed in a bucket while underwater, transferred to the incubation system, and added to the new system while underwater. Power heads (Maxi-Jet PH, Aquarium Systems, USA) were added to each incubation system to increase water circulation. On one occasion an egg was deliberately left in the Shark Reef exhibit, but it disappeared within two weeks. Before eggs were placed in the incubation systems, most of the tendrils were removed to prevent neonate entanglement on hatching. A few tendrils were retained, allowing eggs to be suspended below the surface of the water by tying them to a pipe lying across the top of the tank.

HATCHING

Approximately two to four weeks after the yolk sac was no longer visible, neonates would hatch. If an egg did not hatch within this period, it was generally the result of the neonate getting stuck within the egg-case and dying. On some occasions egg-cases were cut open to aid the emergence of the neonate. The neonates did not appear to be adversely affected by this procedure, even if a small portion of the yolk sac remained. No medical treatments were performed on the external yolk sac or neonates, although these specimens were closely monitored. All aided neonates survived.

DESCRIPTIVE STATISTICS

Throughout the incubation and hatching period, data were recorded for water quality, yolk and embryo development, incubation times, hatch success rates, neonate morphometrics, and neonate gender (Table 34.1).

Water chemistry was maintained at safe and constant levels in accordance with data published by Michael (2001). Water temperatures were measured daily using a dissolved oxygen meter (OxyGuard Handy Meter, Point Four Systems Inc., USA) rounded to the nearest 0.1 °C. Ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) were measured weekly using colorimetric titration (Permachem Reagents, Hach Company World Headquarters, USA). pH was measured weekly using a portable pH meter (Model 410A, Orion Research Inc., USA). During 2001 the incubation systems exhibited signs of pH instability. It was known that elasmobranchs are sensitive to pH shifts (Stoskopf, 1993) so phosphoric acid (H_3PO_4) (Phosphoric Acid 75%, Em Science, USA) was added to the incubation systems to maintain a constant pH of 8.0.

Eggs were checked weekly for embryo development and yolk diameter was measured. Measurements were taken using plastic calipers while illuminating the egg case with an underwater flashlight (UK 400, Underwater Kinetics, USA). To minimize error the egg was placed as level as possible with the flashlight underneath, pointing straight up. This process reduced shadows cast by the yolk and allowed more accurate measurement. On hatching, neonates were weighed using a digital platform scale (XE Series Model 3000, Denver Instrument Company, USA) and TL was measured (as per Compagno,1984).

The period of incubation before first embryos became visible ranged from 23-43 days, with a mean of 33.6±7.5 days. Total incubation time ranged from 123-195 days, with a mean of 152.5±26.5 days. 27.5% of the fertile eggs hatched. The sex ratio of hatched sharks was 10 males to 12 females. Neonates had a TL of 26.7-33.0 cm, with a mean of 30.2±0.8 cm, and their weight ranged from 68.0-117.6 grams with a mean of 92.2±14.0 grams (Table 34.1).

Data recorded during incubation suggested a relationship between temperature and embryo development. This possibility is consistent with observations in other species (Kormanik, 1993; Wourms, 1977). Increasing water temperature resulted in significantly decreased yolk diameters at week 15 (R^2=0.629, n=7) and decreased incubation times (R^2=0.887, n=6), and appeared to have a weaker influence on hatch success rates

and neonate size (Table 34.1). The highest hatch success rate (75%) occurred at a mean temperature of 25.6 °C, while the lowest hatch success rate (0%) occurred at a mean temperature of 23.0 °C. Eggs incubated at this lower temperature produced an embryo but it died after 112 days of incubation. The nature of recorded data introduced artifacts of uncontrolled variables, shifting time-lines, and, in some cases, a degree of measurement subjectivity. Great care should therefore be exercised when interpreting these results.

REARING

After hatching, the neonates would be disoriented and swim in spirals. In some cases this behavior lasted for three to six months and the neonates occasionally injured themselves on exhibit decoration. When maintained in restricted systems neonates were observed accidentally biting each other and rubbing against exhibit walls. When these circumstances arose, the exhibits were appropriately modified to provide sufficient and unimpeded swimming space.

Neonates were indifferent to food when it was initially introduced. A feeding stick was constructed and food presented directly underneath their mouths. This feeding technique induced the sharks to feed with greater enthusiasm, even within minutes of hatching. Neonates were fed twice daily for the first four to six months. A variety of food items were given to the young zebra sharks, including: shrimp (*Metapenaeus dobsoni*), clams (*Mercenaria mercenaria*), little tunny (*Euthynnus alletteratus*), Atlantic mackerel, (*Scomber scombrus*), and squid (*Loligo opalescens*). These food items were cut into bite-sized pieces and soaked in a vitamin supplement (Vita Fish™, Marine Enterprises International, USA). Once neonates were feeding well, they were broadcast fed once per day.

In 1999, the pups were placed in a display tank with fluctuating water temperatures (22.3-29.0 °C). Bluestreak cleaner wrasse (*Labroides dimidiatus*) were later added to the exhibit to control ectoparasites on other tank inhabitants. The wrasse, along with some millet butterflyfish (*Chaetodon miliaris*) and Pacific double-saddle butterflyfish (*Chaetodon ulietensis*), began picking at the eyes of the sharks. Other institutions have reported similar behavior for raccoon butterflyfish (*Chaetodon lunula*), foureye butterflyfish (*Chaetodon capistratus*), and bannerfish (*Heniochus* spp.). The eyes of the neonates clouded over and hemorrhaged. Infection spread to the brain causing meningitis and ultimately death. One of the sharks was moved to a tank with higher water temperatures (>24.0 °C) and survived. Since this incident zebra sharks have never been maintained in water temperatures below 24.0 °C or held with problematic fishes.

Table 34.2. Anti-helminthic treatments administered to the Shark Reef exhibit, Henry Doorly Zoo, between June 1999 and September 2000.

Date of treatment	Medication administered	Treatment duration	Dosage
Jun 1999	Trichlorfon	8 weeks	0.35 mg l^{-1} for week one; 0.45 mg l^{-1} for week two; and 0.55 mg l^{-1} for the final five weeks.
Oct 1999	Trichlorfon	3 weeks	0.55 mg l^{-1}
Feb 2000	Praziquantel	Once only	2.0 mg l^{-1}
May 2000	Trichlorfon	4 weeks	0.25 mg l^{-1} for weeks one and two, and 0.27 mg l^{-1} for weeks three and four.
Sep 2000	Praziquantel	Once only	2.0 mg l^{-1}

Many of the zebra shark pups have been transferred to other institutions and 17 of the original 22 remain alive to this day.

MEDICAL TREATMENTS

During pre-copulatory behavior the pectoral fins of the adult female were frequently injured to the point where medication was deemed necessary. At these times she was treated with oral enrofloxacin (Baytril®, Bayer Corp., USA) at a dosage rate of 408 mg day^{-1} (~8.9 mg kg^{-1} day^{-1}) for a period of two weeks.

Following an outbreak of *Neobenedinia melleni* during June of 1999, a series of dimethyl phosphonate (Trichlorfon or Dylox® 80, Bayer Corp., USA) and praziquantel (Praziquantel 100%, Professional Pharmacy Services Inc., USA) treatments were administered to the Shark Reef exhibit (Table 34.2). These treatments could have influenced reproductive success in the zebra sharks and also influenced the development of embryos incubated within the exhibit. Between September and October of 2000 the adult zebra sharks died. The cause of death is unknown but it is possible that sensitivity to the anti-helminthic treatments may have played a role.

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REFERENCES

Carrier, J. C., H. L. Pratt, Jr., and L. K. Martin. 1994. Group reproductive behaviors in free-living nurse sharks, *Ginglymostoma cirratum*. Copeia 1994(3): 646-656.

Compagno, L. J. V. 1984. Sharks of the World. FAO Species Catalogue, Vol. 4, Parts 1 and 2. Food and Agricultural Organization of the United Nations, United Nations Publications, New York, USA. 655 p.

Castro, J. I., P. M. Bubucis, and N. A. Overstrom. 1988. The reproductive biology of the chain dogfish. Copeia 1988(3): 740-746.

Klimley, A. P. 1980. Observations of courtship and copulation in the nurse shark, *Ginglymostoma cirratum*. Copeia 1980(3): 878-882.

Kormanik, G. A. 1993. Ionic and osmotic environment of developing elasmobranch embryos. Environmental Biology of Fishes 38: 233-240.

Michael, S. W. 2001. Sharks and Rays. T. F. H. Publications, Inc., Neptune City, New Jersey, USA. 256 p.

Stoskopf, M. K. (ed.). 1993. Fish Medicine. W. B. Saunders Company, Harcourt Brace Jovanovich, Inc. Philadelphia, Pennsylvania, USA. 882 p.

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