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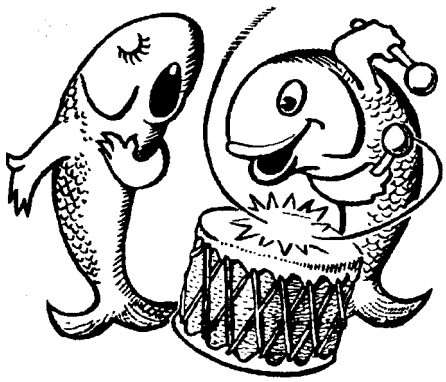

Special Edition No. 3 *May 2018*

The 2nd Shark Supplement

A Companion Volume to the 2nd Elasmobranch Husbandry Manual

Additional Conference Papers from:
The 2nd Elasmobranch Husbandry Symposium.
November 11-13, 2013
Hosted by the Monterey Bay Aquarium

A Bibliography of Elasmobranch Husbandry Articles from:
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2005-2018

Editorial Contributors:

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Elasmobranch Husbandry Manual 2, Ohio Biological Survey, 2017
Provided edited versions of featured Elasmobranch Husbandry Symposium papers.

(*See a complete list of EHS2 and EHM2 organizers, editors,
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Special Thanks

The EHM2 Staff would like to especially thank the team at the Monterey Bay Aquarium for hosting such a successful symposium: Ginger Hopkins, Melissa Leong, Patricia Palacio, Juan M. Ezcurra, Michael J. Murray DVM, Randy Hamilton, Jon Hoech, and John O'Sullivan. We would also like to thank Jennifer Compston for her assistance during the symposium and her continuing support throughout the years of the Elasmobranch Husbandry project. Thank you to Blake Chapman for her assistance as a scientific editor during the development of the Manual II. Thank you to Brian Armitage for his editorial and publishing expertise, and to the Ohio Biological Survey. Thank you to Rolf Williams for the use of the cover illustration. Finally, we would like to thank Peter J. Mohan for his support and for his continued efforts in editing and distributing Drum and Croaker.

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Preface

The Second Shark Supplement is the third special edition of *Drum and Croaker*. It is intended as a companion volume to The Elasmobranch Husbandry Manual 2: Recent Advances in the Care of Sharks, Rays and their Relatives (EHM2), which was published by the Ohio Biological Survey (Columbus, Ohio) in April 2017.

Special Edition #3 is a reservoir for many valuable papers that were presented at the Second Elasmobranch Husbandry Symposium (EHS2), subsequently submitted to the EHM2, but ultimately not included due to space limitations. It also serves as a convenient list of all elasmobranch papers appearing in *Drum and Croaker* since the publication of Special Edition #2 in 2004 (The Shark Supplement). The two *Drum and Croaker* special additions and the pair of Elasombranch Husbandry Manuals together capture 60 years of husbandry information in 4 volumes.

For this special edition, all of the text obtained from the EHM2 editors has been converted into the standard *Drum and Croaker* page format using the spartan editing method traditional to D&C. This is the first publication of these EHS2 papers. All citations of these works should refer directly to this volume and use the page numbers on which they actually appear.

Although many would consider *Drum and Croaker* to be "grey literature", it contains a wealth of otherwise unpublished technical information and observational records that are of tremendous value to aquarists and shark biologists. I would like to express my thanks to all of those who have supported *Drum and Croaker* over the years. Beginning in 2001, current issues of Drum an Croaker, as well as the many historical issues that I continue to scan, restore, and archive, have all resided on the *Drum and Croaker* website, which is hosted by The Columbus Zoo and Aquarium at http://drumandcroaker.org/. Mike Brittsan (Director of Aquatic Services) and Greg Bell (Executive VP/CFO) are the institutional supporters that have made the site possible, while Kevin Bonifas (Director of Technology Services) built and continues to update the pages. Doug Warmolts (VP of Animal Care) was an important advocate for the D&C archiving project.

Pete Mohan
Kent, Ohio, USA
May 1, 2018

FIGHTING WITH FUSARIOSIS DISEASE ON HAMMERHEAD SHARKS, *Sphyrna lewini,* GRIFFITH & SMITH, 1834

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Abstract

In 2011, a group of 20 juvenile hammerhead sharks (*Sphyrna lewini*) about 60 cm long were acquired by Nausicaá and kept in a dedicated 600 m^3 quarantine tank. A few months later 3 sharks showed symptoms of fusariosis. One rapidly died. Immediate actions were decided to try and keep the other ones alive. Since then 8 new occurrences of the disease were observed. Several observational, microbiological, hematological and histological studies were conducted to improve the knowledge of the physiopathology and treatment of this fungal disease. Several drug regimens were tested with various efficiencies.

Introduction

20 hammerhead sharks, 60 cm TL, arrived in Nausicaa between July and October 2011. They were reared in a reserve tank built specifically for them in new quarantine area. Quickly some individuals showed signs of illness, identified as *Fusarium sp.*, which extended over time to other individuals and currently concerns around 50% of the population.

Rearing Conditions and Factors Inducing Stress: Hypotheses

Scalloped hammerhead sharks were provided by De Jong Marinelife in the Netherlands. They were transported by truck between Netherland and Nausicaa, 3 or 4 at a time between July and October 2011. During this entire period, water temperature was maintained between 23 and 24°C to facilitate acclimatization when a group of new sharks was delivered. Once the last sharks were received in October, the temperature was raised to 25°C and then slowly to 27°C. General rearing conditions and initial water quality parameters are shown in Tables 1 and 2.

Table 1. Rearing conditions.

Tank	Circular, black, 570 m^3, 17 m diameter x 2,5 m depth
Day light	6 HQI 2000w, 10000°K, 12h/24
Night light	12 fluorescent tubes, blue, 49w, 24h/24
Filtration turnover	150 m^3/h
Water renewal	12 m^3/week
Streaming pump	300 m^3/h

Table 2. Water quality parameters at sharks' arrival.

Parameter	Unit	Value
Temperature	°C	24
pH		8,32
Conductivity	mS/cm	47,9
Redox	mV	320
O_2	%	98,2
TAC	°F	18
$N\text{-}NH_3$	mg/L	0,038
$N\text{-}NO_2^-$	mg/L	0,006
$N\text{-}NO_3^-$	mg/L	3,97
PO_4^{3-}	mg/L	1,95
Ca^{2+}	mg/L	300

Different hypotheses can be made regarding the factors favoring fusariosis:

A. Low temperature (between 23 & 25°C at the beginning):

Cold temperature seems propitious to *Fusarium* development and reduces the natural ability of the sharks to defend themselves.

The first case of fusariosis appeared in October 2011, when water temperature was 27°C, and that shark died in 2 months. It was the only case where *Fusarium* was effectively found by microbiological culture and on histological analysis. After that, temperature was increased to 28°C and later to 29°C (Figure 1).

B. Low level of mineral salt (Mg^{2+}, Ca^{2+}, K^+, Sr^{2+}):

Mineral salts are analyzed routinely. As a result, we realized in December 2011 that levels of different mineral salts had strongly decreased, because of the use of a poor-quality salt to make artificial seawater with fresh water. We then proceeded to a water change with natural seawater by truck and artificial seawater made with Instant Ocean® salt and fresh water. Composition changes are reflected in Figure 2. The first 3 cases of fusariosis appeared at this time.

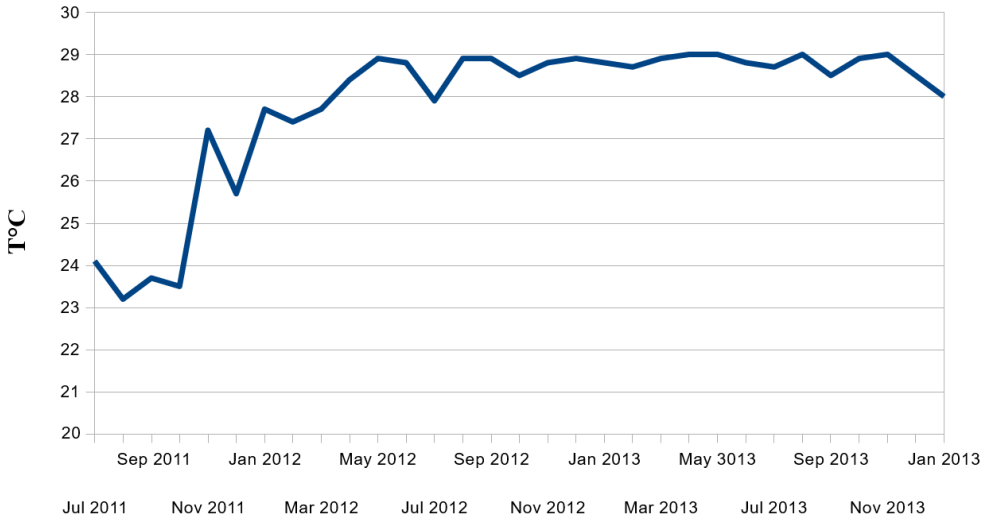

Figure 1. Water temperature evolution after the initial occurance of fusariosis.

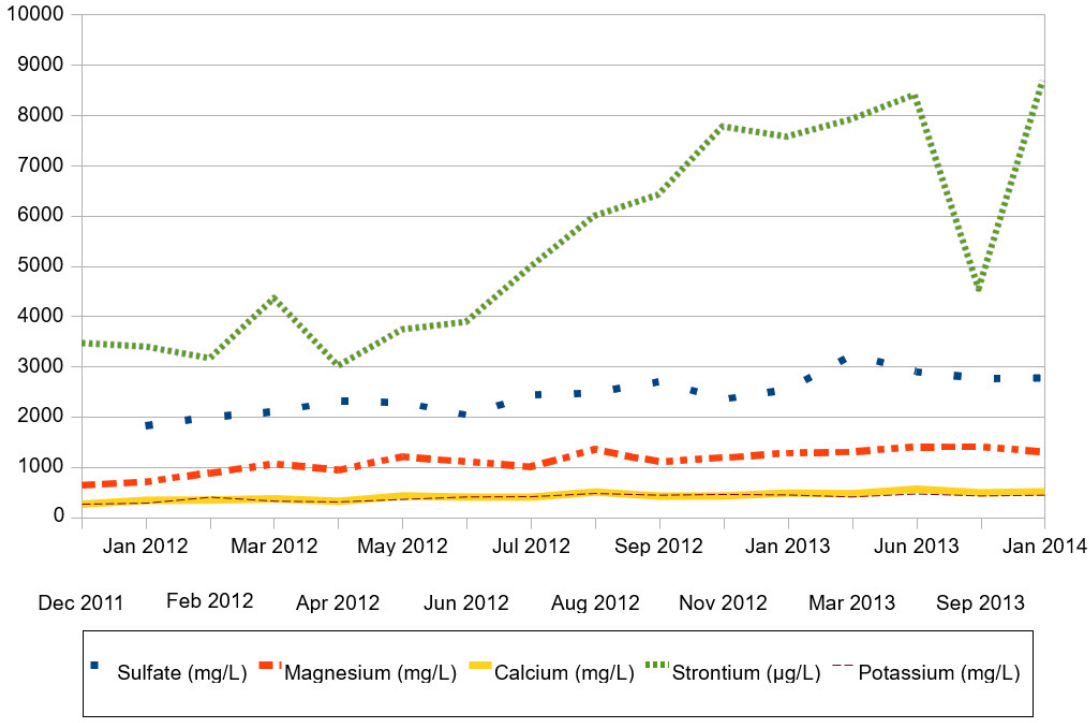

Figure 2. Mineral salt levels related to changes in saltwater sources.

C. High values of magnetic field or heterogeneous distribution of the field in the tank:

The use of 2 stream pumps generating a strong magnetic field was initiated in September 2011. Another 2 pumps were added in August 2012. Each time, several cases appeared in the following weeks. Values of magnetic field were measured directly on the pump itself with a Gaussmeter (Gigahertz ME 3030B®) and were more than 2000 nT. These values decreased away from the pump. They are shown in Table 3 with values to be compared to Table 4, found in others aquariums. The 4 pumps were stopped in June 2013.

Table 3. Magnetic field measured in the hammerhead tank.

On streaming pump (Tunze Turbelle Master Stream®)	>2000 nT
At 1 m from the pump	1500 nT
At 2 m from the pump	400 nT
At 3 m from the pump	150 nT
At 4 m from the pump	50 nT
In the middle of the tank	10 nT

Table 4. Maximum level of magnetic field recorded in other aqauriums.

Shark exhibit tank at Burger's Zoo (Arnhem, NL)	<26 nT
Shark exhibit tank in Nausicaä (Boulogne-sur-Mer, F)	<80 nT
Hammerhead tank with others streaming pumps (Flyght ITT®)	<15 nT

D. Nitrate concentration:

It is known that high nitrate concentration could cause disruption of thyroid metabolism and a weakening of the organism. In our case nitrate rate have doubled in one year resulting of increasing biomass and amount of food distributed.

Other more acute episode, such as the increase of *Vibrio*, lower pH, or peak of nitrite could have also favored this opportunistic disease by generating stress.

All these events (A through D) could combine, amplifying stress.

Fusariosis Symptoms and Disease Evolution

History:

The history of observations and treatments is presented in Table 5.

First stage of the disease:

From a macroscopic point of view, the disease first shows as the occurrence of dark granules along the lateral line (Figure 4). After a few days, they are evolving, forming a small

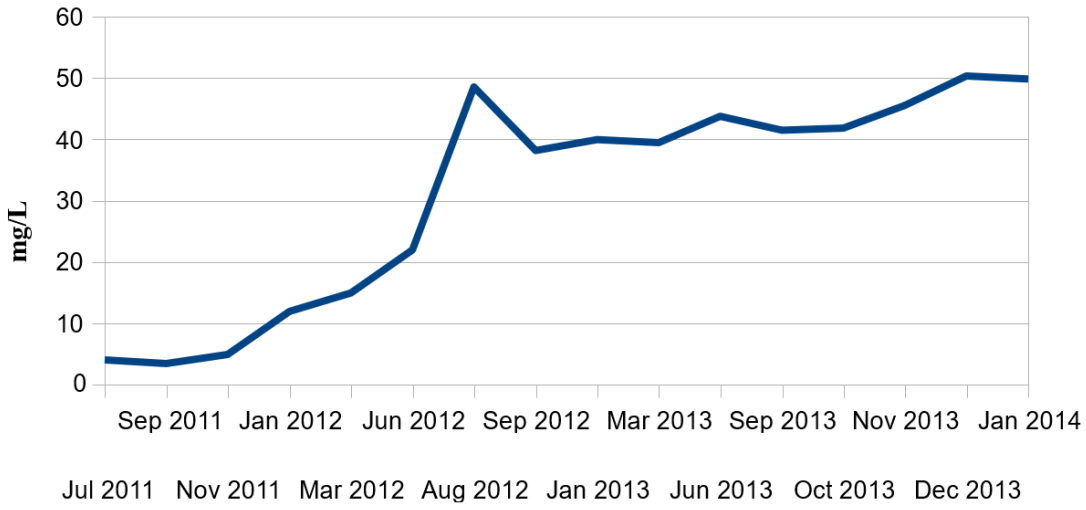

Figure 3. Nitrate nitrogen levels.

white blisters which after bursting are replaced by a small crater (Figure 4). This granulomatous inflammation usually begins along the cephalic part of the lateral line between the pectoral fins and the gills and moves towards the tail causing inflammation of the secondary branches of the lateral line.

Quickly inflammation of the hammer appears with cyst formation (Figure 5) and exudation from the ampullae of Lorenzini pores.

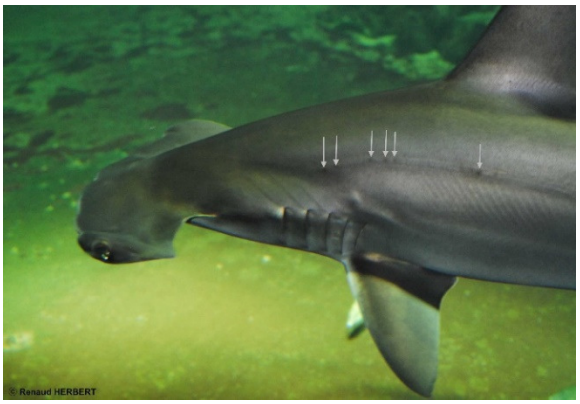

Figure 4. Small dark,granulations appear on lateral line

Figure 5. Extension of granulomatous inflammation the to the hammer and over parts of the body

-Case report of shark No.1:

Between 25 and 27 ° C, the evolution of the disease was very fast and led to the death of the shark in 2 months. 15 days before death, the animal presented large area of deep ulceration, that seemed to reach the cartilage. The underside of the hammer took on a lacy appearance (Figure 6).

After death, the cartilage was so eroded that a cephalofoil broke when the shark was picked up with a landing net from the floor of the tank (Figure 7).

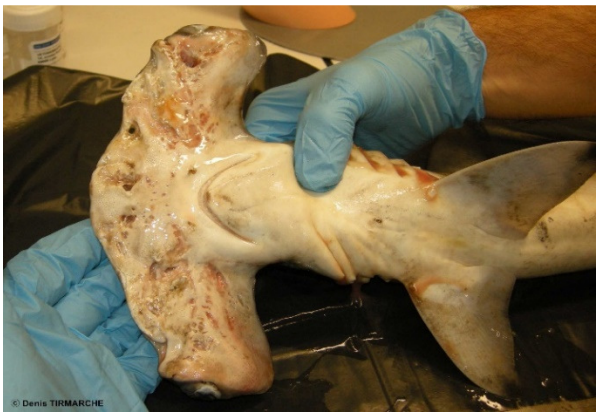

Figure 6. Macroscopic view of the deep ulcerative dermatitis of the hammer.

Figure 7. Failure of the hammer cartilage.

Samples were placed in culture and revealed the presence of *Fusarium sp*. This was then confirmed by histological analyzes by Dr. Karin Lamberger, Laboratory of Veterinary Pathology, Vet Diagnostics in Lyon, France.

Although the samples were partially autolyzed, histological examination revealed two concomitant multi infectious processes. Indeed, the epidermis of the hammer and the lateral line presented big areas of ulceration with proliferation of hyphae from 2 and 4 µm, with irregular shapes and rare budding (Figure 9) extending into the dermis and underlying cartilage (Figure 8). Granulomatous inflammation and eosinophil granules were observed transmurally.

At the same time we noted a septic state characterized by vascular emboli of bacterial colonies. Similarly, the respective parenchyma of the liver, kidney and spleen had numerous fibrin thrombi associated with bacterial colony. Finally, many bacterial colonies were observed circulating in the ovaries, the spiral valve and the heart.

-Case report of the others sharks

At 29°C the course of the disease changed. Growing faster, sepsis dominated the fungus making it non-existent. All fungal cultures performed returned negative.

Depending of the specimen, evolution of the disease was more or less rapid. Some sharks (shark No. 6 and 11) developed a few blisters along the lateral line, but then the symptoms did not evolve further, vesicles regressing by themselves. From time to time a new blister appeared and then disappeared after few days. Although affected by the disease, these animals remained in a relatively stable state.

For other specimens, evolution was faster. Within a few weeks, sepsis developed. Then, we could observe the discharge of purulent exudate (Figure 10) through the pores of the hammer (shark No. 3, 5, 7, 8). Histological analysis showed a sepsis state almost several organs, like spleen (Figure 11) and kidney (Figure 12).

Shark No.	Sex	First symptoms observation (DD/MM/YY)	Death (DD/MM/YY)	Cause of the death	Symptoms	Medication	Observations
1	F	11/10/11	04/12/11	Fusariosis	Granulomatous inflammation Ulcerative dermatitis - Sepsis	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012)	
2	F	21/10/11	Alive		Granulomatous inflammation Ulcerative dermatitis Sepsis	Terbinafine (84 days – dec 2011 to mar 2012) Voriconazole (39 days – jun 2012 to Jul 2012) Terbinafine (33 days - oct 2012 to nov 2012)	Remission
3	F	20/12/11	17/11/13	Sepsis	Granulomatous inflammation Ulcerative dermatitis Keratitis and blindness Sepsis	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012) Enrofloxacine (15 IM – may 2013 to jun 2013) Cefovecine (7 IM – may 2013 to aug 2013) Enrofloxacine (12 IM - Sep 2013) Ceftadizime (12 IM – Sep 2013)	
4	F	27/05/12	28/06/12	Heart failure	Granulomatous inflammation Sepsis	Terbinafine (84 days – dec 2011 to mar 2012) Voriconazole (26 days – Jun 2012) Terbinafine (33 days - oct 2012 to nov 2012)	
5	M	27/09/12	02/01/13	Cerebral hemorrhage	Granulomatous inflammation Ulcerative dermatitis Keratitis and blindness - Sepsis	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012)	
6	M	27/09/12	Alive		Granulomatous inflammation	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012) Voriconazole (8 days – oct 2013 to nov 2013)	Since November 2013, signs of granulamotous inflammation disappear
7	F	06/10/12	09/04/13	Sepsis	Granulomatous inflammation Ulcerative dermatitis Keratitis and blindness - Sepsis	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012) Marbofloxacine (10 IM – feb 2013 to mar 2013)	
8	F	29/04/13	09/01/14	Sepsis	Granulomatous inflammation Ulcerative dermatitis Exophtalmia and blindness Sepsis	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012) Enrofloxacine (14 IM – may 2013 to jun 2013) Cefovecine (7 IM – may 2013 to aug 2013) Enrofloxacine (14 IM - aug 2013 to Sep 2013) Ceftadizime (11 IM – Sep 2013)	
9	F	01/06/13	06/12/13	Sepsis - encephalitis	Granulomatous inflammation Exophtalmia - Sepsis	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012) Enrofloxacine (9 IM - oct 2013 to nov 2013) Ceftadizime (5 IM – oct 2013)	
11	F	11/09/13	Alive		Granulomatous inflammation	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012) Voriconazole (10 days – oct 2013 to Nov 2013)	
14	F	05/02/14	Alive		Granulomatous inflammation	Terbinafine (84 days – dec 2011 to mar 2012) Terbinafine (33 days - oct 2012 to nov 2012)	

Table 5. History of observations and medical treatments realized on the diseased sharks.

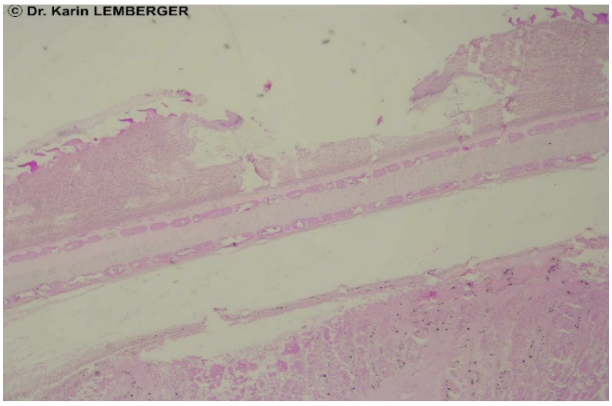

Figure 8. Deep ulcerative dermatitis with fungal Proliferation.

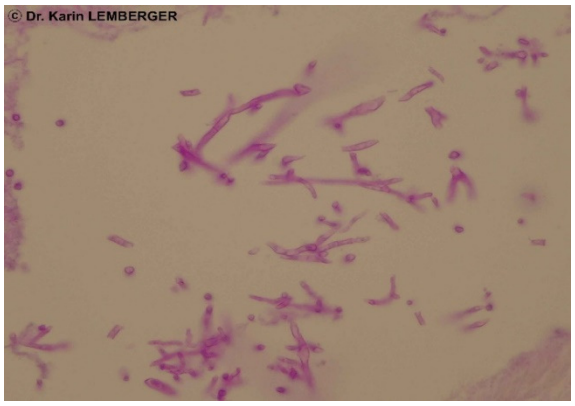

Figure 9. High magnification of the fungal hyphae.

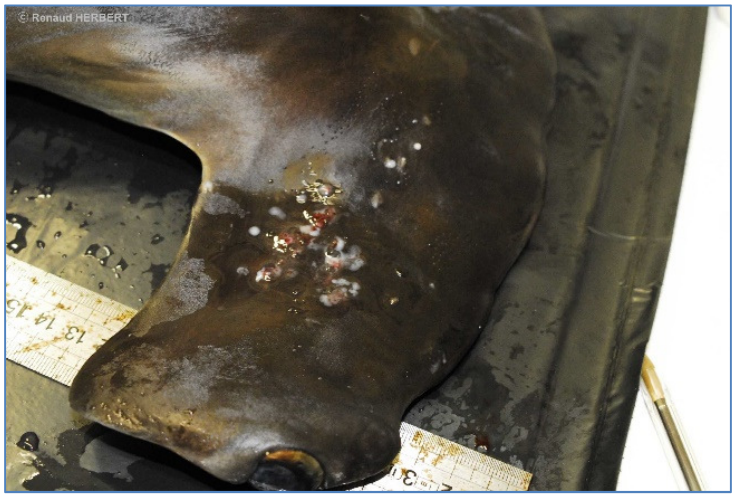

Figure 10. Sepsis – the discharge of purulent exudate through the pores of the hammer.

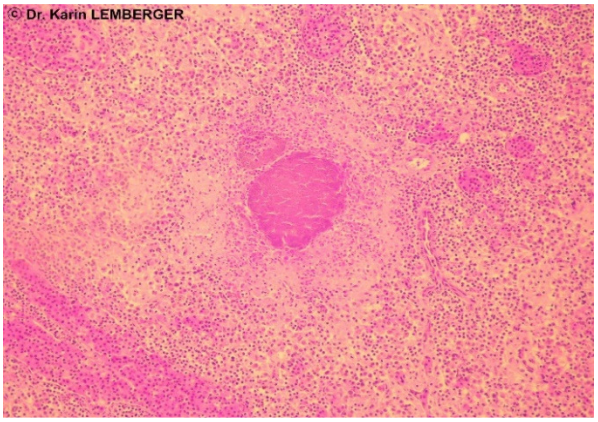

Figure 11. Septic bacterial emboli in the spleen.

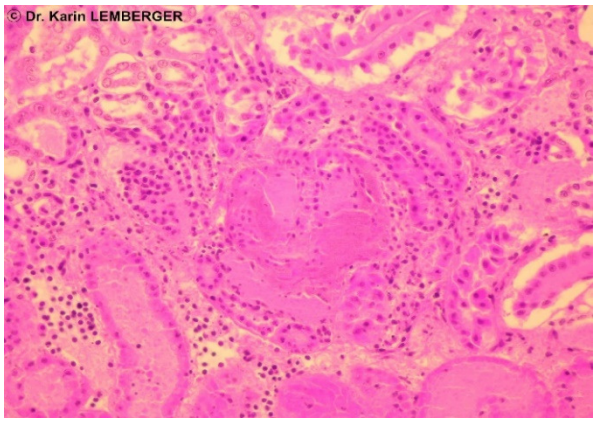

Figure 12. Septic bacterial emboli in the kidney.

Microbiological cultures highlighted various opportunistic pathogens of secondary infection, including *Enterococcus faecalis* in the purulent exudate and *Escherichia coli* (shark No.7) in blood culture. Among the others bacteria present, we could occasionally find *Aeromonas hydrophilica* (shark No.9), *Vibrio alginolyticus* (shark No.9), *Vibrio parahaemolyticus* (shark No.8), *Photobacterium damsela* (shark No.5) and *Chryseobacterium indologenes* (shark No.8). *Enterococcus* was the only bacteria present in all the dead animals. We found it in the exudate, but also in the hammer cartilage and in the cerebral fluid of some shark (sharks No 5, 7, 8, 9). At the same time the full blood panel revealed an almost exclusive neutrophilia (shark No.3), whereas protein electrophoresis shows a dominant inflammatory spectrum (Figure 13).

Fraction	**%**	**g/L**
Total protein		47
Albumin	30,9	14,5
Alpha 1	59	27,8
Alpha 2	2,1	0,9
Beta	0,2	0,1
Gamma	7,8	3,7

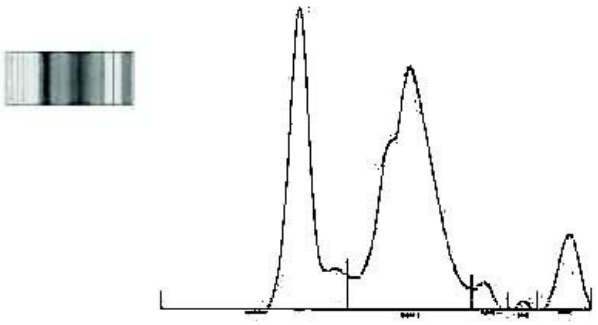

Figure 13. Serum protein electrophoresis on agarose gel (shark No. 3): A/G=0,45

This inflammation of the hammer seemed to be a handicap, preventing the shark to eat normally. Indeed, keeping his appetite, it managed to find and capture food, but seemed to have difficulty ingesting, usually ending by releasing food.

Histologically, the shark No. 5 showed an alteration of the architecture of the hammer with extensive cystic degeneration (Figure 14), however the absence of identified fungal organism is used to send the hypothesis of an old infection of *Fusarium sp*. with pejorative evolution and tissue degeneration.

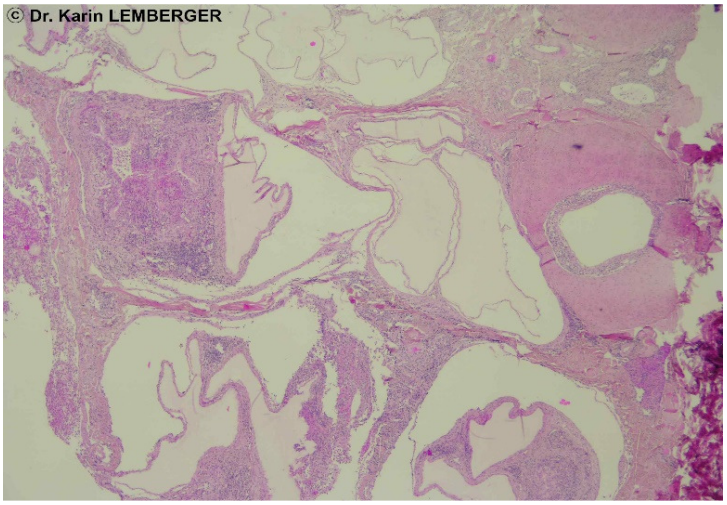

Figure 14. Extensive cystic degeneration and inflammation of the dorsal hammer.

On shark No.7, histological analyses showed an ulcerative and necrotic dermatitis and cellulitis with intra-lesional coccobacilles and Splendor Hoeppli material (Figure 15). This cellular structure is little documented in fishes and sharks but is rather often associated with a fungal infection, which in our case could be a Fusarium. Such a structure was also find in the endomyocarditis on the same animal.

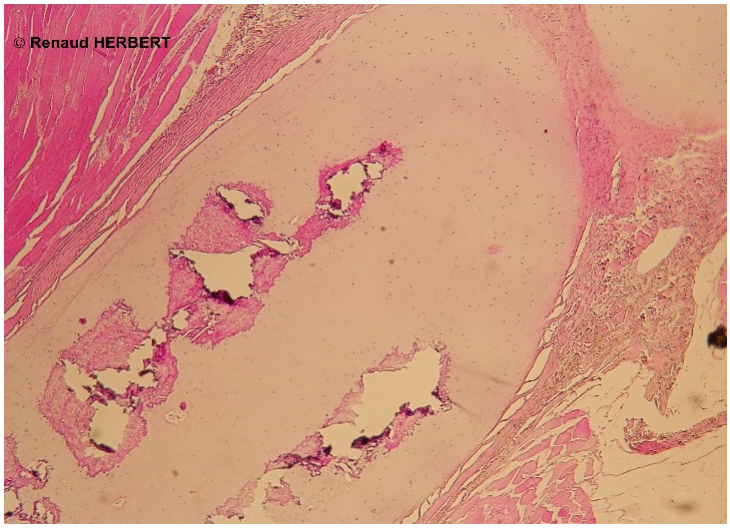

Figure 15. Splendor Hoeppli material in hammer cartilage.

More recently several infected sharks (No. 3 , 5, 7) showed bilateral keratitis evolving in a few days with the loss of the lens and blindness, then progressively extensive ocular fibrosis settled, making all structures of the eye disappear. Two specimens (shark No. 8 and 9) showed a preliminary exophthalmos. It quickly evolved with the loss of the lens in shark No. 8. However, there was a decrease in vision. Indeed, the animal avoided obstacles less easily and took longer to find food. When these sharks became blind, they met even more difficulties to find food. After a few weeks of adjustments, these animals still managed to find something to eat. However, they were subject to strong competition for food from healthy animals and did eat less. This resulted in a more or less marked decrease in liver fat reserves.

Among other symptoms observed, most of these sharks have stopped growing. Their swimming was mostly altered, slightly toned, with a strong lateral inclination. Finally, their mouths were mostly open.

Treatments and Results

Several solutions were put in place to slow the progression of this disease and try to cure the hammerhead sharks.

-Action on salinity (36,5 to 36,9 g/L):

Salinity was slightly increased in order to create unfavorable conditions for the development of the fungus, while remaining in a compatible range with the survival of sharks.

-Action on temperature:

Increasing rearing temperature to 29°C, creates unfavorable conditions to the development of the fungus and the development of immune system of the sharks was optimized. Following this increase in temperature, it was possible to keep sharks No. 2 and 3 alive even though they showed disease shortly after No. 1, a shark that quickly died. Shark No.2 is still alive after 27 months, while No.3 died after 24 months.

However, it is likely that high temperatures promoted the development of sepsis present in most cases. At the end of 2013, most of the ill sharks died of septicemia. It was then decided to decrease the temperature to 27°C with the aim of reducing bacterial development. 2 months later, state of shark No.11 begin to evolve with appearance of new vesicles on the lateral line, and a new case of granulomatous dermatitis appeared (shark No.14)

-Action on dissolved oxygen:

Holding the oxygen saturation of water between 105 and 120% promotes shark metabolism facilitating breathing. In addition, it reduces the risk of temporary hypoxia at mealtime, related to the high temperature.

-Supplementation of food:

To promote the immunity in sharks, about 25% of the diet is supplemented every day with different food supplements.

- Akwavit® sharks: vitamin complex specially formulated for the needs of sharks.
- Lipospheric Vitamin C: around 100 mg/kg BW. Vitamin stimulates the immune system. The lipospheric form slows the oxidation speed of sodium ascorbate in water.
- Ergosan®: immuno stimulant based on seaweed powder (*Laminaria digitata* and *Ascophyllum nodosum*).

-Antimycosic treatments:

Terbinafine (10 mg/kg BW, oral way):

This antimycosic has been distributed for 84 days at the onset of the disease and then again for 33 days in late 2012. No improvement was observed in diseased sharks. Nevertheless, the entire population has benefited and it is possible that there was a preventive effect on healthy animals.

Voriconazole (12 mg/kg BW, oral way):

This is a more potent molecule, which requires precise identification of diseased individuals and target training to obtain treated food. The treatment was administered during daily diving training sessions. Two individuals were able to be treated: No. 2 and 4. In the following months No. 2 started growing again, inflammation of the hammer and ulcerative dermatitis were reabsorbed. Although still smaller, this shark resumed growing. The lateral line seems healthy, though still marked by numerous scars. Actually, it is in remission.

Number 4 died at the end of treatment, due to cardiac decompensation caused by anesthesia. Samples taken showed no trace of *Fusarium sp*., or even sepsis. We can therefore assume that the treatment was effective. However, a glomerulonephritis that can be a side effect of treatment was observed histologically. The kidney failure was confirmed by the high rate of

urea in the blood.

Recent voriconazole treatments on shark No.11 didn't seem to be effective on the disease. Maybe, the duration (only 10 days) was too short. Furthermore, it was difficult to maintain continuity in the daily treatment, and the shark received the treatment 10 times in a period of 18 days.

- Antibacterial treatments:

They are administered intramuscularly using pressurized syringe and pole. The aim is to fight the secondary infection and slow the progression of sepsis that appears to be the cause of most deaths.

Marbofloxacin (fluoroquinolone - 12 mg/kg BW, 3 times/week):

Only No. 7 got this drug. No effects were observed. He became totally blind during treatment and died shortly after.

Enrofloxacin (fluoroquinolone - 10 mg/kg BW, 3 times/week):

Sharks No. 3 and No. 8 were treated several times in 4-weeks. Each time the drug was associated with a cephalosporin either Cefovecin (8 mg/kg BW every 14 days) or Ceftadizime (30 mg/kg BW, 3 times/week). In two cases, the treatment was partially effective. Visually pores of the hammer stopped discharging purulent exudate and sharks started to feed again. The sample showed that the treatment was able to stop the peripheral infection in the dermis but blood sepsis continues to be present. Once the treatment was stopped, the infection returned stronger.

On shark No.9, the combination of enrofloxacine and ceftazidime didn't show any effects. *Enterococcus* present in all these sharks appear to be resistant to most antibacterial molecules of which cephalosporine and fluoroquinolone.

New antibacterials, such as amoxicillin, must be investigated on sharks No.11 and 14 to fight against *Enterococcus* infection.

After over 2 years of breeding, morbidity was 55% and the mortality rate is 35%, however mortality directly related to the disease is only 25%, since two sharks that were sick died because of other causes (No. 4: cardiac decompensation due to anesthesia and No. 5: cerebral hemorrhage after isolation in a too smaller pool). Only one shark died directly from *Fusarium*. The other ones died from sepsis probably originated by an old infection of *Fusarium*. However, biomass continues to increase. Today, sharks weight an average of 16 kg and about 1.6 m TL.

Photo credits

Figures 4, 5 & 10: Renaud HERBERT – Nausicaä, Boulogne sur Mer, France.
Figures 6 & 7: Denis Tirmarche – Nausicaä, Boulogne sur Mer, France.
Figures 8, 9, 11, 12 & 14: Doctor Karin Lamberger – Vet Diagnostics, Lyon, France.

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INTRA- AND INTER-SPECIFIC FOOD-RELATED INTERACTIONS IN A GROUP OF SHARKS AT ACQUARIO DI GENOVA (ITALY)

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Abstract

The shark tank built in 1992 at Acquario di Genova (Italy) has a volume of 1.2 million liters and hosts different species of Elasmobranchs. From 2006 to 2012 the food data records include food type, quantity, weight, and the order of arrival of each specimen to the skimmer to feed. These data might reveal possible hierarchy between individuals and species and its changes influenced by the group variations. During this seven-year period, the shark group varied eight times due to different factors such as new arrivals and deaths. The preliminary data analysis revealed a correlation between behavioral pattern and the size of the animals.

Introduction

Competition occurs when two or more specimens demand the same use of a resource, normally limited, and the access to this resource is established by agonistic behaviors. Such behaviors can be considered social in the sense that they can lead to complex relationships and structures. Hierarchy structures are well known among insects (Wilson, 1971), teleost fishes (Croft et al., 2005), birds (Guhl, 1975) and mammals (Connor et al., 1998, 2006; Lusseau et al., 2006), but relatively few studies have examined the hierarchy and sociality among sharks (Allee and Dickinson, 1954; Myrberg, 1991; Bres, 1993; Sims et al 2000; Ritter, 2009; Sperone et Al. 2010).

The Acquario di Genova was built in 1992 and the shark tank has been one of the main attractions from the very beginning. Over the years the population and the décor of the tank has varied slightly, but basically the sandbar sharks *Carcharhinus plumbeus* (Nardo, 1827) and for a long while the sand tiger shark *Carcharias taurus* (Rafinesque, 1810) have been the star species. These are considered to be wide-ranging coastal species in tropical and temperate regions (Froese and Pauly, 2013).

As we all know, feeding is of critical importance to health and survival, therefore each specimen is fed individually. Feeding time is one of the major moments of interaction between specimens and an exciting moment for visitors. During feeding time, it is possible to record food-type, quantity, and also to observe different specific and individual attitudes of the specimens. We decided to record the arrival order during the feeding events to estimate if the inter- and intra-specific interactions were random or the result of an internal hierarchy. During this seven-year

period, the group composition of the shark tank has changed due to natural mortality and new arrivals and the data analysis has allowed us to highlight how these changes have altered the internal balance between individuals and species.

Materials and methods

The Acquario di Genova shark tank is 20 years old with a closed-circuit water system and a volume of 1200 cubic meters. It is of polygonal shape with the central part of the acrylic convex. The tank dimensions are: 23.5 x 10 x 4.8m. The Life Support System (Figure 1) includes 4 pumps (300m^3/h), 4 pressure sand filters of 3.6m diameter, a heat exchanger and ozone tower (ORP340-380mV); the flow is 900 m^3/h for a complete turn over every 1.5 hours.

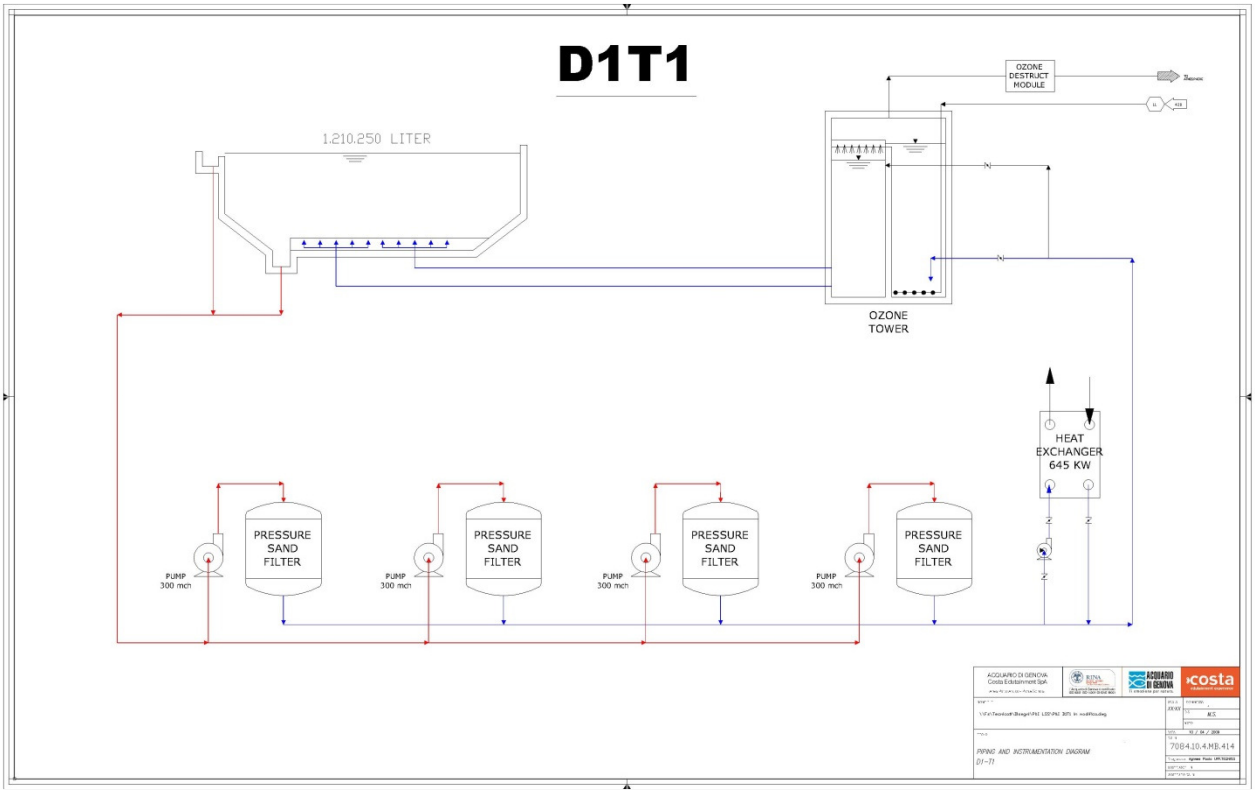


Figure 1. Life Support System for the Acquario di Genova shark tank.

The photoperiod is 12 h day/12 h night as provided by two circuits one from 8.00 am to 20.00 pm and one from 9.30 am to 17.30 pm. Temperature varies from 22 to 26°C according to a protocol adopted from the Antibes aquarium (Catteau, 2007) for *C. plumbeus* husbandry and reproduction. Water parameters are: S=34-38 ppt, 8<pH<8.3, NH_4<0.1 ppm, NO_2<0.05 ppm, NO_3<40 ppm, PO_4<2 ppm. Water changes can vary from a minimum of 10% per week to maximum 20% 3 times per week depending on water availability.

Food is provided 3 times per week by an operator on the central part of the skimmer, who offers food with a long aluminum tong to each specimen. The food types include teleost (herring and hake) and cephalopods (squid and cuttlefish) and the food body weight ratio varies from 1-2% per week for *C. taurus* to 2-4% per week for *C. plumbeus* (Janse et al., 2004). Once a week, KI

and Mazuri vitamin tablets are added to the food of each individual as a function of its weight. The data collected in this study included: the type of food, quantity and order of arrival to the food station of the two main species, *C. plumbeus* and *C. taurus*.

Figure 2 summarizes species, the specimen's nick-name, birth date, sex, origin (WC: wild caught or CB: captive bred), weight and the residence period of each specimen in the tank. In 7 years, 8 different population scenarios were observed. The periods of time when the sharks' composition was stable are indicated as pop1 to pop8. From pop1 to pop6 (Jan 2006 - Dec 2011) both studied species were present in the tank, while in pop7 and pop8 (Dec 2011 - Dec 2012) *C. plumbeus* was the only species. The periods: pop5 and pop7, extended for too short a period (1 month) to be examined.

Sandbar and sand tiger sharks are not the only cartilaginous fishes present in the tank. All the species are reported in Fig.2 to provide a complete picture of the elasmobranch assemblage and to better interpret and understand the interactions between species.

Table 1. Elasmobranch species in the Acquario di Genova Shark Tank 2006-2012.

species	Nick name	Birth date	Sex	Origin	Weight	01/01/06	28/02/07	08/11/07	19/06/08	18/03/09	17/04/09	05/05/09	08/09/09	22/10/10	01/02/11		09/03/11	08/12/11		17/01/12	31/12/12
Carcharhinus plumbeus	Due	?	M	WC	45 kg	from 1992															
Carcharhinus plumbeus	Sei	?	M	WC	42 kg	from 1992															
Carcharhinus plumbeus	Sette	?	M	WC	49 kg	from 1992															
Carcharhinus plumbeus	Nove	?	F	WC	65 kg	from 1992															
Carcharhinus plumbeus	BB	2006	F	CB	45 kg																
Carcharhinus plumbeus	Alba	2008	F	CB	35kg																
Carcharhinus plumbeus	Aurora	2008	F	CB	35kg																
Carcharhinus plumbeus	Biagio	2008	M	CB	35 kg																
Carcharhinus plumbeus	Stella	2009	F	CB	15 kg																
Carcharhinus plumbeus	Nuvola	2009	F	CB	15 kg																
Carcharhinus plumbeus	Cosmo	2009	M	CB	15 kg																
Carcharhinus plumbeus	Paperino	2009	M	CB	15 kg																
Carcharias taurus	Ciro	?	M	WC	65 kg	from 1996															
Carcharias taurus	Bianca	?	F	WC	94 kg	from 1996															
						pop1			pop2		pop3				pop4	pop5	pop6		pop7	pop8	
						C. plumbeus and *C. taurus*														*C. plumbeus*	
Carcharhinus melanopterus																					
Carcharhinus melanopterus																					
Pristis zijsron						from 2002															
Pristis zijsron						from 2002															
Stegostoma fasciatum																					
Ginglymostoma cirratum																					
Ginglymostoma cirratum																					
Ginglymostoma cirratum																					
Rhinobatos rhinobatos																					
Rhinobatos rhinobatos																					
Dasyatis centroura																					

Results

The data analysis reports the "First shark eating index" (number of times eating 1st divided by the total number of times eating), and the "Second shark eating index" (number of times eating 2nd divided by the total number of times eating). This was calculated for each specimen in the population and the significance was tested using the Chi-square.

In Figure 2 the graphs A-C-E-G-I-M show the "First shark eating index" and the graphs B–D-F-H-L-N show the "Second shark eating index". The double asterisk (**) indicates the category which gives the maximum contribution to the Chi-square total value; the single asterisk

(*) indicates the category which gives the second maximum contribution to the Chi-square total value. The numbers on the top of the bars indicate the total feeding events for each individual.

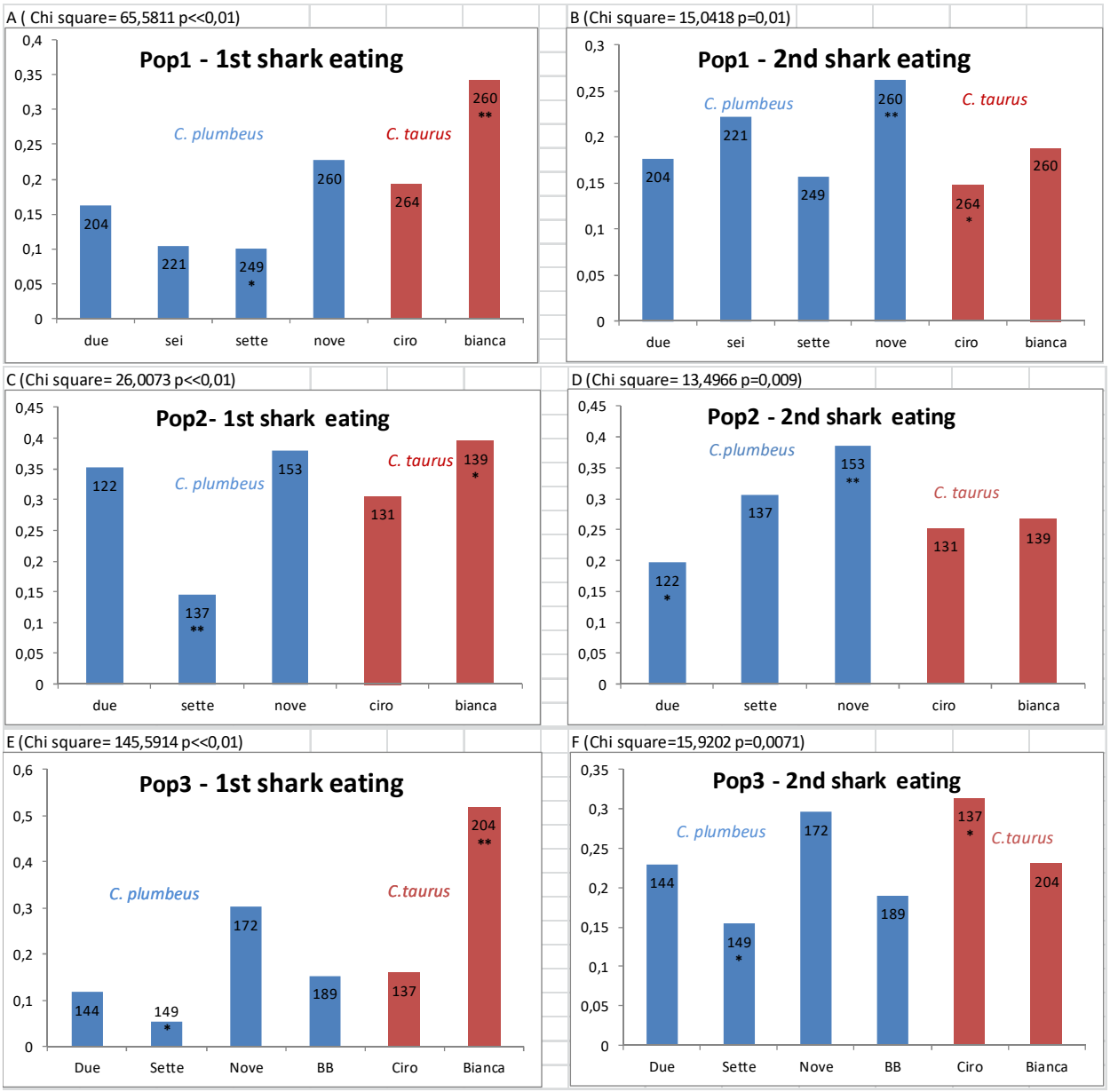
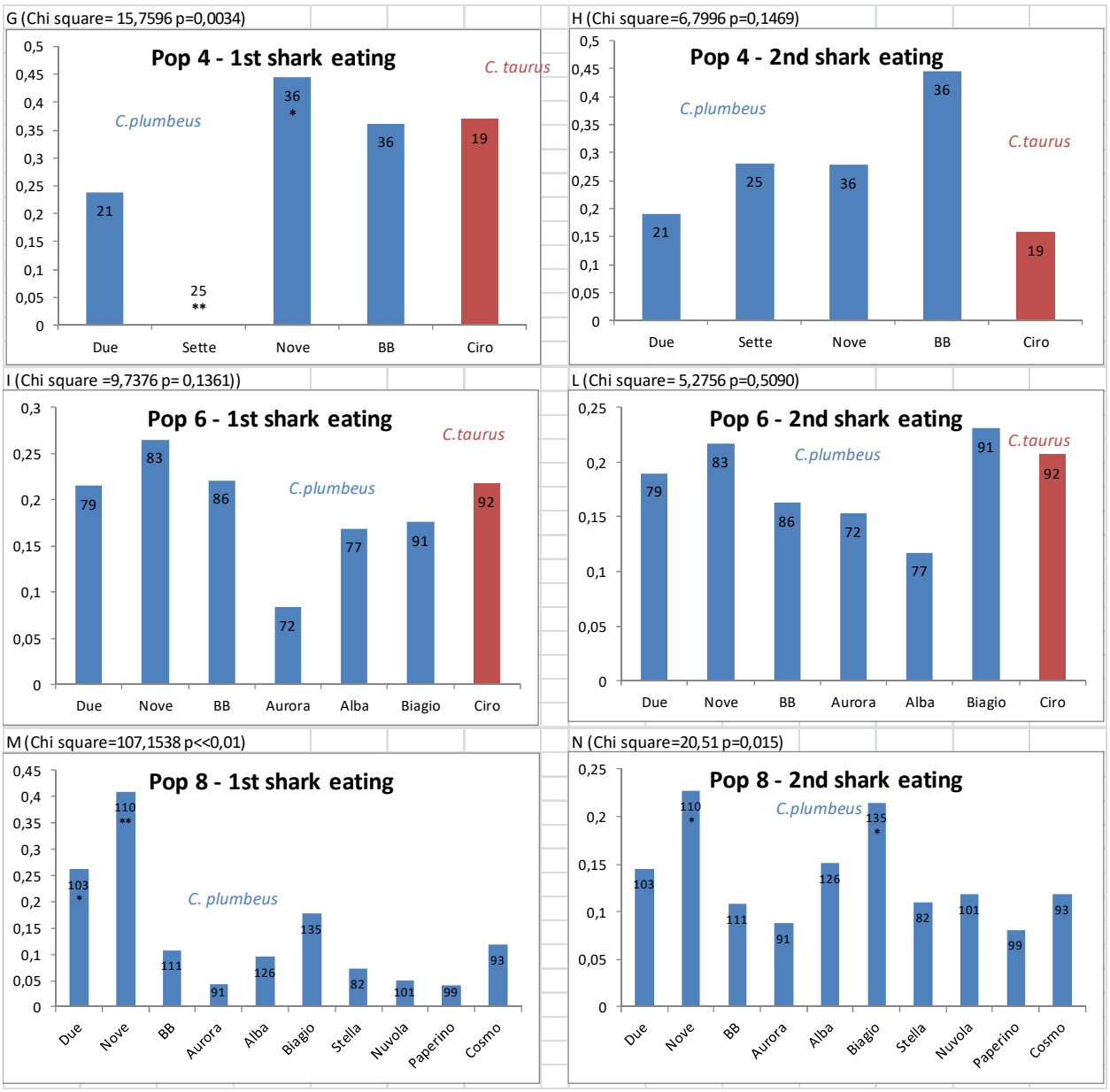


Figure 2 (A through F). Order of feeding for sharks in the various populations described in Table 1.



<u>Figure 2</u> (G through N). Order of feeding for sharks in the various populations described in Table 1.

The graphs A and C show a significant dominant position of the Sand tiger female "Bianca" for a long period of 38 months (pop1 and pop2) as the first shark eating, followed by the sub-dominant female sandbar "Nove" as the second shark eating. The periods: pop1 and pop2, differ because of the death of the sandbar male "Sei". The statistical analysis indicates a significant difference in the first and second positions (i.e. the first and second shark eating, respectively) in pop1 and the second position in pop2. While the 1st shark eating graph significance in pop2 is more related to the shark eating for less time during the first feeding event.

In pop3 (18 months, graphs E and F), when the young sand bar female BB arrived in the tank, the dominance of the sand tiger female "Bianca" is significant, but the second position is

taken by the sand tiger male "Ciro". However, this second position significance is indicated by its second maximum contribution to the Chi-square total value.

After the death of "Bianca" at the end of pop3, a change in the behavior is indicated for the subsequent period, pop4 (graphs G and H). The sandbar female "Nove" was significant as the first shark eating (became dominant), while the second position was taken by "BB". However, while having the highest index, the Chi-sqaure test was not significant for the second position. Similarly, in pop6 (graphs I and L) no significant difference in the first and second positions was detected by the test. In pop8, where both the sand tiger specimens were absent, and new young *C. plumbeus* specimens "Stella", "Nuvola", "Paperino" and "Cosmo" entered the tank, the first position of the feeding hierarchy was significant and occupied by the old female "Nove" (graph M), and the second shark eating was the male "Biagio" (graph N), born in 2008 and arrived in the tank 9 months before the youngest sandbars.

Discussion and conclusion

The *C. plumbeus* and *C. taurus* species are considered scavengers and reef-associated predators (Mecloy et al, 2006; Froese and Pauly, 2013; Smale, 2005). In the tank both species occupy the water column, while most others present in the tank are benthic, except for the two male black tip reef sharks (*Carcharhinus melanopterus*). These latter two sharks are significantly smaller and not well adapted to the tank, and therefore are not considered as competitive species with *C. plumbeus* and *C. taurus*.

The data analysis suggests that a type of hierarchy exists between the specimens of both species, and this hierarchy changes in response to variations in the tank's population composition. The statistical analysis indicates that in at least 4 of the 6 periods there was significant dominance indicated.

For the first and second period the sand tiger female "Bianca", the biggest animal in the tank, can be considered the dominant feeder, and the second is the big sandbar female "Nove", while the sand tiger male "Ciro" participated slightly in the competition despite its considerable size compared to that of the sandbar female "Nove". In this long period the sandbar male "Sei" died, and two new species were introduced: one female zebra shark (*Stegostoma fasciatum*) which occupied the bottom of the tank but was very present during feeding time to the skimmer, and the above-mentioned black tip reef sharks, who occupied the upper part of the water column but were intimidated and frightened by all the other species, especially during feeding section. However, none of these events seemed to have interfered with the tank hierarchy during these long periods.

In the third period lasting 18 months the new entry of the sandbar shark female "BB" seems to have affected the second level of the hierarchy. In fact, "Bianca" remains dominant but followed by "Ciro" who takes the position of "Nove", probably disturbed by the new young sandbar female. In this period a black tip shark died and shortly after three nurse sharks entered in the tank.

With the death of the big sand tiger shark female "Bianca", the sandbar female "Nove" now apparently accustomed to the presence of the young female "BB", becomes the dominant and unchallenged individual in the tank. It is interesting to note that in the last periods examined the

second position seems to be taken by a young sandbar male “Biagio”, despite the presence of the older and bigger male “Due” who apparently never competes for dominance.

These results suggest that the behavioral pattern for dominance is significantly related with the size of animals as for white sharks in South Africa (Sperone et al., 2010).

Interesting to note that the sandbar male, ”Due”, despite being larger and older than the young sandbar male “Biagio” was definitely dominated by the latter. This seems to be in contrast with the previously statement that dominance is closely linked to the size of the animals, even if the behavioral patterns may be different when considering animals who occupy the lower levels in the hierarchy.

No evidence of a dominant species can be detected but the sample numbers of both species in this study are obviously too small to reveal this effect. However, between the two species it’s interesting to note the behavior among “Nove” and “Ciro” who were of similar size, but were different species and sex. The sand tiger male “Ciro” seems to have been increasingly shy, and was not able to establish itself in the hierarchy, except for a brief moment.

It is difficult to say if the behavioral patterns are related to the shark’s sex. Apparently there is female-oriented dominance in the tank, but the sample numbers are too small in this study to even advance an hypothesis, and it would be in contrast to what has been observed in the wild for white sharks (Sperone et al., 2010), and also for bonnet head sharks where females tend to shy away from males regardless of size (Allee and Dickinson, 1954; Myrberg, 1981)

This study, despite its limits due to the low number of species, specimens and space, supports the hypothesis that in a shark tank, after a certain time, a kind of hierarchy is established that may change according to new entrants and deaths. This hierarchy appears to be size-based.

This finding can be of interest for tank management and when there is a need to add new specimens in a group of sharks. It would be of great interest to compare these data with similar studies in other public aquariums to be able to confirm or deny these preliminary findings.

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USE AND BENEFITS OF TARGET TRAINING IN TWO SPECIES OF STINGRAY, *Myliobatis californica* AND *Dasyatis (Pteroplatytrygon) violacea*

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Abstract

Target training with food reinforcement was used in 2 species of stingray, *Myliobatis californica* and *Dasyatis (Pteroplatytrygon) violacea* to aid in ease of transition from smaller tank to large multi-species exhibit. Use of a target for feeding *M. californica*, while in aquarium touch pool allowed rays to have a familiar reference object when transferred to a larger exhibit. Rays picked up feeding from a diver with a target quicker in the new exhibit than historical accounts without prior conditioning. Training *D. violacea* in holding prior to transfer to the Open Seas exhibit aided in ease of feeding.

Introduction

It has been determined that many elasmobranchs possess the cognitive ability to be easily trained to a target, or station. Maintaining a trained feeding station can ultimately aid husbandry staff in daily care of the animal. Training has proven beneficial in two species of stingray on display at the Monterey Bay Aquarium (MBA). Two of MBA's larger multispecies exhibits display a variety of elasmobranchs in addition to teleost fishes. The Open Seas exhibit is a 1.2 million gallon (3.8-million-liter) exhibit displaying northeast Pacific pelagic fishes including the pelagic stingray, *Dasyatis (Pteroplatytrygon) violacea*. The Monterey Bay Habitats exhibit is a 334,000 gal (1,181,468-liter) exhibit depicting the local marine habitats of Monterey Bay, one of 2 exhibits where we display bat rays, *Myliobatis californica*. The other exhibit housing bat rays is the 11,500 gal (43,532-liter) Bat Ray Touch Pool. Feeding and particularly ensuring each animal is getting their adequate nutrition in a communal exhibit can be challenging when there are many animals and diverse feeding modes. Through the use of operant conditioning we have been able to train these two species of stingray to approach specific targets with food as their positive reinforcement. The use of target training has also been observed to ease stress when transferring rays from one exhibit to another. Picking up on a target, a familiar object in an otherwise unknown environment and one that they have been conditioned to for receiving food has allowed them to adapt more quickly to a feeding regime in a new exhibit.

Training *Dasyatis (Pteroplatytrygon) violacea*

We display anywhere from 2 to 4 pelagic rays, *D. violacea* at one time in the Open Seas exhibit. Our pelagic rays are collected from the wild as needed and are first housed in our quarantine facility. In addition to health observation, this quarantine period is an opportunity to condition the rays to be fed by hand at a targeted station. Feeding at a station is imperative to ensure adequate nutrition when the rays are moved to the Open Seas exhibit. In this tank, they would otherwise be competing for food with a multitude of other fishes such as tunas and sharks in a large volume of water. The targeting process is fairly simple. Training is started shortly, if not immediately, upon being acquired. While in quarantine rays are kept in an 11ft - diameter, roughly 2,200gal (8300liter), cylindrical tank. The target consists of a white rectangular plastic board with a bold red X. The target is placed in the tank against the wall when food is offered. Initially the

food is broadcast in the tank and eventually rays are only reinforced with food when they actually swim right up to the target. The natural feeding behavior of a pelagic ray's midwater swimming and flipping upside down is conducive to their training. The trainer can easily drop food anywhere within their ventral region once they have approached the target displayed and turned. The ray is thus able to manipulate food with their wings to their mouth (Figure1). The pelagic rays pick up this behavior quickly and can hit the target within 2 weeks of training, on average. Target training has proven successful with animals approaching target and feeding in Open Seas within 1 to 2 days of being transferred from quarantine holding to display (Figure 2). On occasion pelagic rays may need to be transferred from exhibit back to a holding pool for a variety of reasons. The consistency of having a familiar target wherever they are being housed has reduced transfer stress with rays targeting and eating almost immediately upon being moved.

Figure 1. *D. violacea* stationed in holding.

Figure 2. *D. violacea* stationed in Open Seas exhibit.

Training *Myliobatis californica*

The training of bat rays, *M. californica*, to a target with a food reinforcer has been conducted in a similar way to the conditioning of pelagic rays. The bat ray target consists of a weighted white plastic disc, 9in (23cm) diameter, dissected by a black cross made with electrical tape. Bat rays were typically acquired from the wild and conditioned in quarantine prior to going on display in the Monterey Bay Habitats exhibit (MBT). This exhibit typically has two bat rays on display at any given time. Unlike the behavior of the pelagic rays, the demersal feeding behavior of bat rays would make it difficult to feed a bat ray at the surface of a deep water exhibit. It also poses a challenge for the bat rays to compete with other midwater fishes in the communal exhibit. In addition to being conditioned to associating food with a target, the bat rays must also become desensitized to being hand fed by a scuba diver underwater. This desensitization process has historically been very time and staff consuming. The process takes an average of 6 weeks in a holding pool to gradually get the bat rays comfortable enough to approach a diver with a target and take food by hand. After this desensitization and target training process the bat rays are able to go on exhibit and continue to be fed by hand at target during our semi-weekly public feeding show (Figure 3).

With stricter regulations on collecting and releasing of animals under our institutional collecting permit it has become slightly more challenging to routinely acquire and release bat rays for both the MBT exhibit and the Bat Ray Touch Pool. Bat rays that have outgrown the touch pool

would have been historically released back to the wild without issue. Now a more viable option would be to transfer the bat ray to the MBT exhibit if there is a need for one in that display, first by way of quarantine for diver desensitization and target training. With this process happening on a more regular basis, the idea of using the same exact target to train the bat rays to a feeding station at the touch pool may make it easier to transition rays to the MBT. Training of bat rays in the touch pool using the same target commenced in 2011. Bat rays at the touch pool were already conditioned to feeding by hand anywhere along the edge of the pool. The rays were first desensitized to the target by placing it in different places along touch pool wall during feeding. Rays were fed by hand opportunistically as they swam along the wall as they had done previously. When the rays were in close proximity to the target they would be fed immediately and if they touched the target they would be fed their favorite food item. Eventually the rays are only fed when they touch the target (Figure 4).

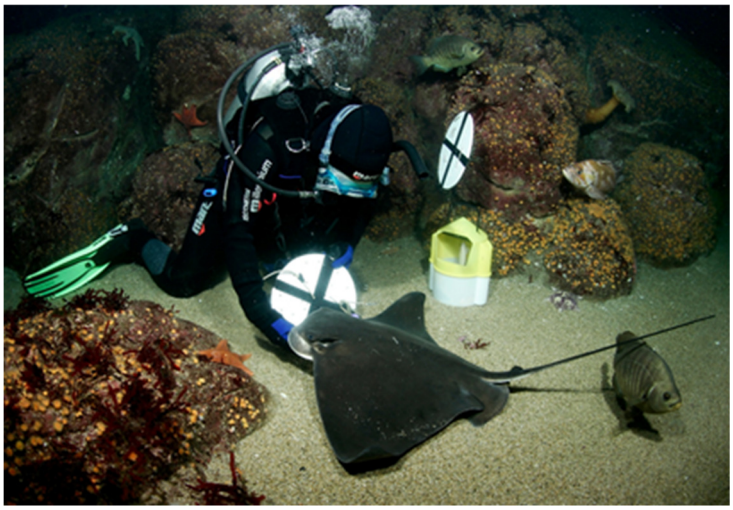

Figure 3. *M. californica* stationed at a diver.

Figure 4. *M. californica* stationed in the touch pool.

With only 6-8 rays in the touch pool at any given time trainers are able to distinguish individuals and reinforce approximations to the target appropriately as individuals may be in different stages of training. Since we started the prior target training in the touch pool the bat rays moving to MBT took an average of 3 weeks in quarantine for scuba diver desensitization, an improvement over the previous time of 6 weeks. Scuba diver de-sense in quarantine is still staff, time, and space consuming. Starting in the fall of 2013 we are currently working to forego the quarantine diver de-sense and instead placed a target trained touch pool bat ray directly into the MBT exhibit. We are conducting diver de-sense on exhibit by adding 2-3 additional dives to the normal twice weekly feeding show dives specifically targeting this ray and getting it used to the presence of divers. With the retention of seeing the familiar target the ray already appeared to show less stress when approached by divers than rays previously placed on exhibit and picked up feeding from a diver within 3 weeks.

Conclusions

Training stingrays to a target has proved very useful in aiding husbandry efforts at MBA. In addition to the benefits of being able to ensure the stingrays get their adequate diet in a multi

species exhibit, it has also eased stress when having to transfer them from one place to another. The addition of target training bat rays at the touch pool has also allowed for better health observations and stinging barb re-growth checks when they are calmly stationed on target and feeding. If elasmobranchs possess the ability to retain learnt behaviors we should utilize the opportunity when applicable for better husbandry care.

Acknowledgments

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Carcharhinus plumbeus: HUSBANDRY AND REPRODUCTION

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Abstract

The first of April 1996 saw the opening of a two million litre sea water aquarium in Marineland Antibes. In March of the same year, seven wild sandbar sharks (*Carcharhinus plumbeus*) and four sand tiger sharks arrived from the waters of Florida.

Seventeen years later, twenty-six reproductive cycles have produced more than two hundred *Carcharhinus plumbeus* pups and our first F2 sharks arrived in 2010.

Today more than seventy sandbar sharks born in Marineland have been transferred to aquariums around the world.

Topics covered during the presentation will include husbandry, reproduction (mating, gestation, delivery) and animal care.

Introduction

The sandbar shark (*Carcharhinus plumbeus*, Nardo, 1827) occurs world-wide in tropical and warm temperate waters. The species is a relatively common aquarium-held species and there has been some reproductive success in Europe, notably at Marineland Antibes, France and Madrid Zoo Aquarium, Spain. Although late maturing, *Carcharhinus plumbeus* is a relatively fecund shark giving livebirth to between 2 and 15 young every other year. The species is listed as Vulnerable on the IUCN Red List and there are conservation concerns for this species (recovery programme for the wild population managed by US officials).

Husbandry

Holding conditions

The shark exhibit at Marineland Antibes is a figure 8-shaped tank with a volume of 2,000 m^3 (27 m x 16 m x 4.5 m). Artificial concrete decoration covers part of the periphery walls and central area.

Diet

Sharks were fed three times per week (1-3% of body weight) using 40% white fish (conger) and 60% cephalopods (calamari). In addition, we supplemented food with poly-vitamin tablets and IK.

Water quality

From the beginning in 1996, we decided to run our “closed” system as a “semi-open” system, using natural Mediterranean seawater. Our daily routine includes water changes (2 to 5 % daily, or 15 % to 20 % / week). New seawater at 14°C is easily available all year round (pumped from 600 m off shore and 70 m deep (below the thermocline).

Water parameters

- temperature: 23 to 26 °C
- salinity: 30 to 37 g/L
- pH: 7.9 to 8.1
- ammonia: 0.0 ppm
- nitrite: 0.0 ppm
- nitrate: < 15 ppm

LSS

Ozonated protein skimmers + rapid sand filters. Flow rate: 1 volume / 90mn.

Artificial lighting

We use 16 HQI sources with illumination hours varying with the opening hours of the park; 13 hours in low season (8am-9pm), and15 hours in high season (10am-1am)

Population

In March 1996, seven (4.3.0) wild *C. plumbeus*, (adults, close to two metres) arrived from Florida. In addition; sand tiger sharks (*Carcharias taurus*), nurse sharks (*Ginglymostoma cirratum*), rays and Caribbean reef fish completed the "shark exhibit".

Reproduction

Mating

First courtship occurred in May 1996, just two months after arrival. Two males were observed chasing a female for several days without mating.

In May 1997, after a few days of courtship, we were able to observe our fist mating event. Since then, we have observed mating behavior each year, and 27 reproductive cycles have been recorded.

- Mating at present occurs in early November.In 1997 mating was in May and has progressively moved forward over the year.
- The males begin courtship by following the females around the tank. They then start to gently bite the females on the back, in the region of the second dorsal fin, moving progressively up the back. This behaviour finishes with severe biting of the pectoral fin and the introduction of one of the claspers into the cloaca.
- Courtship and mating behaviour is spread over 15 days (first days, chasing only, with one or more males participating).
- All our C. *plumbeus* mate (3.4.0) Males have only one mating episode with any given female (courtship and mating) during the annual mating period. Males can mate successively with several different females.
- Wounds heal completely within three weeks.
- Mating could have dramatic consequences: in 2010, our biggest female was found dead two days after copulation. Necropsy noted massive blood loss due to wounds inflicted by males.

Pregnancy

Pregnancy duration (12 months in the wild) depends on tank temperature regulation.

Tank mate constraints: sand tiger sharks cannot be maintained at 26°C all year round. We must adopt an empiric temperature regulation pattern following sandbar shark reproduction cycles.

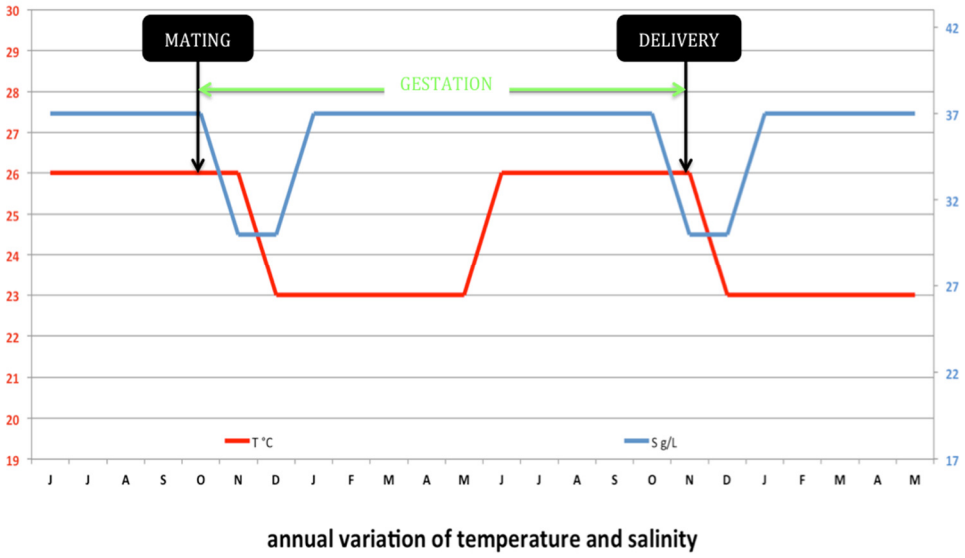

Figure 1. Shark tank temperature and salinity at Marineland

We lower water temperature from 26°C to 23°C two months after the end of the mating period, then six month later, we increase it to 26°C until delivery (Figure 1). Pregnancy duration is 14 months with 23°C for 6 months and 26 °C for 8 months. In 1998, lower temperatures (23°C all year round) caused longer pregnancies (16 months) and may have induced delivery problems (all pups stillborn).

Additional observations related to pregnancies include:

- Food consumption of pregnant females increases (to 5 % of body weight).
- Towards the end of pregnancy, females show a faster, laborious, open-mouthed swim pattern.
- Females sometimes stop eating a few days before delivery, but in most cases they eat up till the day before delivery.
- The day before delivery, we observe a slight movement of the bulge in the belly away from the pectoral fins.

Delivery

Normal delivery occurs in the early hours of the morning (between 1 am and 4 am). Pups are released one by one, tail first.

- Placental tissue is very often found months or days prior to delivery (abortions?).
- Normal delivery time between 1 and 3 hours.
- Litter size: 1 to 15 pups
- Last born often stillborn and smaller

- Newborn pups begin by swimming to the surface thus making them easy to catch.
- Mothers are watched until they return to a normal swim pattern.
- Special attention is given to mothers' feeding behaviour.

Medical issues

At the end of July 1998, after 16 months of gestation, our pregnant female seemed ready to give birth and we began watching. Few days later (first of august) three tails appeared and we were waiting for normal delivery. Unfortunately, one hour later, intervention seemed to be inevitable (no contractions, no delivery advance). After light anaesthesia using Medetomidine / Ketamine, the female was removed from the pool and we proceeded to the extraction of seven pups that appeared to have been dead for several days. Reanimation of the female took place half an hour later; the female awoke violently and was released in our hospital tank, after antibiotic IM (Baytril 5mg/Kg once daily for seven days).

One week later we moved the female back to the shark exhibit where two males immediately chased her. This female produced live pups every other year until January 2010 (6 reproductive cycles and 73 pups) and was unfortunately killed in November 2010 during a new mating event.

On the 10th of January 2011 another pregnant female was found on the bottom of the tank. Necropsy found seven pups in advanced decomposition state. It seemed to be a similar delivery problem but without any obvious signal leading to an intervention.

Pup Husbandry

Our pup facility (hospital tank – 56 m^3) is connected to the main pool. Just after delivery, pups are moved to the "hospital" tank to avert predation. One hour after birth, young sharks can swim faster and are able to avoid the collecting net. They will stay there until they reach the minimum length of one meter (between 12 and 16 months).

Identification

We try to identify each individual using photo ID and abrasion marks caused by friction on tank decorations in the first minutes of life. Those marks will disappear in a few days but were useful to follow individual feeding. Pups will be chipped two weeks later.

Food and feeding

Over the years, our experience has shown that pups may and must eat on their first day of life. In the past we had registered numerous cases of anorexia linked with inappropriate food and feeding plan. We now offer appetent food (scallops and prawn) on the first afternoon of life, and then three times a day during two weeks before adding fish (mackerel, herring, sprats) and calamari. For a starting period (two months), we manage with over-feeding to avoid dominance and collect all untaken food one hour later. Early anorexia cases are now rare and no linked with feeding.

Medical issues

The first problem in a predator tank is predation. Without a secure separate delivery tank, you must be present and catch pups as soon as possible. In our case, predation events, increases

with addition of young sandbar sharks (2-3 YO), who always search actively for prey. Adults are more opportunistic but due to larger mouth often killed pups by biting. Pup survival depended on bite location and Marbocyl injection (10mg/Kg/ 3 times every 48H) in the case of a "lucky" pup bite.

Abrasion against the walls is the second concern, and for this species, directly linked to the facility. Sandbar shark are not able to turn in small area without injured. For example, our 56 m^3 hospital tank is suitable for newborn pups until they reach one metre, then we must move them to a bigger facility to avoid nose abrasion.

Results

Since 1998 27 reproductive cycles have provided us with new data. The Marineland Sandbar shark breeding program had produced 208 pups (Table 1). This total includes stillborns (22.60%), victims of predation (10.57 %) and deaths due to early anorexia (6.73 %).

We record homogeneous litters with pup size dependant on the mother. Over deliveries, certain females always produce litters with more than ten 'small' pups, as other females always produce smaller litters with bigger pups.

- Sex ratio: 52% male and 48% female.
- Pup measurements: males = females.
- TL range = 47 - 63 cm.
  average TL = 56.5 cm
- Weight range = 780 g - 1400 g
  average body weight = 1134 g

Propagation

Marineland sandbar shark breeding program is a new sustainable animal source for aquarium collections. In Europe, Marineland Antibes and Madrid Zoo Aquarium have regularly produced captive bred *Carcharhinus plumbeus*. Pups are available each year thus reducing or eliminating the necessity of taking animals from the wild.

Aquarium collections must take sustainability into consideration, and several aquariums had already choosen captive bred Sandbar shark for their collection. **72** young *Carcharhinus plumbeus* have moved from Marineland to 20 aquariums across Europe (France, Italy, Greece, Spain, Hungary, Sweden) and Asia (South Korea).

Conclusion

European population management using EAZA tools is under supervision of an *Carcharhinus plumbeus* ESB (Elasmobranch StudBook) and the demographics are presented in Figure 2. At present, the European captive Sandbar shark population is clearly divided in two slots: The 'old' (20 to 40 YO) wild caught sharks and the 'young' captive bred and propagated animals mainly produced by Marineland Sandbar shark breeding program.

Table 1. *C plumbeus* births (Marineland Antibes)

Litter	DATE	Total Pups	Stillborn	Predation	Anorexia
27	**14/12/2013**	8	0	0	0
26	**07/12/2012**	2	0	0	0
25	**11/01/2012**	9	1	0	1
24	**04/02/2011**	4	1	1	0
23	**31/01/2011**	7	7	0	0
22	**10/01/2011**	7	0	1	0
21	**12/04/2010**	1	1	0	0
20	**17/01/2010**	11	2	0	0
19	**20/03/2009**	1	0	0	0
18	**08/03/2009**	15	1	0	0
17	**27/04/2008**	12	3	3	0
16	**19/04/2008**	4	3	1	0
15	**09/05/2007**	11	1	2	0
14	**28/07/2006**	3	1	1	0
13	**26/07/2006**	4	0	1	0
12	**04/06/2006**	11	1	2	1
11	**23/06/2005**	6	5	0	1
10	**08/08/2004**	5	1	2	2
9	**07/07/2004**	15	2	3	1
8	**17/07/2003**	12	1	2	0
7	**11/10/2002**	1	0	0	1
6	**29/09/2002**	9	7	0	0
5	**22/07/2002**	14	0	1	4
4	**11/08/2001**	7	1	0	0
3	**08/11/2000**	10	1	2	1
2	**23/08/1999**	12	0	0	2
1	**27/07/1998**	7	7	0	0
	Total	**208**	**47**	**22**	**14**

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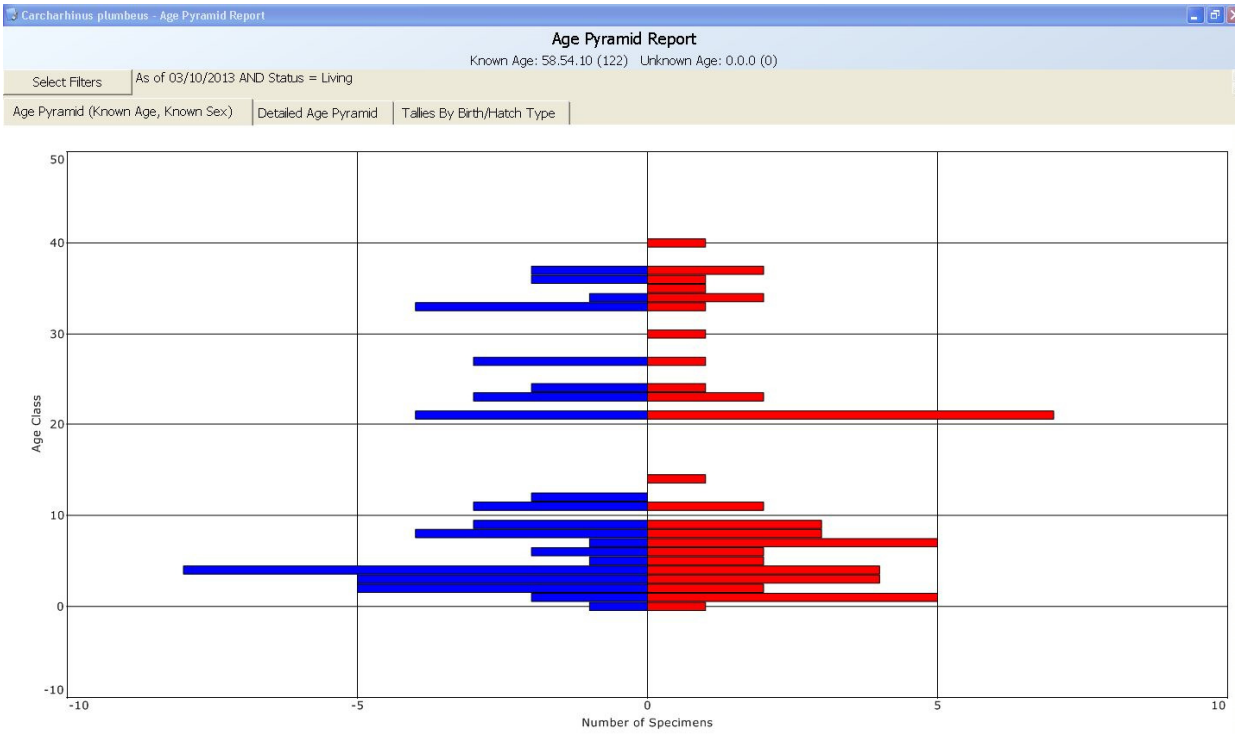

Figure 2. European *C. plumbeus* captive population (2013)

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CAPTIVE BREEDING OF THE SANDBAR SHARK, *Carcharhinus plumbeus*

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Abstract

The captive breeding of elasmobranchs has been a desired goal in public aquaria for many years. Recent advances in exhibit spaces and increased holding along with advances in husbandry techniques and animal health care have increasingly made this possible. The births of 28 sandbar shark pups at Shark Reef, since 2009, has allowed us to investigate factors that may provide a platform for a better understanding of the captive reproduction of carcharhinid species. The use of temperature fluctuation, early capture, ultrasound, suitable holding of adult females as well as feeding and husbandry of neonates (including housing spatial requirements) are valuable tools in this process. Conditioning and transport of pups is another critical topic and with the placement of 17 pups at facilities in the Western U.S. this is the first step in providing sustainable collections for AZA facilities.

Introduction

Since 2009 Shark Reef has had good success in the captive reproduction, rearing and transport of the sandbar sharks, *Carcharhinus plumbeus*. This paper looks at the factors that are believed to have contributed to this success. Temperature fluctuation, social grouping, male female ratio as well as early capture and ultrasound identification of pregnant females appear to be significant. The rearing of neonate sandbar pups with respect to feeding, housing and transport is also addressed in this paper in an effort to provide a brief review of the information gathered to enable others to duplicate these conditions in their facilities.

The value of producing, placing and displaying captive bred elasmobranchs in our facilities is discussed with attention to the conservation and collection sustainability messaging for our constituents.

Reproductive Physiology

It appears that there is evidence to suggest a biennial reproductive pattern in sandbar sharks similar to other carcharhinid species. Females may display synchronous or asynchronous reproductive cycles. Some of these patterns have a phylogenetic basis. For example, squaloid sharks have biennial cycles with concurrent vitellogenesis and gestation, while sharks of the genus *Carcharhinus* generally have biennial cycles with consecutive, year-long vitellogenesis and gestation (Castro 1993, 1996).

The pattern seems to vary dependent upon a number of factors including species food availability and water temperature.

Sandbar sharks at Shark Reef appear to follow an asynchronous biennial cycle as described by Castro (2009).

Sandbar History at Shark Reef

Shark Reef has housed sandbar sharks, *C. plumbeus* since its opening in 2000. The numbers have varied from a high of 16 animals to a low of eight. Currently the display houses 10 animals 8.0.2. The first evidence of reproductive activity was observed as far back as 2005 and in 2007 a deceased pup was removed from the display. Since this time births have occurred in 2009, 2011, 2012, and 2013. This pattern seems to support the asynchronous biennial reproductive cycle.

Temperature and Reproduction

In 2003 temperature "cycling" was introduced into the system management protocol. The range was 73 to 76 degrees Fahrenheit. After this change a marked behavioral change in the animals was observed with a significant increase in breeding behaviors and breeding wounds on females. While is it impossible to determine if this change resulted from the temperature change or the age/sexual maturity of the animals – the change in behavior was significant and appears to have been a "landmark" in the onset of reproduction.

Reproductive History of Females at Shark Reef

Two female sandbar sharks (SR ID # 47 and # 166) have produced all of the surviving pups since 2009. One other female gave birth to three pups but none survived.

- Shark # 166 was the first to give birth in 2009. On 31-Oct-08 this animal was moved from display to holding after it was captured and imaged with ultrasound (pups confirmed on this date)
- On 13-March-09 this animal was captured for examination including ultrasound and blood draw. Ultrasound revealed two pups of ~ 35 cm (avg. birth length = 40 cm) both pups visualized moving, gilling with heartbeat. Our veterinarian Christopher Yach was able to palpate tail-fin on one pup.
- On13-March-09 at ~ 1:30 pm # 166 gave birth to five-pups (one pup was badly bitten, and sutured by our vet team.) The remaining animals all appeared to be in good health. The animal that was bitten was sexed (female) and measured TL = 53 cm cross-section at gills 6 cm, Rostrum to pre-dorsal length = 17 cm rostrum dorsal length = 23 cm weight = 1.0 kg # 166 was moved to separate holding from the pups for recovery and to avoid any further injuries.
- On 19-Mar-09 at ~ 1:30 am the injured pup expired likely as a result of its wounds.
- On 17-Dec-10 # 166 was transferred from display to holding after positive ultrasound. Confirmed one heartbeat but a very crowded reproductive tract
- On 26-Jan-11 this animal gave birth to eight shark pups - between ~ 2:30 am and 4:30 am. # 166 moved to H1 to avoid aggression or injury to pups. Pup # 7 was given 0.25 mg/kg (estimated weight 1.0 kg) of Dexamethasone due to difficulty with placenta and "bobbing" at the surface. Fed ~ 100 grams of S. Krill and ~ 75 grams of mackerel filet - only visualized one pup eating.
- On 16-April-11 one pup was weighed at random - weight = 1.8 kg. The six surviving pups of this litter were transferred from the birthing system to another holding system make room for another possible litter.
- This was female # 13 who did in fact give birth. This resulted in two stillborn pups and one pup that expired at a later date.
- On 18-Jan-13 # 166 was transferred from A13 after positive ultrasound.

- On 8-Feb-13 this animal was captured for ultrasound and blood work; at least four large pups visualized.
- On 25-May-13 at ~ 11:00 pm # 166 gave birth to five pups - all looked good with the exception of one pup that had a constricted pupil. Some of the pups had initial mottling but this seemed to subside. Fed Superba Krill on this night and did not observe and feeding. # 166 moved to separate holding at ~ 11:45
- On 26-May-13 one pup was weighed on this date weight was ~ 1 kg.
- Shark # 47 – a larger (and likely older female than # 166) was the third animal to give birth and the second to successfully produce pups.
- On 30-Jan-04 animal was caught for ultrasound to see if any pups could be visualized (based on weight gain.) No pups were visualized at this time.
- On 18-May-07 animal was captured for ultrasound and again no pups were visualized at this time. This was the same year that an expired sandbar pup was found in the display system overflow skimmer.
- On 10-April-09 this animal was captured for a regular physical and ultrasound. Ultrasound performed and no pups were visualized (though significant follicular activity)
- On 23-July-10 this animal was captured for ultrasound - this animal had gained significant weight and based on earlier ultrasound in 2009 along with biennial reproductive information and previous birth of pups in 2009 from # 166 was thought to be pregnant. Animal was not feeding and previous capture attempt was unsuccessful. Animal lost very significant weight and began eating again and was captured on this date.
- Ultrasound of abdomen revealed huge normal liver but not pregnant nor any evidence of pregnancy or follicular development. Normal ultrasound
- On 5-Aug-11 this animal was captured for ultrasound and confirmed pregnant
- Based on ultrasound it was estimated that # 47 was 40% to term so perhaps due in December. Ultrasound confirmed multiple live pups, possibly three to four, as well as could be measured length = 18 to 19 cm.
- On 16-Dec-11 animal captured and U/S revealed multiple large pups ~ 45 to 50 cm TL and 5 cm at gill width. Animal transferred from H1 to H2 on this date.
- On 3-March-12 at ~ 11:00 pm this animal gave birth to 7 pups - five doing well one in very poor condition and one with some amount of difficulty. All pups born alive and adult female relocated to separate holding without incident. At ~ 12:30 am, pup in very poor condition isolated and given I.M. Dexamethasone at 0.25 mg/kg (two injections) and Vitamin B at 0.1 mg kg. A second pup was given one injection of Dex at 0.25 mg/kg and Vitamin B at 0.1 mg/kg. Pup isolated was a male ~ 950 grams and TL = 53 cm. All pups survived.

Pup Care and Growth

Sadly, we have not acquired a tremendous amount of data on size at birth and growth rates. On 17-Mar-09 (birth date 13-Mar-09) pups were transferred to a larger system for better care and grow out. On this date the following information was collected:

1, Female, ~ 50 cm TL
2, Female, ~ 53cm TL
3, Male, ~ 55 cm TL
4, Female, ~ 56 cm TL
5, Male, ~ 56 cm T

On 10-April-09 two of the pups were captured, measured and weighed on this date the following information was collected:
#1, Female, ~ 61 cm TL, weight = 1.3 kg
#2, Male, ~ 59 cm TL, weight = 1.2 kg

On 24-April-09 two of the pups (randomly chosen) were captured, measured and weighed:
#1, Female, ~ 60 cm TL, 49 cm FL, weight = 1.3 kg
#2, Male, ~ 62 cm TL, 50 cm FL, weight = 1.4 kg

On 10-Sept-09 two pups were captured:
1, Male, ~ 84 cm TL, weight ~ 4.2 kg
2, Male, 75 cm TL, weight ~ 2.9 kg

On 18-Dec-09 two animals were transferred from H4 to H2 for growout and to reduce rostral deformity:
1, Male, 94 cm TL, 79 cm FL, weight = 5.3 kg
2, Female, 91 cm TL, 76 cm FL

On 31-Dec-09 two animals were transferred to a larger system for growout and to reduce rostral deformity:
1, Male, 88 cm TL, 74 cm FL, weight = 5.5 kg
2, Female, 88 cm TL, 75 cm FL, weight = 6.5 kg

On 4-June-10 two pups were captured, blood drawn, weighed and measured in preparation for de-accession:
1, Female, 108 cm TL, 92 cm FL, weight = 10.0 kg
2, Female, 92 cm TL, 82 cm FL, weight = 6.5 kg

On 24-April-12 (birth date 3-Mar-12) 7 pups were captured and weighed:
1, Male, 65 cm TL, 64 cm FL, weight = 1.5 kg
2, Male, 59 cm TL, 54 cm FL, weight = 1.3 kg
3, Male, 60 cm TL, 52 cm FL, weight = 1.9 kg
4, Male, 53cm TL, 48.5 cm FL, weight = 1.9 kg
5, Female, 69 cm TL, 55 cm FL, weight = 1.9 kg
6, Female, 70 cm TL, 55 cm FL, weight = 2.0 kg
7, Male, 66 cm TL, 52 cm FL, weight = 1.7 kg

On 26-May-13 (birth date 25-May-13) one pup was captured and found to weigh 1.0 kg.

On 27-July-13 the following information was collected:
1, Female, 60 cm TL, 48 cm FL, weight = 1.4 kg
2, Female, 64 cm TL, 52 cm FL, weight = 1.5 kg
3, Female, 62 cm TL, 50 cm FL, weight = 2.5(?) kg
4, Male, 65 cm TL, 51 cm FL, weight = 1.6 kg
5, Male, 66 cm TL, 53 cm FL, weight = 1.6 kg

The number of births during this period from these two females appears to indicate a stable pattern of biennial reproduction in our facility.

Sandbar Pup Care and Growth

We have not acquired sufficient data to be able to provide a detail of growth. During the first 16 months – animal gained 50 cm to 100 cm TL and weight increased by a factor of up to 8.

Among the challenges in maintaining these animals has been feeding. With this species (and others) good results were obtained by broadcasting Superba krill a minimum of three times daily. If an animal was feeding sporadically – we would add a fourth feeding after "lights out." All broadcast was left in the tank overnight and then removed. This was provided at ~ 10% BW/day per animal. We have observed animals feeding on this item within hours of birth. We have used this as the first feed item on all of our pups and they take it well as a first feed and grow well. This is followed by the introduction of fin fish (filets cut in cubes) along with the broadcast of krill. Once acclimated to fin fish – pups were weaned from krill and after ~ two months were feeding exclusively on fin fish. Pups were fed three times daily for the first two to three months at 10% BW/day per animal and then feed reduced to twice daily. Feeding was less predictable at this stage and dependent upon numbers and staffing pups were conditioned to feed off tongs at this point and feed amounts could be more carefully measured. Food taken by smaller and larger individuals is presented in Figures 1 and 2.

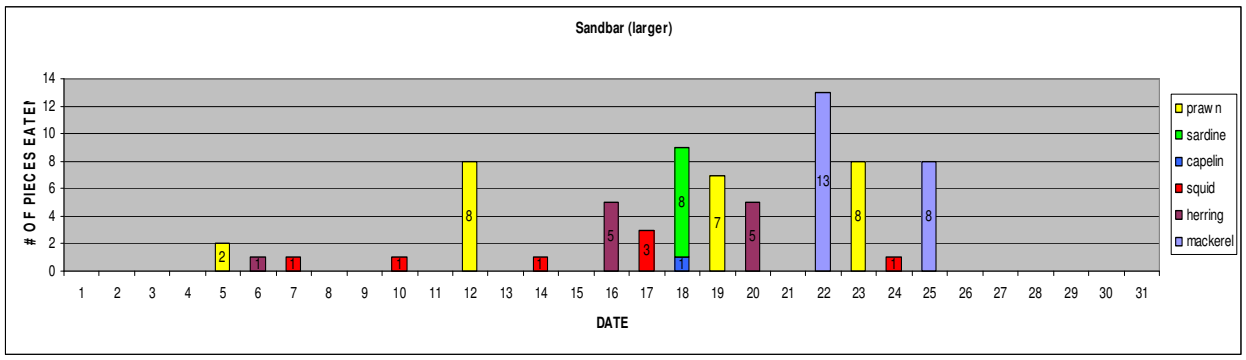

Figure 1. Food items consumed by larger sandbar sharks.

Conditioning and Enclosure Issues

Each animal was unique in its adaptation to target feeding with some animal quickly taking to feeding from a plastic feeding stick (rigid airline tubing) and others shying away from any tool introduced into the enclosure. Once acclimated to the feed stick conditioning to feeding off of plastic tongs was readily achieved with all pups. The only issue with the use of the feeding tongs was the number of pups and the inability to effectively identify and target feed animals. As a result, on occasion some pups were still broadcast feed to assure equitable distribution of feed. We encountered several enclosure issues with the pups as they grew. With most of pups we experienced the beginning of rostral deformity if maintained in our smaller holding system 6 meters in diameter X two meters in depth ~ 60,000 liters. The result was a dorsal curve of the rostrum at times associated with abrasions as well. When pups of three months were placed in a

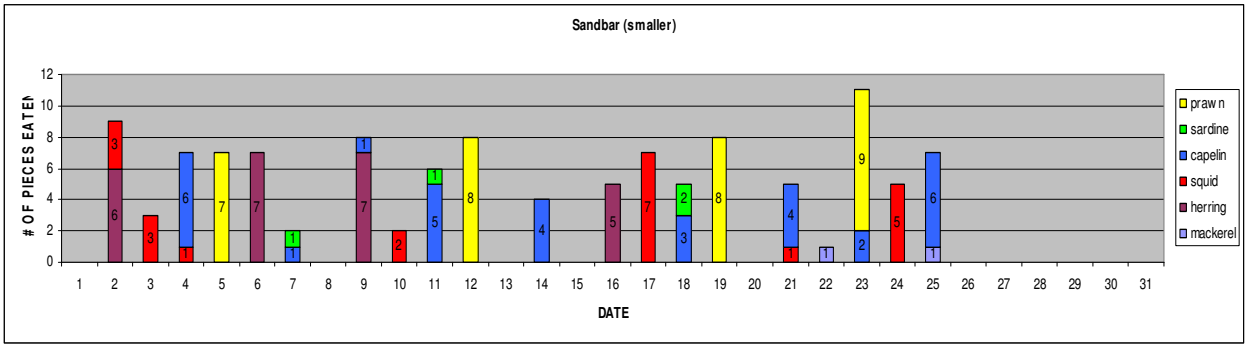

Figure 2. Food items consumed by smaller sandbar sharks.

five meter by three meter by three meter ~ 120,000 liter rectangular display with artificial coral décor the animals were much challenged in navigating the system. This resulted in the rapid onset of rostral and ventral abrasions and the aforementioned rostral deformity. In holding moving the pups to our 9-meter diameter X three-meter depth ~ 160,000 liter holding system not only resulted in no further deformity but in the resolution of the existing deformity as well. Based on our experience with the pups in our facility we now place all pups in a larger system as soon as it is available and preferably within the first three to four months of growth.

Transport

The pups at our facility have proven to be very tolerant of transport (both short-term and long-term.) Pups were transported on numerous occasions for up to eight hours in 86-inch diameter X 44-inch depth plastic blue well tubs (Pentair Aquatic Eco-systems) with oxygen at 130 to 150% saturation. No filtration was used in these transports and animals arrived in good condition. At or facility it is protocol to administer I.M. Dexamethasone at 0.25 mg/Kg and Amikacin at 4 mg/Kg prior to transport. More recently two female pups were transported by Shedd Aquarium in similar transports with oxygen and supplemental canister filtration for ~ 30 hours and arrived in excellent condition (pers. comm. Lise Watson) The relative simplicity of captive reproduction along with tolerance for transport make this a good choice for facilities seeking to display this species.

Future Considerations and Challenges

The key to success with this species and likely others is the ability to easily capture and isolate gravid females. This can be the single greatest challenge for many facilities where display design and limited holding pose obstacles to both. The investment in dedicated space to hold gravid female and rear young is essential for the success of future captive breeding programs. This is true of species already successfully bred but is also the key for those target species not yet commonly reproduced.

Summary

In overview the successful rearing of this species is hopefully the first step in programs that target some of our most charismatic species i.e. sandtigers, sawfish, shark rays, and other carcharhinid sharks. Collectively we need to share information on our successes and failures as well as resources. Facilities that can house breeder adults and provide for the rearing of young may need to be exploited for this potential. This may require financial support from facilities that ultimately plan to display the species targeted in their facilities. One very important piece of the

puzzle is access to quality transport at a reasonable price to encourage facilities "sitting on the fence" with respect to acquiring captive bred young to move forward with their plans. We must also have strong interpretation of displays that message to our visitors the value of captive bred collections to our conservation and sustainability missions. In new facilities as well as expansions of existing facilities providing dedicated display and holding for the less charismatic "small" elasmobranch young will also encourage others to invest limited resources in captive breeding research and efforts. The future of aquariums demands that we walk the walk of sustainable collections and this can only be achieved by our collaborative effort and commitment to this goal.

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HUSBANDRY AND BREEDING MANAGEMENT OF *Rhinobatos cemiculus,* GEOFFROY SAINT HILAIRE,1817

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Abstract

Eight juvenile guitarfish, *Rhinobatos cemiculus,* were acquired by Nausicaá in 2006. Within 4 years these elasmobranchs became sexually mature and a first birth was recorded in 2009. Since then, three female guitarfish gave birth to babies every year, which brought the total number of offspring up to 45 by the end of 2012. Husbandry techniques, biometry on adults and juveniles, ultrasonography and regular blood sampling are now included in the regular husbandry routine in order to evaluate reproductive physiology of this species, that is not common in aquariums.

Introduction

In 2006, Nausicaà opened a new exhibit named "Cap au Sud". It takes the visitor on a trip along the east coast of the Atlantic Ocean from North Norway to Cape Town in South Africa. During this trip, visitor discovers various biotopes and impacts of human activities on this environment. During a stopover in Lagos, Nigeria, visitors are introduced to the blackchin guitarfish (*Rhinobatos cemiculus*, Geoffroy Saint-Hilaire, 1817). They become aware of the issues with water pollution from plastics that over time breakdown into fine particles that can be ingested by fishes, making fish sterile.

This exhibit is an opportunity for Nausicaà to introduce a new species into its elasmobranch collection: the blackchin guitarfish. These captive animals have been given special attention in order to optimize breeding. Monitoring of growth is preformed through regular biometric measurements. After a few years, guitarfish reach sexual maturity and begin to reproduce on an annual basis. Regular monitoring of broodstock is occurs using ultrasonography and blood sampling for hormonal changes. Juveniles also are followed through development (ie., growth, first feeding, feeding rate, malformations, etc).

1- Characteristics of the species

The blackchin guitarfish (*Rhinobatos cemiculus*) is a demersal species living on sandy-muddy soft bottom of the coastal zone to about 100 m depth. It is found along the eastern Atlantic coast, North of Portugal to Angola, and in the Mediterranean Sea. It feeds primarily on benthic invertebrates (60% crustaceans) and occasionally small fishes, especially as adults. It is considered as an endangered specie by the IUCN (2012) due to overfishing for finning.

This species rarely occurs in aquariums. In a study conducted among European aquariums in 2011, only two aquariums were reported having this species in their collection: the aquarium of Hanover in Germany and Nausicaà in France. However, the juvenile stage of this species can easily be confused with common guitarfish: *Rhinobatos rhinobatos,* Linaeus, 1758, with a similar distribution area as *R. cemiculus*. Once adult, *R. cemiculus* is the only species to reach 265 cm in length, while *R. rhinobatos* remains small and does not exceed 100 cm. To identify juveniles, other criteria must be used, rather than general morphology of the two species, as described by Fischer et. al. (1987). However, these criteria are subjective and sometimes difficult to understand if the two species cannot be compared.

The criteria to differentiate *R. cemiculus* of *R. rhinobatos* are mainly:

- A triangular nose with a closed angle: 60-65° against 65° in *R. rhinobatos.*
- A narrow rostral cartilage.
- The rostral ridges very close.
- A preorbital length less than or equal to the distance between the orbit posterior edge and the posterior insertion of pectoral fin.
- A preorbital length equal to 6-8 times the diameter of the eye (against 5 times in *R. rhinobatos*).
- A black pigmentation at the ventral tip of the rostrum.
- A beige to brown color (khaki brown in *R. rhinobatos*).
- A small anterior nasal lobe of the nostril.

It seems that even fishermen and aquarium suppliers have trouble distinguishing these two species. Indeed, individuals provided to Nausicaà in 2006, were sold as *R. rhinobatos.* Proper identification was made with certainty after checking morphological criteria specific to each of the two species. The identification was further confirmed once they matured.

2- Maintenance conditions

Eight individuals are held at Nausicaà with a sex ratio of 1.1. Wild-caught, they were provided by "Fauna Marina" a Spanish company based in Cadiz specialized in the capture and fish supply for aquariums. On their arrival in Boulogne-sur-Mer, the animals weighed 3.5 to 4 kg and measured approximately one meter in length.

The stock was then divided into two groups. One half was placed in the exhibit while the other half was maintained in quarantine. In both situations, the LSS is similar. It is a closed system with a turnover of about one hour, with mechanical and biological filtration, an UV sterilizer, and a heat exchanger. The seawater renewal varies between 1-3%/day. Seasonal temperature variation of a few degrees Celsius was observed. Water in the exhibition is slightly colder by about 2 to 3°C than that in quarantine (Figure 1). Nevertheless, temperature curves parallel each other and are subject to similar variations. Observed water parameters for both systems are presented in Table 1. In the wild, temperature ranges are similar, but with greater amplitude.

The quarantine tank is a 70 m^3 circular tank in black GRP, with a floor area of 50 m^2. The exhibition tank is a shallow rectangular pool (30 cm), giving animals a floor area of 12 m^2, with a volume of 3.6 m^3. The available bottom area is more important, than the shape or the volume, for this animal.

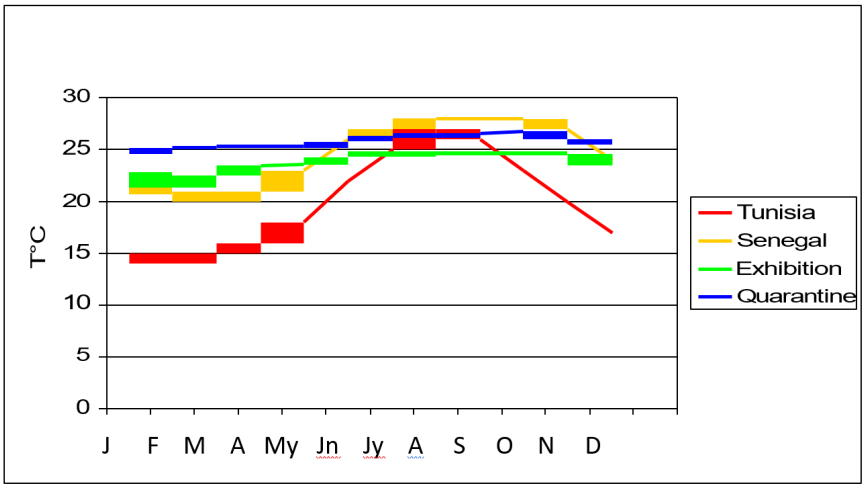

Figure 1. Annual temperature profile in the different rearing tank and in the wild.

In both holding situations, animals were exposed to artificial lighting with a photoperiod of 12:12; however, the photoperiods were reversed on the two systems. Quarantine lighting consists of six fluorescent tubes and 400 watts HQI spotlight that operates during the day. This tank is dark during the night. In exhibition five 400 watt HQI spotlights illuminate the tank overnight. During the day it is kept dark.

Table 1. Water quality

Parameter	Unit	Quarantine		Exhibition	
		Average	Range	Average	Range
Temperature	°C	25,2	22,6 – 27,4	23	19,3 – 25,7
Conductivity	mS/cm	45,7	43,3 - 49,9	48,2	45,2 - 50,7
pH		7,94	7,67 - 8,17	7,91	7,55 - 8,15
TAC	French degree	14,1	12,2 - 15,5	14,0	12,5 - 19,5
N-NH4	mg/L	0,037	0 - 0,165	0,048	0 - 0,121
N-NO2	mg/L	0,002	4,3 - 14,8	3,63	0,92 - 6,32
N-NO3	mg/L	9,49	0 - 0,012	0,007	0,001 - 0,08
PO4	mg/L	4,23	2,57 - 5,41	2,67	1,16 - 8,78
Vibrio spp.	CFU	65	0 - 260	90	0 – 560

In Nausicaà, both sexes occur in the same tank. Sometimes in the quarantine area, they are mixed with other species such as humphead wrasse (*Cheilinus ondulatus*, Rüppel, 1835), zebra shark (*Stegostoma fasciatum*, Hermann, 1783), Blacktip reef shark (*Carcharhinus melanopterus*, Quoy & Gaimard, 1824) and/or juvenile sandbar shark (*Carcharhinus plumbeus*, Nardo, 1827).

3- Feeding and growth of adult animals

Broodstock were fed three times a week. Feeding rate is 2 to 2.5% BW / day. Diet consisted mainly of various fishes (Figure 2) between 50 to 100 grams. Half of the species had high lipid content.

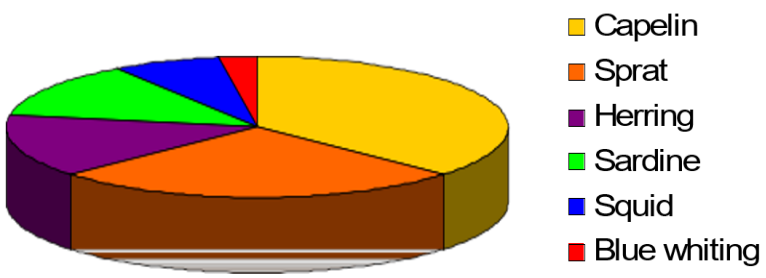

Figure 2. Diet items provided to adult blackchin guitarfish by relative percentage.

There is a weight gain during pregnancy, resulting from development of the embryo. However, this gain does not seem to be proportional to the number of embryos. In fact, the female with the largest litter usually weighs the least. Once parturition takes place, there is a sudden net weight loss, which is reflected in Figure 3.

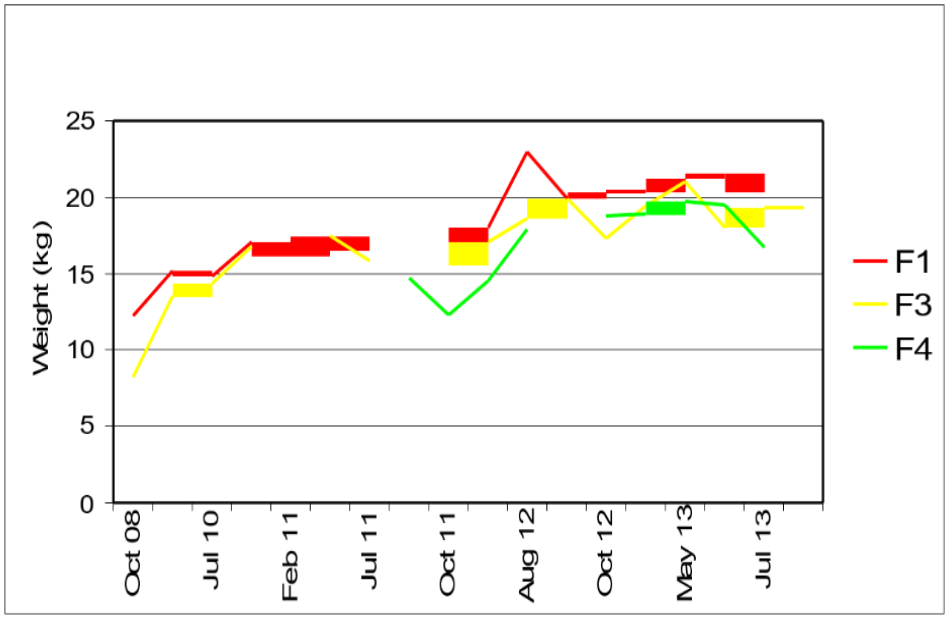

Figure 3. Growth of the 3 adult females blackchin guitarfish in kilograms from 2008 to 2013.

4- Reproduction

4-1- Sexual cycle

4-1-1- Sexual dimorphism

In *R. cemiculus*, there is little sexual dimorphism. Besides the presence of claspers in males, the only dimorphism observed is the difference in size between the two genders, which is common in elasmobranchs. Females are generally larger than males.

4-1-2- Sexual maturity

The first birth was observed in 2009. Since then, three females gave birth annually, resulting in 11 litters. The females have reached a total length of 144 to 150 cm and an average weight of 12 kg. Seck et al (2004) estimated that females reach 163 cm and 14.4 kg in length at maturity for those caught off the coast of. However, Capapé & Zaouali (1981) fixed maturity at 110 cm, in a study in Tunisia.

For males the size of first reproduction has been observed between 125 and 140 cm and a weight of more than 7.5 kg. For females, Seck et al. (2004) describes juveniles being less than 152 cm.

The age of sexual maturity is difficult to determine. The age of our animals at Nausicaa upon arrival in 2006 was not known. However, given the total length of the animals, their age could be estimated between 2 and 3 years old. These animals bred after three years of captivity, bringing their age of maturity between 5 and 6 years.

4-1-3- Folliculogenesis

In the wild, *R. cemiculus* occurs annually. Only one of the ovaries is productive, follicles from other waves undergo atresia. In the wild, vitellogenesis takes place from April to August. Oocytes reached a maximum size of more than 40mm in the stroma, ready to be released. Gestation runs parallel to vitellogenesis. According to Seck et al (1991), newly formed eggs can be found encapsulated in the uterus from December to March. Embryos can be found from April to August reaching a size greater than 20 cm. These processes are equally distributed between both uteri. Parturition usually takes place in August, to coincide with the age of maturity for the new oocytes. Gestation lasts a duration of 5 to 8 months (Lessa & Lahaye, 1982; Capapé & Zaouali, 1994; Seck et al, 2004).

Embryonic diapause could be environmentally influenced. It has not been described in *R. rhinobatos* nor *R. cemiculus* in the Tunisian warm waters, but could be possible for the same species in waters with more variable temperature such as on the coast of Senegal (Capapé & Zaouali, 1994; Seck et al, 2004). In his study, Seck et al encountered relatively homogeneous values between 20 and 22 eggs in both ovaries. In Tunisian waters , Capapé estimates ovocytes to be between six and 16 (Capapé & Zaouali, 1994; Seck et al, 2004). Similarly, uterine fecundity counts the number of elements in the two uteri, eggs or embryos. Seck et al's results did not statistically differ from those of ovarian fertility and were between 16 and 24 elements, whereas in the Capapé study, uterine fecundity was statistically different witha mean of 7.52 versus 9.16 (Capapé & Zaouali, 1994; Seck et al, 2004).

4-1-5- Mating and fertilization

A female may mate with several males, resulting in sperm competition. A copulatory plug is usually produced by alkaline gland to prevent futher introduction of sperm. Fertilization occurs before encapsulation. No evidence suggests that sperm storage occurs *R. cemiculus* (Hamlett, 1999a; Carrier et al, 2004).

At present, no mating has ever been observed in captivity. The only observable coupling clues are bite marks on the edges of the pectoral fins. The animals of both sexes have bite marks. It was found that the coupling takes place in the days following parturition, when the two sexes occur together.

4-1-6- Pregnancy

In the Rhinobatidae, the common way of gestation is aplacental viviparity by maternotrophy. *Rhinobatos cemiculus* is no exception to the rule, the pregnancy begins with embryos consuming yolk reserves. Halfway through gestation, embryos become free in the uterus and receive, in addition to the yolk, provision of " uterine milk" secreted into the lumen of the uterus (Dulvy & Reynolds, 1997). Uterine epithelium develops villi on the entire surface, that can measure several centimetres in length. These are called trophonemata. Villi are narrow to their base, wide at their distal end and wrap the fetus closely. Surface secretion is increased allowing a large supply of nutrients. This partly explains why the species with that type of gestation produce juveniles that are proportionately larger. The secreted fluid contains proteins, lipids, and mucus. Fetuses absorb secretions either by ingestion or gill absorption. By three quarters of the way through gestation, the yolk sac is almost depleted and will begin to resorb as the cord vitellin (Wood-Mason & Alcock, 1891; Bearden, 1959; Hamlett & al, 1985; Hamlett, 1999b).

About 2 months before parturition, the dorsal part of the animal begins to widen on each side of the rachis between the gills and the pelvic girdle. This area expands more and more throughout gestation. Ventrally, although difficult to observe, there is a swelling in the same area.

Shortly before parturition, the females are captured and isolated in a circular tank of smaller size (4 m diameter - 12 m^3) to reduce the risk of predation on juveniles at birth. Isolation is estimated based on the date, the size of the females, and the observations made by ultrasonography. One week before parturition, a swelling of cloacal papilla can be observed.

4-1-7- Parturition

Little is known about parturition in *R. cemiculus*. From birth pups are autonomous and appear as miniature adults with a ventral scar, where the external yolk sac was located. At necropsy, we observed the presence of an internal yolk vesicle.

In most cases, parturition occurs during the months of July and August as has been observed in the wild. However, the birth of three litters in 2012 occurred between September and November. The same temporal shift occurred in 2013.

Although we have been able to observe the birth, it has not been possible to observe the expulsion of neonates. The female stays almost motionless on the bottom after a few minutes juveniles appear one by one from the female underside and begin to swim immediately. Pups are expelled one by one. The time interval between the birth of two individuals is ranges from two to five minutes. Staff quickly isolate young in a $12m^3$ lack circular pool.

Within 24 to 48 hours after birth, the female is captured, anesthetized with eugenol (30 ppm), weighed and a blood sample is collected. The female is then placed in the presence of the male in the main culture tank.

When birth happened in 2011, three females were isolated for several months after parturition, which is the only case where the females were not placed immediately with males. The delay in placing the two sexes together may explain the shift in the time of birth of 2012 (born September-November 2012 instead of July-August in other years).

4-2-Reproduction monitoring

4-2-1- Population growth

All the broodstock (3 males and 3 females) are marked with microchip transponders, for the purpose of individual monitoring of each animal. The usual transponder size is 2 mm diameter x 12 mm long. Young animals are marked one month after birth with smaller transponders with the idea to follow each individual's growth and also to separate brothers from sisters, avoiding inbreeding when they will be able to reproduce. Liter demographics (Table 2, Figure 5) and population growth (Figure 4) are presented below.

4-2-2- Identification method

On some animals, an external plastic tag was placed through one of the dorsal fins. However, these tags stand out and must be renewed regularly. Over time, the passage of the tag through the fin often generates an inflammatory reaction.

Table 2. Reproduction of female *R. cemiculus* in Nausicaâ since 2009 (litter composition and mortality)

Lot	Birth	Progenitor ID	Litter size	Sex ratio (F/M)	Stillborn	% survival
2009	10/08/2009	?	1	1/0	1	0
2010-F3	09/07/2010	F3	2	1/1	1	0
2010-F1	13/07/2010	F1	7	¾	2	0
2010-F4	17/08/2010	F4	2	1/1	0	100
2011-F1	02/07/2011	F1	4	1/3	0	100
2011-F3	28/07/2011	F3	2	1/1	1	50
2011-F4	22/08/2011	F4	3	½	1	33,3
2012-F1	01-02/09/12	F1	9	2/1	0	88,9
2012-F3	29/10/2012	F3	12	5/7	8	33,3
2012-F4	11/11/2012	F4	4	1/1	0	75
2013-F1	27/06/13	F1	1	1/0	1	0
2013-F4	15/07/13	F1	4	1/1	0	50
2013-F3	07/10/13	F3	4	1/3	0	100

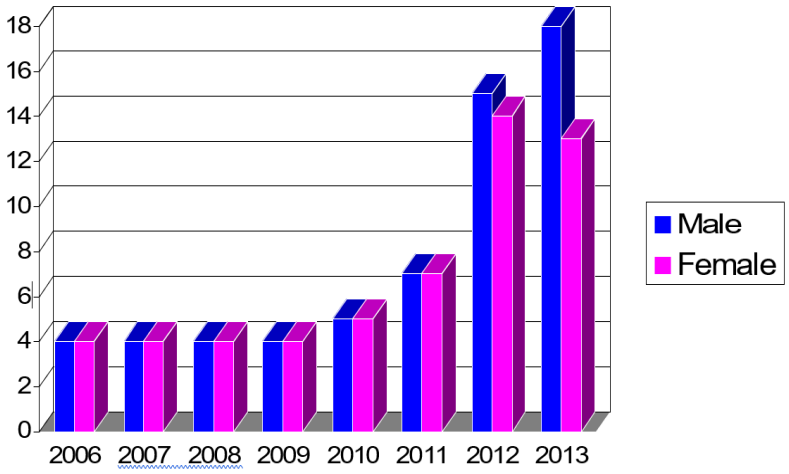

Figure 4. Growth of the blackchin guitarfish population (in number of individuals) at Nausicaä between 2006 and 2013.

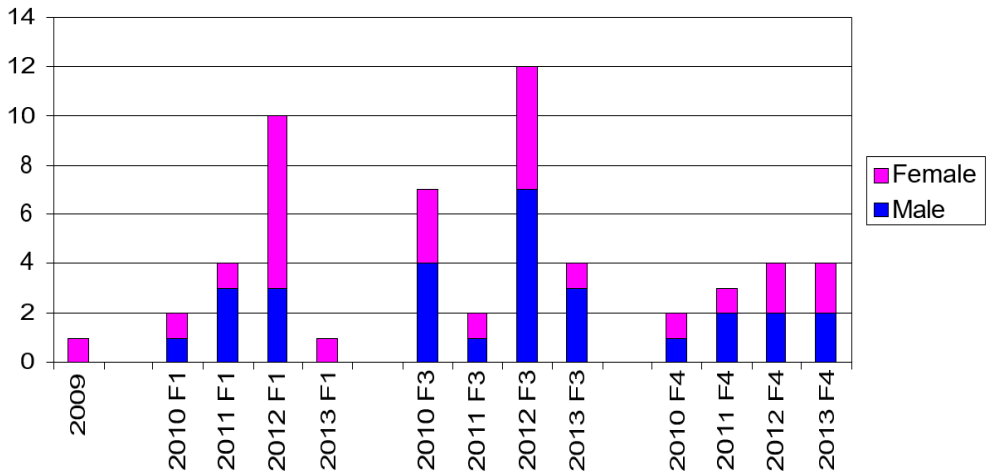

Figure 5. Gender composition of each litter in number of individuals.

4-2-3- Pregnancy monitoring method

Monitoring to follow pregnancy is implemented by ultrasonography and blood sampling. For this examination, individuals are anesthetized using a bath of 35 ppm eugenol (99%) diluted in ethanol in a 1/10 proportion. Prior to anesthesia, the oxygen saturation in the water bath is raised to 200%, in order to increase the partial pressure of oxygen in the blood of the animal. About 15 minutes is required to reach stage III of anesthesia. The ray can then be taken out of the water for

monitoring. Examination takes about 20 minutes outside of water. Recovery takes around 10 minutes. Ultrasonography allows one to follow development of ovary, uterus and embryos, but also allows measurement of the heartbeat of adult and fetus.

Blood samples are collected via a caudal puncture for:

- Smears.
- Cell blood counts (collected in 7 cc tube with EDTA in which we add 0,5 cc heparin).
- Biochemistry (usual parameters like BUN, Total Protein).
- Hormone assay in serum (blood is collected in 7 cc tube with clot activator.
- Plasma is collected passively after 12h and conserved in a dry tube at -10°C. The deep frozen tube with plasma is then sent in a carbo-ice by carrier to the lab which will perform the analysis.

Three steroid hormones (estradiol (E2), progesterone (P) and testosterone (T)) are assayed every month with the aim to establish the annual hormonal cycle for this species and correlate it with the ultrasonographic observations.

Measurement technique is via Radio Immuno Assay (RIA), where shark samples are in competition with mammal serum, using radioactive labeled progesterone. Then radioactivity is assayed and is inversely proportional to the amount of shark hormone. For P measurement, the laboratory uses a progesterone RIA Kit from Beckman Coulter Compagny, and for E2 and T, a Spectria IDS method is used.

4-2-4- Hormone assay observations

When the curves of three hormones overlap, we observe at the time of birth a fall of the oestradiol (Figure 6). Two months after birth, the quantity of oestradiol is always weak but a new peak of progesterone appears, this one lasts 3 months. Finally, 6 months before birth, progesterone drops while the rate of oestradiol increases to > 5.0 nmol / L, as well as in duration of >4mo). This period corresponds to the gestation. The variation of concentration in testosterone seems to follow the oestradiol concentration. The role of testosterone, except for being a precursor of the oestradiol, could not be determined.

There is a:

- seasonal progesterone peak (September, December / January)
- low progesterone concentration observed at delivery time
- high concentration of oestradiol throughout gestation
- punctual decrease of oestradiol at delivery time (could be delivery inductor)
- return to the basal concentration of estradiol, 2 months after delivery

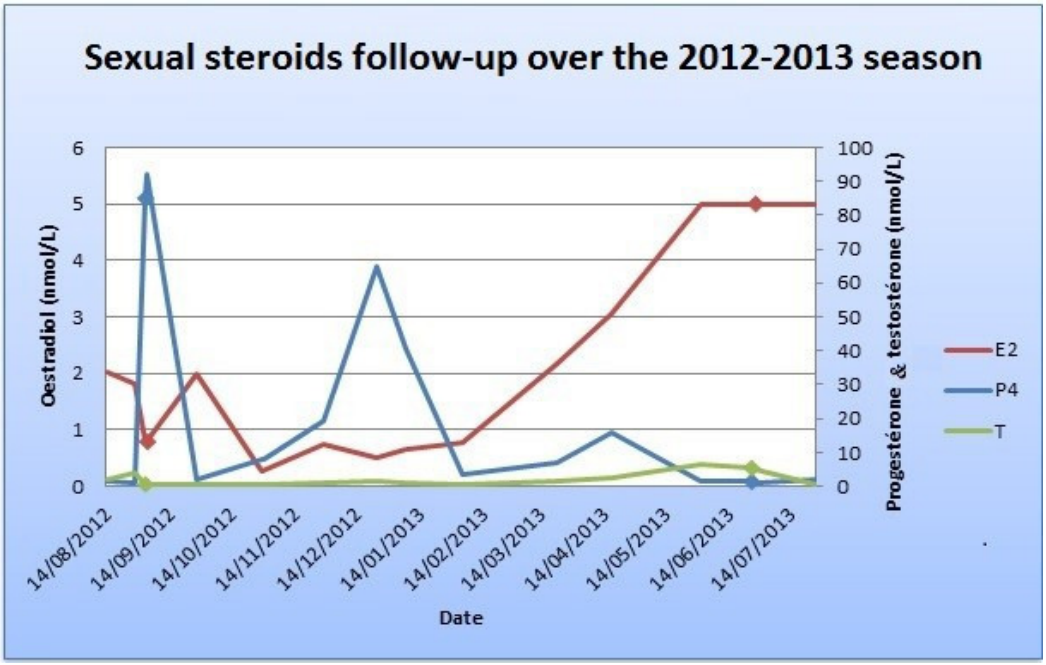

Figure 6: Rate of estradiol, progesterone and testosterone in *R. cemiculus* female during the study (the dots indicate the dates of birth).

4-2-5- Ultrasonography observations

From ultrasonography monitoring, we can observed the following sequences:

- In one female, the observation of an atresic follicle suggests the presence of non-ovulatory follicular waves, 1 to 4 months postpartum.
- Ovulatory follicle wave and fertilization almost six months after parturition (February – April).
- No vitellogenesis observed during gestation: on the contrary to Seck et al (2004) and Capapé and Zaouali (1994) observations, we did not highlight a folliculogenesis during the gestation. Gestation seems to be a low ovarian activity period, and ovulation after delivery appears unlikely. No vitellogenesis observed during gestation.
- The ovulatory follicle wave ends when mature follicles of 30 cm are released.
- Before ovulation, uterus wall thickens (Figure 7).
- The first uterine images confirming gestation are images of segmented uterus. The presence of encapsulated eggs was also described by Seck et al (2004), however he described an encapsulated form during 3 to 4 months: that is much longer than what we observed in our study (1 to 2 months maximum).(Figure 8)
- The thickening of the uterine mucosa, which continues during the first stages of the gestation, will lead to the formation of folds. These are getting organized in individual villi making protrusion in the lumen. It is the appearance of trophonemata that will provide nourishement to the developing embryos in the form of a secretion called histotroph or uterine milk. (Figure 8)

- Trophomata suspended in the uterine fluid not associated with the embryo (Figure 10): The developed trophomata was observed throughout the rest of gestation period. They become spatulate, are in suspension in the uterine liquid, and do not seem to have particular links with the embryos which are very mobile.
- The chronological development of embryos is difficult to determine. The liberation of the egg happens when between 3 to 10 cm(See photo 5).
- We suppose that the limit is situated towards 3 centimeters, because in the size of 10 cm, the foetus is almost autonomous.
- The heart rate is measurable, the breath is active, the nutrition also, and a kind of intra uterine swimming exists. The continuation of the growth seems to be a proportional enlargement of the model existing in 10 cm (Figure 12,13, & 14).
- Gestation lasts approximately 100 days.
- Quick involution of the uterus postpartum (approximately 10 days).

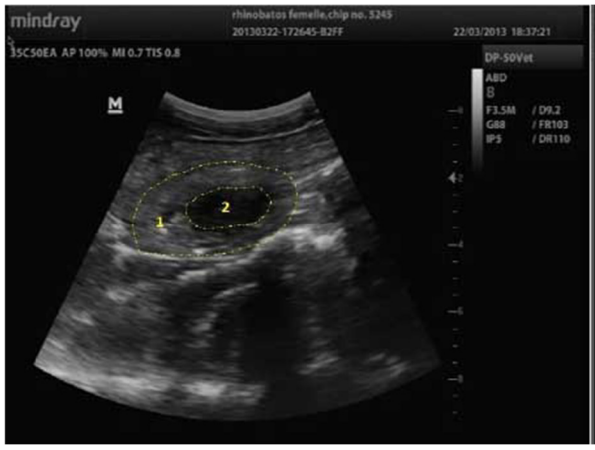

Figure 7. Uterus during thickening in March: female 5245. 1 = uterus mucosa, 2 = uterus lumen

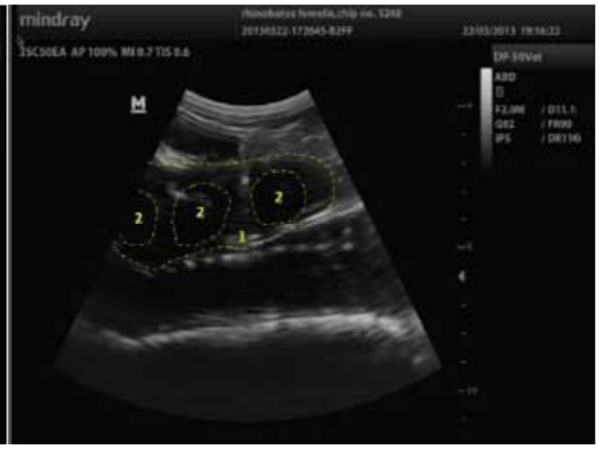

Figure 8. Uterus picture in "lodges" signs of the presence of eggs in the uterus. 1= uterus mucosa, 2 = “lodges” of the egg (diameter = 2.8 cm).

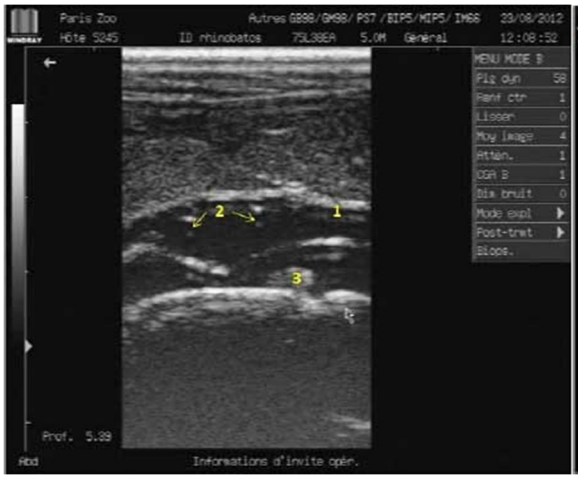

Figure 9. Apparition of trophomata in August 2012 in female 5245. 1 = uterus mucosa, 2 = small trophomata, 3 = embryo

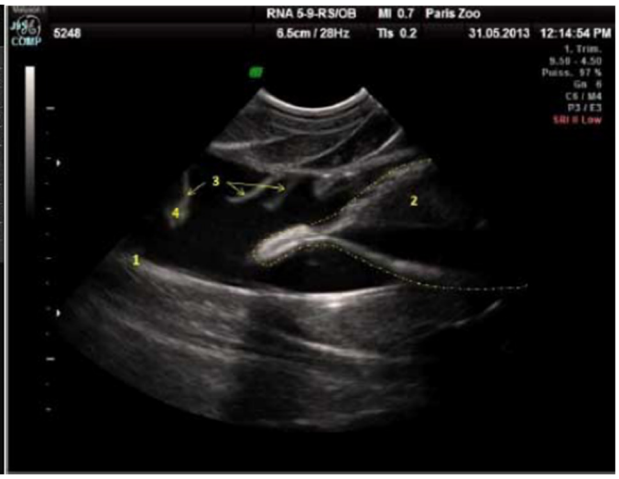

Figure 10. Trophomata developed in female 5248 in May 2013. 1 = uterus mucosa, 2 = fœtus, 3= trophomata, 4 = trophomata extremity in "spatula"

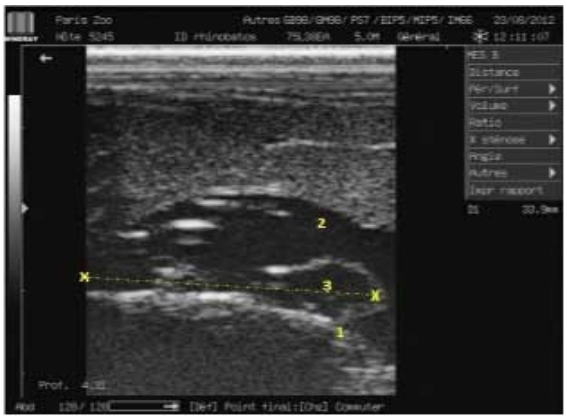

Figure 11. three cm embryo still in the egg in female 5245 in August 2012. 1 = uterus mucosa, 2 = uterus lumen, 3 = embryo.

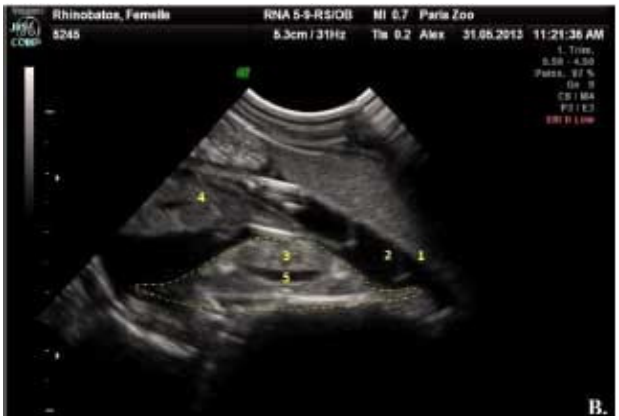

Figure 12. 11cm fetus in transversal section in female 5245 in May 2013. 1 = uterus mucosa, 2 = uterus lumen, 3 = embryo, 4 = second embryo, 5 = pharynx.

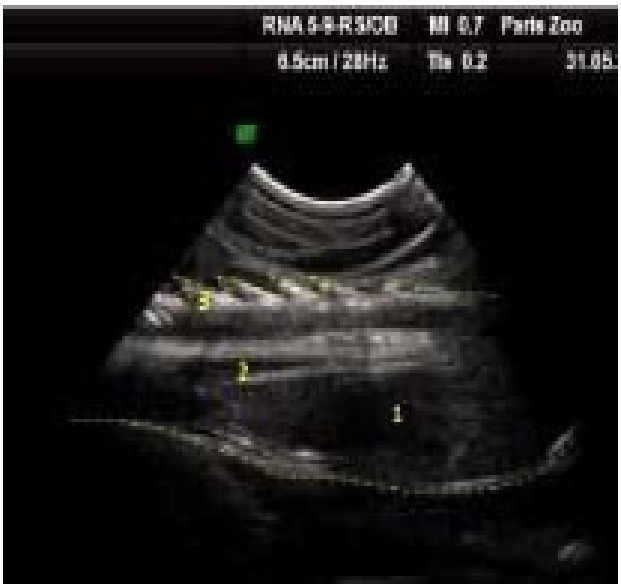

Figure 13. Foetus of 13 cm in female 5248 in May 2013. 1 = abdominal cavity of the foetus, 2 = rachis, 3 = dorsal denticles.

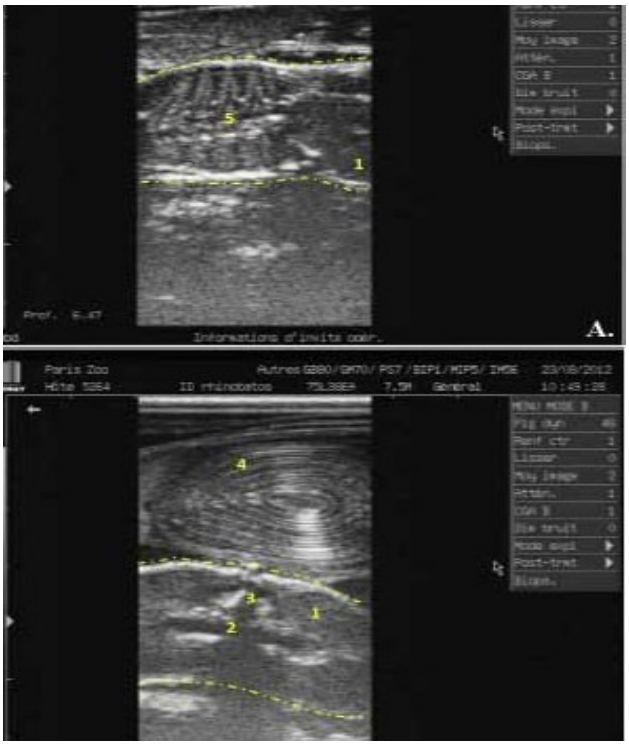

Figure 14. Near to final size foetus [25-30cm]. A: longitudinal section. B: cross section. 1 = foetus, 2 = pharynx, 3 = rachis, 4 = spiral valve, 5 = gill arches.

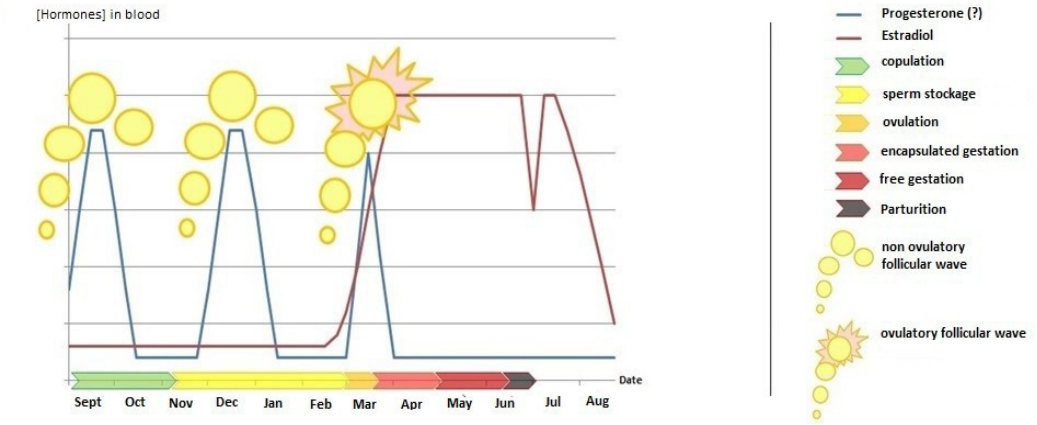

Figure 15. Proposal for a general pattern of annual reproductive cycle observed in captive *Rhinobatos cemiculus* (Boitard, 2013).

4-2-6- Proposal for a general pattern

By considering all of the data a general pattern can be described for the reproductive cycle of *Rhinobatos cemiculus*, even if information does not match perfectly. The main point of difference concerns the ovulatory wave of ovocytes. Indeed, peaks of progesterone were described although not corresponding in ovulatory phases observed by ultrasonography. These peaks seem characteristic of *R.cemiculus* and could be associated with the non-ovulatory follicular waves. On the other hand, ultrasonography placed ovulation for every female, despite the fact that no peak of progesterone resulting from a luteal activity was observed. The variations in progesterone concentrations during ovulation may not be evident if they were too short.

Observations of animals place the mating a short time after the reintroduction of the male, approximately 15 days. But the observations of gestation in the uterus and the estimations of ovulation were done much later (approximately 5 to 6 months): Contrary to Seck, who describes an embryonic diapause to the Senegalese animals, no uterine storage of eggs was observed before the beginning of the gestation. So it seems possible that, like with other species of elasmobranchs, that sperm storage occurs in the female. This would last over winter.

5- Juveniles Feeding

First feeding shortly after delivery is very important. Generally new born babies don't have any internal yolk reserve and need to eat soon after parturition. The best first food is live food such as shrimps which stimulate hunting behavior by their constant motion. They also can be left in self-service in the rearing tank without pollution risk.

Weaning on inert food can be tried after one week. But weight gain must be managed. In some cases, we tried to start with not live food like chopped herring. Although young guitarfish showed a great activity in the presence of this food and seemed to eat it, we have to note that after 3 weeks of this diet without living food, all the animals had lost around 30% of body weight and some individuals died.

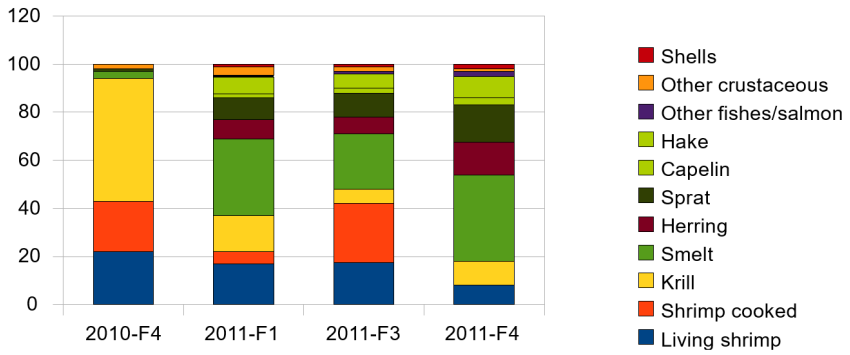

Figure 16. Proportion (%) of different food quality eaten by 4 different litter during the first 6 months.

According to the considered litters, weaning and food diversification is more or less fast. Once the youngsters eat and grow well, feeding frequency is reduced to once a day with a rate between 5-10% of body weight per day.

6- Mortality at Birth and Malformations

Of a total 56 births since 2009, 26,8% were stillborn and another quarter of the survivors died within 1 month after birth.

Causes of death identified:

- First feeding is very important and food must be always available in the tank. At this age the loss of weight is very fast and becomes rapidly lethal.
- Embryos vary in development. Some may still retain an internal yolk reserve at delivery, while others have consumed it all and have no hepatic fat reserve. These individuals are generally smaller and are stillborn.
- Embryos abnormality represent a small part of the new born.
- Close to the delivery the gestating female must be isolated, to prevent predation and attack by other adults.
- Bad rearing conditions with low temperatures may have caused mortality of the first litters.

Among the abnormalities identified most of them are lethal:

- The pectoral fins are not attached to the head (2009).
- The anterior part of the esophagus may not be connected to the pharynx and may have a free extremity in the pericardiac cavity(2010-F1).
- Lordosis (2010-F1).
- Cranioschizia (2012-F4): The young individual lived 3 days presenting strong disorientation.

Some defects, including separation of the pectoral fin with the head have been observed in other species such as *Raja sp.* (Templeman, 1965) or the South American freshwater stingray

(*Potamotrygon motoro*, Muller & Henle, 1841) (Rosa et al, 1996). The separation of pectoral fins from the head seems to be a normal state during the early stages of embryonic development. The same applies to the closure of the neural groove. The fact that embryogenesis is not coming to the end, may have a congenital cause, but according to Heupel et al (1999), beyond the congenital aspect, many defects causing a deformation of the skeleton may be associated with other factors such as parasitism, injury or tumors, or nutritional deficiencies.

Another abnormality is the presence of depigmentation area on the body. This is not lethal, and these areas generally become pigmented after few weeks.

7- Health

Health examinations could be conducted during pregnancy monitoring by ultrasonography or blood sampling.

The most current health problem are bacterial infections that lead to ascites. This condition can usually be cured by treatment with an intramuscular injection of fluoroquinolone (marbofloxacin: 2,5 mg/kg BW every 2 days or enrofloxacin: 10 mg/kg BW every 2 days. Enrofloxacin also can be administered orally if the animal still has an appetite. Infections of *Vibrio vulnificus* caused the death of two guitarfishes three years old. Sometimes the cause of ascites is only traumatic. Such a case occurred on an adult male after he was bitten by sandbar shark. Half of the pectoral fin was removed by the shark resulting in a hemorrhage at the origin of the ascites. Only a microbiological culture of the ascitic fluid can determine the origin whether infection or trauma. Fluid can be collected using a needle guided by ultrasonography.

On one female, small vesicles appeared on the dorsal rostral cartilage. The venous system in this area became very apparent as if an infection spread along the vessels. Attempts to remove the fluid from the vesicles using a small needle failed. The animal was treated by enrofloxacin per os (5 mg/kg) every day for 10 days and the problem disappeared.

An adult female (F2) died few months after her transfer to another installation. A necropsy revealed a bacterial infection of the endometria? maybe related to eggs retention.

Worms were found following anesthesia baths. The worms were not identified. They seemed to be monogenean probably located in gills or skin. No treatment was given.

Regular ultrasonography and blood smear examination made on a regular base, can help to prevent various infectious disease.

Conclusion

The reproductive pattern in captivity will have to be confirmed by additional observations. It is possible that the captive conditions (temperature, photoperiod, and availability of the food) may modified the reproductive cycle.

Since 2013, the blackchin guitarfish is included in the Elasmobranchs Regional Collection Plan of EAZA. The species is subject to a monitoring program that will ultimately lead to the development of a studbook. Currently few aquariums hold this species in captivity and Nausicaa seems to hold most of the European stock. An inquiry should be completed in order to ensure proper identification of the genus *Rhinobatos* held by other aquariums. A future objective will be to develop a genetic study of the species. Several aspects can be explored, like the genetic identification of the species in order to avoid confusion with other closely related species, the characterization of the different populations (Atlantic and mediterranean), and finally a genetic test to determine filiation of each newborn but also to make sure that there is no inbreeding in the broodstock.

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ACQUISITION, TRANSPORTATION AND HUSBANDRY OF SCALLOPED HAMMERHEAD SHARKS *(Sphyrna lewini)* WITHIN A MULTISPECIES EXHIBIT

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Abstract

Nine male and fourteen female scalloped hammerhead sharks (*Sphyrna lewini*) were acquired from Kagoshima, Japan, and transported to Ocean Park, Hong Kong, for display. The five-day transport resulted in a survival rate of 91.3%. After a 14-month quarantine period, the hammerhead sharks were transported to their display tank at the Grand Aquarium. The exhibit was specifically designed to facilitate the compatible housing of scalloped hammerhead sharks with over 160 teleost species. Abnormal behavior of scalloped hammerhead sharks was observed during an incident involving ozone in November 2013, which ultimately resulted in the death of six female sharks. The surviving female sharks appeared to be more affected by the incident than the male sharks. The growth rate of sharks in the aquarium was higher than that of wild conspecifics in the same age range.

Background

The Grand Aquarium in Ocean Park, Hong Kong, was opened in 2011, with the mission of providing visitors with a new way to experience the ocean. The aquarium further aimed to highlight the importance of the sustainable use of marine resources, by presenting an ecologically-realistic, multispecies community that resembled natural oceanic habitat.

The scalloped hammerhead shark, *Sphyrna lewini* (Griffiths & Smith, 1834) (family Sphyrnidae) was one of the iconic species chosen for display to help present the aquarium's core message. This shark is named for its distinctive head structure (cephalofoil), which is dorso-ventrally flattened and laterally extended. The unique, but highly-recognizable appearance of this species, along with its energetic swimming behavior, provides excellent exhibition value, and the display of this charismatic shark was expected to make a strong impression on aquarium visitors.

Shark Acquisition and Transportation

The scalloped hammerhead sharks exhibited in the Grand Aquarium were caught off Kasasa Kagoshima prefecture, Japan, by pole fishing and set net. While construction of the Grand Aquarium continued, and to acclimatize sharks to human care, 23 scalloped hammerhead sharks were kept in a sea pen in Kagoshima for nine months. The sharks were then transported to Hong Kong by vessel. The vessel contained 10 holding containers, 4.5 m x 3.5 m x 4.5 m (deep), with a volume of 60 tons, each (Figures 1 and 2).

To allow continuous water exchange in the holding containers, and to minimize instability within the containers during rough seas, the transport route was carefully planned, taking into account various factors, including weather, sea condition and ambient water quality. During the

voyage, water quality (within the containers and from the ambient seawater intake) and shark condition were closely monitored.

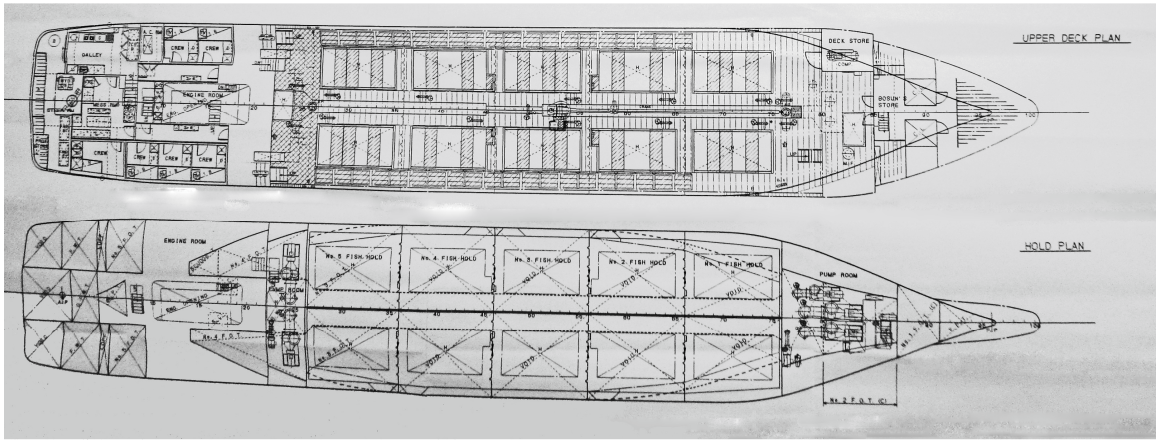

Figure 1. Plan of the transportation vessel for the long-distance sea transport of scalloped hammerhead sharks (*Sphyrna lewini*).

Prolonged transportation is one of the challenging aspects for an aquarium that wishes to display hammerhead sharks. According to published data (Young et al., 2002), the longest documented transport of hammerhead sharks was 70 h, and resulted in a survival rate of just 33% two weeks following transport. In the present case, hammerhead sharks successfully arrived at Ocean Park after a five day ocean voyage, with a 91.3% survival rate at one month post-transport.

Figure 2. The transport vessel with live fish holding containers for scalloped hammerhead shark (*Sphyrna lewini*) transportation.

Quarantine

Upon arrival at Ocean Park in November 2009, the hammerhead sharks were kept in an isolated, cylindrical fiberglass tank (9.14 m diameter, 2.94 m deep, with total volume of 192.8 m^3) for 14 months for quarantine and acclimatization (until the construction of the Grand Aquarium was completed in January 2011). The quarantine tank was outdoors, but covered with a canvas roof. During the first nine months of the quarantine period, there were eight mortalities, which were attributed to ulceration, lateral line disease, fungal infection and poor appetite. Lateral line disease in hammerhead sharks is a known condition, and often results from *Fusarium* infection (Austwick, 1984; Crow et al., 1995).

Gerald et al. (1995) suggested that sharks became immunocompromised after prolonged transportation and quarantine periods, so the aquarium veterinary team prescribed a supplement of 1-6-Beta-glucan to be added to food, in addition to the usual Mazuri Shark and Ray vitamin supplement given. For the remaining five months of quarantine, and subsequently while on exhibit, no further occurrence of lateral line disease was detected.

Transportation from the quarantine facility to the exhibit

When transporting hammerhead sharks, special consideration was given to the head structure, and in particular, the laterally positioned eyes. To minimize handling stress, a canvas partition was placed in the quarantine tank, which was used to gently guide the animals towards the transport tank (Figure 3a). Instead of using traditional netting, sharks were introduced into the transportation tank with a custom-made nylon stretcher (Figure 3b and 3c). This stretcher prevented the sharks from becoming entangled, or causing damage to their eyes. The transportation container was marked with a cross-hatched pattern, in an effort to alert the sharks to the tank walls. To further prevent injury, only one shark was transported at a time (Figure 3d). The transportation tank was fitted with a window to allow observation of shark during transport (Figure 3e) and a water pump and oxygen tank to maintain water quality during the transfer from quarantine to the display tank.

At the aquarium, the entire transportation tank was hoisted by crane and submerged into the exhibit tank, then a sliding door on the side of tank was opened to allow the shark to swim out freely. Thus, no further handling was required (Figure 3f).

Husbandry in a multispecies tank

To facilitate successful husbandry of the hammerhead sharks, the composition of other species in the exhibit was carefully considered. Also, to ensure adequate area for swimming and gliding in the 5,200-ton tank, the rockwork was designed to minimize obstruction. Apart from the rockwork located at the deep, central area of the tank, no other rockwork was present along the tank walls (Figure 4).

To minimize competition, and to ensure that each shark was receiving an adequate amount of food, temporal and spatial segregation during surface feeding was employed. This involved the occurrence of two feeding per day. Hammerhead sharks were offered food at the beginning, and again at the end, of each session, after all other surface-feeding was done. This method ensured

Figure 3. (A) To minimize handling stress, a canvas partition was placed in the quarantine tank to allow scalloped hammerhead sharks (*Sphyrna lewini*) to be gently guided into the transport tank. (B) Design of the custom-made nylon shark stretcher. (C) The sharks were handled and delivered to transportation tank with a custom-made, nylon stretcher. (D) The transportation container was marked with a net-like pattern in an effort to alert the shark to the distance from the tank wall. (E) The transportation tank was fitted with a window to allow observation of shark during transport. (F) Upon arrival at the exhibit, the entire transportation tank was hoisted by crane and submerged into the tank. A sliding door on the side of tank was opened, and the shark was able to swim out on their own.

close monitoring and recording of hammerhead shark feeding behavior. It also kept the sharks to their own feeding site to minimize competition with other species, which were feed at a different feeding location. Although previous reports have suggested that hammerhead sharks prefer certain food items, such as Japanese jack mackerel (*Trachurus japonicas*), bonito (Sardini) and squid (Teuthida), a range of food items was offered at the Grand Aquarium to ensure a broad spectrum of nutrients (Figure 5).

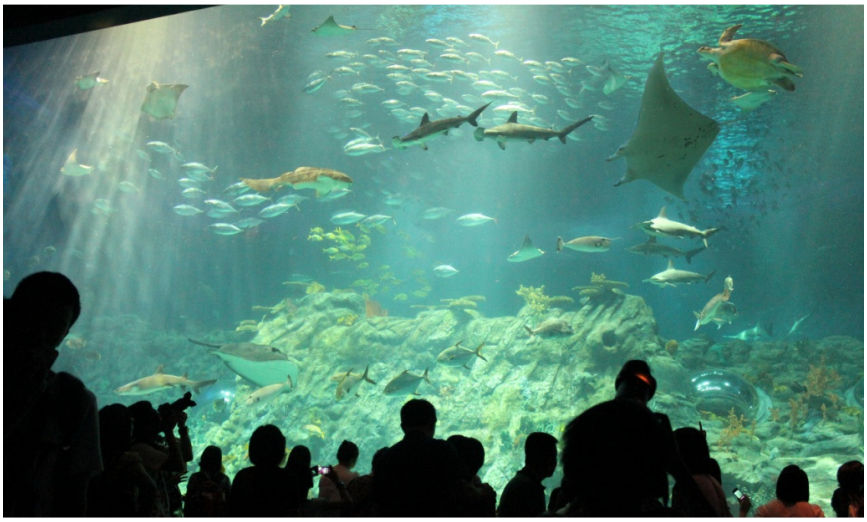

Figure 4. Apart from the rockwork located at the deep, central area of the main tank, no other rockwork was present along the tank walls to maximize unobstructed area for swimming and navigating.

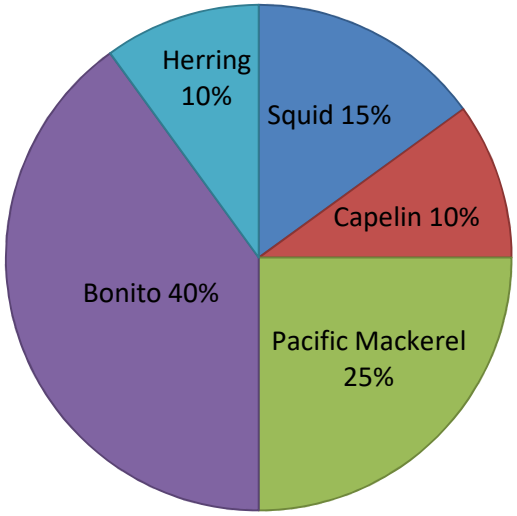

Figure 5. Food type and proportion offered to scalloped hammerhead sharks (*Sphyrna lewini*) at the Grand Aquarium.

Response to ozone residue

Except for one individual that died within a month of introduction to the Grand Aquarium exhibit, the remaining five male and nine female hammerhead sharks acclimated well to the exhibit environment, and continued to grow. However, an unfortunate ozone toxicity incident occurred in

November, 2013. Hammerhead sharks were first observed to be behaving abnormally. They continuously swam with their mouths and gills agape, and they appeared to be disoriented (Figure 6). Over the course of one day, six female hammerhead sharks died. Following the incident, investigation showed that the sensor regulating ozone output was faulty, and this allowed increased levels of ozone by-products to enter the tank. Notably, the hammerhead sharks were the only the only animals affected by the increased ozone level. Previous studies (Morris et al., 2012) suggested that response to ozone exposure could be varied, and species-specific amongst elasmobranchs. Yet, the response rapidity and lethality of this event with regard to the hammerhead sharks still remains surprising, and questionable. Also interesting, was that all mortalities were females and the three surviving female sharks appeared more affected than the five males, taking longer for feeding to resume in the surviving females, compared to the males (Figure 7). Necropsy examinations and histopathology found signs of gill injury (branchiopathy) and systemic congestion, but no theory as to the difference in responses between sexes was conceived.

Figure 6 (A, B). During an incidence of increased ozonation, scalloped hammerhead sharks (*Sphyrna lewini*) were observed to behave abnormally. They swam with mouths and gills agape and appeared disorientated.

Growth data

Total length (TL) measurements of hammerhead sharks were taken three times during their display: on arrival at Ocean Park in November 2009, after relocation from the quarantine facility to the exhibit in January 2011, and finally in November 2013 (Table 1), following the ozone incident. TL at sexual maturity for female and male scalloped hammerhead sharks has been reported to be 210 cm and 198 cm, respectively (Chen et al., 1988). Thus, female sharks at the Grand Aquarium were approaching, or had reached, sexual maturity by January 2011.

The growth rate of sharks in quarantine was slightly lower than that of wild conspecifics (Chen et al., 1990); however, once on exhibit, hammerhead sharks at the Grand Aquarium grew faster than wild conspecifics (Table 2). Such a finding suggests that these sharks were well-adapted to the exhibit environment.

More frequent measurement of the scalloped hammerhead sharks would have been advantageous; however, to achieve this, while not causing unnecessary (and potentially damaging) stress to the animals, individuals must be easily identifiable. This level of identification in this species remained a challenge at the Grand Aquarium.

Table 1. Comparison between average total length (TL) of male and female scalloped hammerhead sharks (*Sphyrna lewini*) in Ocean Park, including published data on length at maturity. *a*: n = 9 males, 14 females. *b*: n = 6 males, 9 females. *c*: n = 6 females; measurements from newly-dead sharks. *: data not available. 1: data from Chen *et al.* 1988.

Date of Measurement	Time Under Human Care (Months)	Male Average TL (cm) (Range)	Female Average TL (cm) (Range)
November 2009[a]	0	123.1 (111-141)	122.1 (110-136)
January 2011[b]	14	152.7 (148-158)	142.1 (123-160)
November 2013[c]	48	*	216.33 (203-226)
Total Length at Maturity [1]		198	210

Table 2. Comparison of average growth rates (centimeters per year) of scalloped hammerhead sharks (*Sphyrna lewini*) at Ocean Park, Hong Kong, with published data of wild specimens. *: data not available; 1: based on assumed age of 1-3 months when initially collected.

Data Source	Male	Female
Ocean Park Quarantine (aged 1-2)[1]	25.3	17.2
Ocean Park Exhibition (aged 2-4)[1]	*	26.2
Chen et al., 1990[2]		
Aged 0-1	54	63
Aged 2-5	22-42	23-25
Aged 6-8	3-15	-
Aged 6-13	-	3-19

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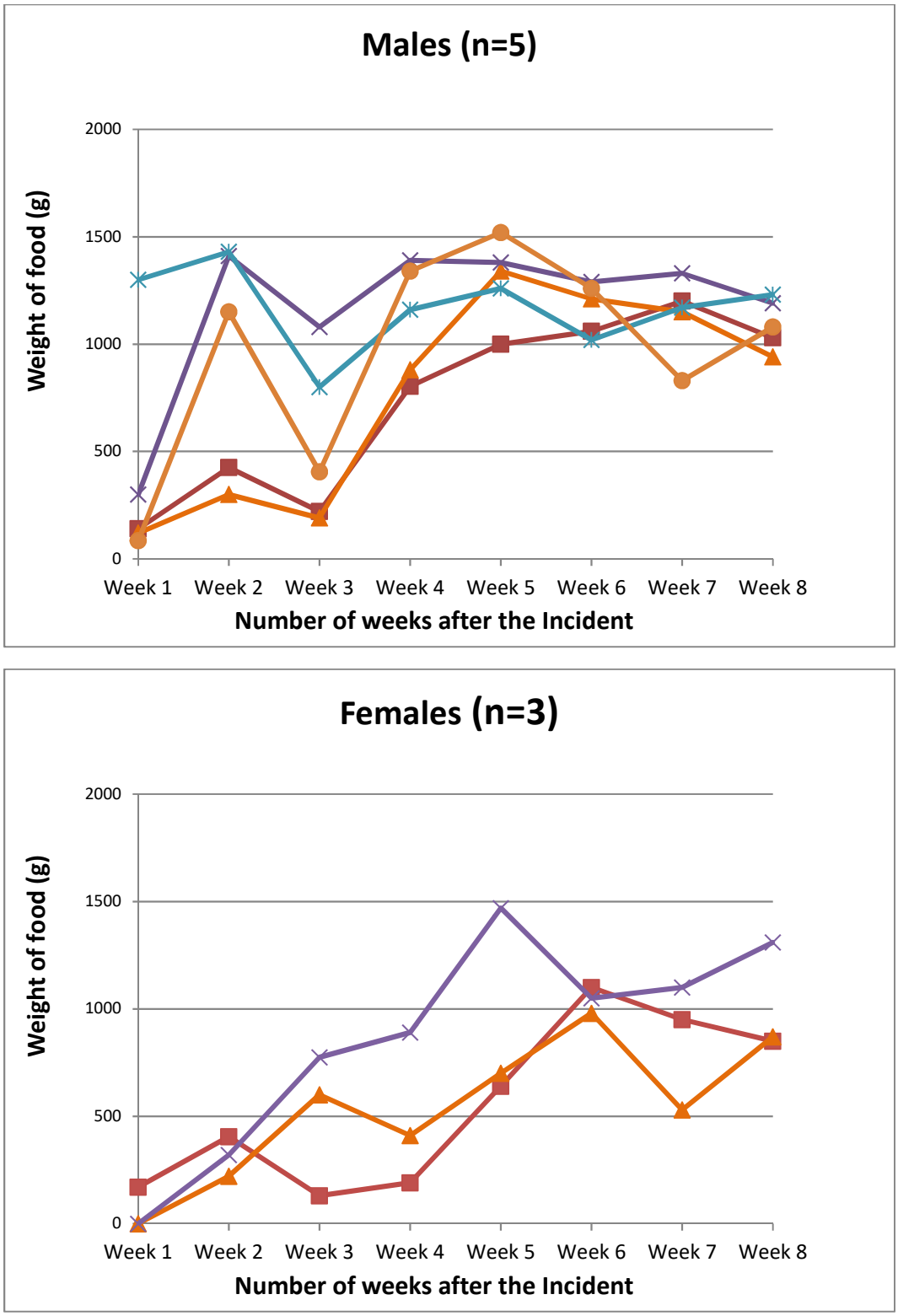

Figure 7. Weekly feeding rate of male vs. female scalloped hammerhead sharks (*Sphyrna lewini*) after the ozone incident.

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FRESHWATER TREATMENTS ON TWO MYLIOBATID RAYS: THE BAT RAY (*Myliobatis californica*) AND THE SPOTTED EAGLE RAY (*Aetobatus narinari*)

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Abstract

Freshwater treatments have proven to be a successful treatment to control monogenean parasites on the bat ray, *Myliobatis californica*, at the Monterey Bay Aquarium. These rays have been displayed for multiple years in temperate (11.6°C), semi-open seawater systems containing silica sand substrate. Freshwater is a good alternative treatment for ectoparasites because it is non-toxic, affordable, accessible and can be disposed of easily. Freshwater treatments have been effective on all life stages of monogeneans and repeated treatments do not lead to drug resistant strains of parasites. Freshwater treatments are so effective that they are now part of a regular, prophylactic treatment regimen at the Monterey Bay Aquarium. This paper outlines the protocols for conducting freshwater treatments and compares the results of freshwater bathes given to control monogeneans and copepod parasites on another Myliobatid ray, the spotted eagle ray, *Aetobatus narinari*. These spotted eagle rays have been displayed for many years in a tropical (22.2°C) open seawater system at the Ihilani resort and are part of a daily visitor interactive experience. The purpose of this chapter is to describe an effective, environmentally safe and simple protocol for freshwater bathes as a treatment of monogenean parasites on Myliobatid rays.

Introduction

The 1.2 million-liter Monterey Bay Habitats exhibit is an open seawater system with a 30-micron sand substrate. This exhibit receives filtered and unfiltered seawater indirectly from the ocean, which introduces naturally occurring parasites. Two bat rays are currently on exhibit and are hand-fed by divers two times a week.

The Ihilani resort is located on Oahu, Hawaii and has a 340,000-liter exhibit, which is divided into two pools: one where the spotted eagle rays reside, and another where visitors are able to interact with the eagle rays (overseen by the staff of Reef and Ray, LLC). Both of these exhibit pools receive unfiltered seawater and neither has substrate, since the substrate was intentionally removed to decrease the density of monogenean parasites. There are three spotted eagle rays held in this system and they are fed daily during the visitor interactions.

Therapeutic freshwater treatments specifically target ectoparasites which can cause epidermal damage and potential eye damage by scarring the cornea. The parasites we are most concerned with are Monogeneans (Family Monocotylidea) which are thin, flat or cylindrical, soft-bodied, bi-laterally symmetrical and semitransparent or opaque worms that are typically 1 – 20 mm long when relaxed. Monogeneans are flexible and possess distinct anterior and posterior ends of the body. The mouth is located at the anterior end of the body and the posterior end of the body is an attachment structure known as the haptor (Bychowsky, 1961; Yamaguti, 1963 a; Schell, 1985;

Hendrix, 1994; Kearn, 1998). Monogeneans attach to teleosts and elasmobranchs with the haptor, a sucker with claw-like hooks (Benz, G. and Bullard, S. 2004), and this attachment point is the cause of damage on the animal's epidermis. The soft, scale-less epidermis of Myliobatoid rays is an ideal place for parasites to attach, feed, grow and reproduce. Monogeneans have high reproductive rates (Kearn 1986) and are protrandrous hermaphrodites that produce large number of eggs (over 80/day in some species; Thoney 1990). Oviparous Monogeneans either lay their eggs in the sand or broadcast them in the water column, which then drift down to the sand. Some monogenean eggs can remain viable for over three months so it is hard to eradicate all of the lifecycle stages of the infestation. Demersal elasmobranchs, which are slow moving and in frequent contact with the bottom substrate, are more prone to parasitic infection.

As a result of monogenean infection, the bat ray can display cloudy patches on the dark epidermis, erosion of the pectoral disc margins, exhibit 'flashing' behavior and a decrease in appetite. The cloudiness on the bat ray's skin is a result of excess mucus production in response to the monogenean infestation (Noga 1996). Monogeneans feeding on fish epithelium cause erosion and the resulting exposure of the underlying dermis (Paperna and Overstreet 1981). Untreated infections can lead to blindness, scale loss and the development of open wounds that expose underlying muscle and connective tissue. Secondary bacterial infections and death may result if the infestation is not addressed. Several thousand parasites have been found on intensely infected fish (Jahn and Kuhn, 1932). Infestation in the spotted eagle ray results in the ray becoming lethargic, exhibiting a notable increase in respiration rates ("flutter breathing") and changes in coloration ("stress stripes"). When these symptoms are present, we propose the use of a freshwater treatment for monogeneans on the skin and gills.

Freshwater Treatments

Even though live food (oysters) and multivitamin supplements (Mazuri Shark/Ray II tablet) are given to enhance the elasmobranch diet and help them combat parasite infection, a prophylactic bath is required for the bat rays in the Monterey Bay Habitats exhibit every two months. The frequency is due to our operational use of unfiltered sea water, high parasite load in our substrate and recruitment onto this species in particular. The staff at Reef & Ray LLC has also been successful at prophylactically treating their spotted eagle rays on a bimonthly basis using this freshwater bath protocol. They note that this freshwater treatment is also effective against prevalent parasitic copepods.

After an exposure of four minutes to freshwater, the cell membranes of the Monogeneans rupture due to osmotic shock, causing cell death. Most of the parasites will fall off of the infected fish in the freshwater bath during treatment, and the rest will fall off within the next few hours.

All species being treated must be observed throughout the entire bath and removed from the freshwater immediately if the animals seem to be overly stressed. Most aquarists spend a great amount of time observing their rays and can determine slight agitation from rapid decline. Some signs of stress may be losing the ability to navigate the treatment tank, flipping over dorsally and being unable to return itself to ventral resting position or having respiration rates drop until spiracle movement is barely detectable.

A ray that is heavily infested with parasites or appears compromised may recover faster if given a shorter initial freshwater bath to help eliminate a portion of the parasite load and allowing it and its immune system to recover before subjecting the animal to a full term (four minute) bath. Though alternative chemical treatments exist that could be used to treat fishes with monogenean infestations, the drugs may be expensive and the parasites could potentially become immune to chemical treatment over time (Goven et al., 1980). Some monogenean eggs are relatively resistant to chemical exposure, including exposure to Praziquantel (Thoney, 1990). This procedure for freshwater bathes has also been successful in treating monogeneans on giant black sea bass (*Stereolepis gigas*), California halibut (*Paralichthys californicus*) and California sheephead (*Semicossyphus pulcher*) displayed at the Monterey Bay Aquarium.

Gear used for Freshwater Treatments

- Dissolved Oxygen (DO) meter.
- pH meter.
- Large transport tank (light bottom preferable) for treatment with cover/jump guard.
- Saltwater recovery bath.
- Sodium thiosulfate or Carbon filter.
- Sodium bicarbonate or 10% HCL/muriatic acid.
- Appropriate nylon slings or rubber nets for restraining and transferring anima.l
- Non-knotted Koi nets for divers.
- Oxygen cylinder and air stone.
- Salt water recovery bath.
- Ice, chiller or heater.
- Digital clock.
- Aprons.
- If measurements are desired: metric tape or calipers, hanging scale and bridle.
- AVID reader and extra PIT tags.
- Optional: Camera, flashlight, sample vials.

Steps for preparing and performing a freshwater bath:

1. Fill transport/treatment tank with freshwater. Use carbon filter or add sodium thiosulfate for removal of chloramines.
2. Measure temperature and pH of exhibit seawater and freshwater bath.
3. Balance temperature and pH of freshwater to match that of exhibit seawat.er
   a. Temperature; use a chiller or heater, add ice.
   b. pH; aerate, add sodium bicarbonate, 10% HCL or RO water based on need and availability.
4. Add oxygen to treatment and recovery tank (120 – 150 % saturation).
5. Remove animals from exhibit and perform four-minute freshwater bath.
6. Observe animal health and behavior while in freshwater bath.
7. Move animals into a salt water recovery tank.
8. Move animals back on exhibit.

Balancing pH and temperature

Use a pH meter and thermometer to measure the pH and temperature of the exhibit seawater. Fill a suitably sized fiberglass transport tank with freshwater. It is easier to use a transport

tank with a light blue Rhinoliner® finish or gel coat to monitor dark colored rays. There are numerous ways to increase the pH of freshwater. If the domestic freshwater source is high in alkalinity ("hard"), a freshwater bath can be aerated for a couple of hours prior to the immersion treatment. Aeration is generally used in water with a high concentration of carbon dioxide. At concentrations greater than 10 ppm, the carbon dioxide is loosely bound to the water and can easily be stripped by aeration, raising the pH to a more normal level. At lower concentrations, carbon dioxide is neutralized through the addition of an alkali, such as lime or soda ash. Lime or sodium bicarbonate (Ca(OH)2) reacts with carbon dioxide, removing the carbon dioxide from the water: CO2 + Ca(OH)2 <===> CaCO3 + H2O.

Domestic freshwater can be variable; at times if it is low in alkalinity ("soft"), you can use a small amount of diluted muriatic acid (10% HCL) to decrease pH (I have used 400 – 500 ml for 1700 L of water to lower pH by 0.8). If you have the time and the access to a source of freshwater produced by reverse osmosis (RO water), you can use a combination of domestic water and RO water which usually has a pH of five. This involves measuring pH and balancing water sources.

Domestic freshwater is usually high in concentrations of chloramines (individuals can use test strips to test (HACH, Aquatic Ecosystems, etc) the levels of chloramines in their local freshwater supply). Sodium thiosulfate MUST be used to buffer chlorine or a carbon filter can be used to decrease the use of chemical buffers.

Buckets of ice can be used to drop the temperature of the freshwater bath but may affect the pH, a chiller is the best way to manage lowering temperature if one is available. Some facilities wanting to treat tropical rays may need to use a heater to balance temperature. The dissolved oxygen of City freshwater can be low and Monterey city freshwater is usually around 50 ppm and can/should be increased by aeration with an oxygen bottle and an air stone. Use the oxygen cylinder and designated air stone to bring the dissolved oxygen up to 120 - 150 ppm. This level of dissolved oxygen acts as a therapeutic agent, calming the animal and, in personal observations, helps to facilitate faster recovery when returned to the salt water recovery tank.

Once the temperature, pH and dissolved oxygen levels of the freshwater bath are within the correct range, you can remove the animals from exhibit.

Removing Animals from Exhibit

In the Monterey Bay Habitats exhibit this procedure requires two divers to enter the exhibit and remove the bat rays one at a time. Each diver uses two koi nets, to capture and restrain the bat rays or guide them over to the diver platform. Two topside staff will use a round nylon sling to receive the bat ray from the divers and carry the ray up into the service area where the bath is located.

Reef & Ray, LLC uses a gate to partition off the pools and two aquarists capture the spotted eagle rays with deep pocketed, soft rubber nets. They use a soft, self- supported, dark blue fish bag for their treatments that can be zipped closed to darken the enclosure and calm the rays.

Observe, Return and Reinforce

Note the time when the ray is introduced to the freshwater bath, time for four minutes. Have a designated husbandry member standing by to watch for animal stress (specifically respiration rates) and to ensure the animal doesn't escape the transport tank. The freshwater bath should be deep enough for the animal to be completely immersed but not so deep that the animal can escape over the sides and out of the transport container. That staff member should observe the animal for the entirety of the bath. The monogeneans (and copepods) will turn white and begin to fall off the animal. At four minutes prepare the nylon sling/rubber net to move the ray from the freshwater bath to the salt water recovery tank. Allow the rays to recover five to ten minutes before returning to exhibit if time and space allow, take any measurements (lengths, weights) at this time. Siphon or sump pump freshwater and parasites to sewer. Reef & Ray LLC feed their spotted eagle rays fresh clams post-freshwater bath for positive reinforcement, all of their rays eat immediately post-bath.

Parasite collection, counting and species identification

Day 1: One of the two bat rays from the Monterey Bay Habitats exhibit was anesthetized using MS222 (75 mg l^{-1}) and buffered with sodium bicarbonate (ratio of 1:2 MS222 to sodium bicarbonate by weight) and ventilated while it was in a round nylon sling. At this time parasites were manually collected with forceps, relaxed with hot salt water and then fixed in 10% formalin in order to be sent out for species identification (collection method based on "A Primer for the Collection and Study of Mongeneans" by Dr. Stephen A. Bullard).

Parasites were sent to Dr. Ash Bullard at Auburn University for identification. The large, visible monogenean found on the epidermis of the bat ray was identified as *Dendromonocotyle californica* (family Monocotylidae). There was a second, microscopic monogenean which we believe is an unidentified species of *Decacotyle sp* (family Monocotylidae). We continue to work on collecting and identifying the second monogenean though it has been originally thought that this species lives on the gill or nares. Reef & Ray, LLC has witnessed that the freshwater was also effective against parasitic copepods as there were hundreds of deceased copepods lining the bottom of their bag post-treatment (Kevin Izumi personal communication), the copepods were not identified for the purpose of this paper. Kevin Izumi believes that the monogeneans affecting their spotted eagle rays could be microscopic and living on the gill which makes it extremely difficult to collect on live animals without a lab or hospital setting.

A 15 cm x 15 cm PVC-square transect was placed on the animal's dorsal epidermis to count the number of parasites located within the square and compare throughout the treatment (Figure 1). The bat ray was then transferred to a transport tank containing filtered seawater and moved to a 12 ft. round Quarantine tank for holding and recovery. The Quarantine tank was filled with and received only filtered seawater. No substrate or other species were kept with the bat ray while it resided in Quarantine.

Day 2: 24 hours later, with the bat ray fully recovered from the anesthesia, it received the standard four-minute freshwater treatment in a gel coated transport tank. The bat ray was returned to the designated Quarantine holding tank for another 24 hours.

Day 3: The bat ray did not show any signs of distress and was transported to the Animal Health Lab for a follow-up using the 15 cm x 15 cm to count the number of parasites remaining on the dorsal epidermis post-freshwater bath.

Figure 1. Performing parasite counts using a 15 x 15 cm PVC square.

Results

Two people took two counts of monogeneans within the 15 cm x 15 cm PVC square. The number of parasites counted within the transect pre-freshwater treatment were 72, 60, 63, 71. Post-freshwater bath there were no parasites detected within the transect square or anywhere on the dermis of the animal. It appears that 100% of all visible monogeneans on the epidermis were successfully eradicated with the freshwater bath.

Freshwater Exposure Experiments

Twenty monogeneans were collected while the bat ray was anesthetized and placed in salt water from the exhibit. In a lab setting, the parasites were divided into petri dishes which were then flushed with freshwater (sample from the freshwater bath) and parasites were observed over time and then re-introduced to salt water environment.

In this first set of trials, the monogeneans were thoroughly attached to the sides and bottom of the glass petri dish and it was very difficult to pipette them off, in some instances, the parasite adhered itself to the inside of the pipette as well. All of the monogeneans were exposed to at least

four minutes of freshwater exposure and then all parasites were re-introduced to saltwater and monitored on a wet table (11°C) for 24 hours.

After four minutes of freshwater exposure all the parasites either perished or were permanently impaired. The cell rupture due to osmotic shock causes the inversion of the haptor and after four minutes of submersion the monogenean appears to lose the ability to attach itself to any surface. The parasites were not able to create suction/attach to any surface be it a soft, fine paint brush or the glass sides of the petri dish.

From this experiment it is determined that, though most of monogeneans lose their ability to maintain attachment during the four-minute freshwater bath and fall off in the treatment tank, the few that remain attached do eventually fall off as all monogeneans are permanently affected by their exposure to the freshwater.

Unfortunately, during the next round of similar experiments, different forceps were used and the collection vessel was a plastic weighing sled instead of a glass petri dish. All of the parasites in the plastic weighing sled had perished within minutes of collection. Since these experiments can only be done when the rays need a freshwater treatment, there is a lapse of two months between every trial. We will continue to expand the experiments on these monogeneans in the future.

Conclusion

Freshwater bathes are an effective means to control and treat monogenean infestations on bat rays at the Monterey Bay Aquarium. Currently, this is the only prophylactic treatment used. Reef & Ray, LLC have permanently added prophylactic freshwater bathes into their treatment regimen for the spotted eagle ray and it has completely negated their demand and use of Praziquantel.

From an informal survey on AquaticInfo list serve, there are at least six institutions that routinely perform prophylactic treatments on six different species of rays (including *Dasyatis americana, Dasyatis Sabina, Rhioptera bonasus, Neotrygon kuhiliis and M. californica and A. narinari* at institutions other than that of the authors) and for a variety of parasites (including trematodes, ciliates and leeches).

An animal that is already heavily parasitized or immunocompromised is less able to tolerate the stress of freshwater exposure. It is important to carefully observe ray populations and immediate take action when there are any signs of stress or immune response to parasitism. Freshwater bathes are most effective when done prophylactically. If an animal is heavily infested with parasites it is best to start with a short duration bath (1 – 2 minutes) to reduce the number of parasites. If the animal recovers well, then gradually work up to a full (four minute) duration bath. If an animal has open wounds from the parasites it may be prudent, while the animal is removed from exhibit, to take a blood sample and treat for any potential secondary bacterial infection before using freshwater treatments.

Combining careful monitoring of ray health and routine freshwater treatments could lead to facilities treating more parasites using fewer chemical treatments.

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EXTRAORDINARY PRESENCE OF A GIANT DEVILRAY *Mobula mobular* (BONNATERRE, 1788) SPECIMEN IN THE COASTAL WATERS OF THE LIGURIAN SEA (ITALY) IN SUMMER 2012

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The Giant Devilray *Mobula mobuar* (Bonaterre, 1788) occurs in offshore, deep waters and, occasionally, in shallow waters (Bradai and Capapé 2001) throughout the Mediterranean Sea from the surface to a depth of several thousand metres. Outside the Mediterranean, it occurs along the coast of Africa from Morocco to Senegal, the Canary Islands, Madeira, the Azores, Portugal, and as a vagrant, off southern Ireland (Notarbartolo di Sciara 1987).

Like all mobulids, the Giant Devilray is an epipelagic batoid, feeding on planktonic crustaceans and small schooling fishes, which are trapped on its specialised branchial filter plates. Mobulids are aplacental viviparous matrotroph rays, in that the pups receive their nourishment from uterine milk secretion (Wourms 1977). They give birth to a single huge pup.

It is not apparent from the literature whether *M. mobular* has a restricted reproductive season in the Mediterranean. The observations of Notarbartolo di Sciara & Serena (1988) suggest that in the northern Mediterranean the species gives birth in summer and that the pup could be up to 1,660 mm disc width at birth. The gestation period is still largely conjectural, but could be one of the longest known in Chondrichthyans (Serena 2000).

On the IUCN red list this species is listed as *Endangered.* It is also listed in the Berne Convention Appendix 2, and in Barcelona Convention Appendix 2, to which it is a protected species.

From June to August 2012, a young female of *Mobula mobular* entered the port and the coastal waters of Liguria among the cities of Spotorno and Arenzano, inhabiting mainly the areas between the port of Savona and the beaches of Albisola Marina, swimming quietly between the boats, swimmers and anglers arousing much clamor, hysteria and misinformation. In fact the first reaction to the presence of the animal from the local population was amazement and a strong need to intervene to save her even though there was nothing to suggest she was hurt or in trouble.

The specimen was a young female of about 2 m wingspan and the local population called her “Samanta”. Her unusual behavior and close proximity to the coast caused her some superficial injuries with hooks and lines becoming anchored on her skin, hindering her movements.

It took a considerable effort on the part of biologists of the Acquario of Genoa along with the Port Authorities and the Scientific Community to convince the public that the best approach was the observation and the monitoring of the animal in its environment, rather than an intervention of capture and transportation to a tank .

Thanks to the close collaboration between Acquario di Genova, Port Authority, ISPRA and all the National Scientific Community (GRIS), it was possible to adopt a common strategy to monitor the condition of the animal, its movements and properly inform the local population that was alarmed for its own safety than that of the animal itself, and to make people accept and respect the decision of scientists, specialists and Authorities not to capture but to leave the specimen in the wild. After a period, people began to cooperate with authorities and scientists by providing useful information on the status of the animal. Thanks to these reports, Biologists and Veterinarians of the Acquario di Genova could intervene twice to remove hooks and fishing lines trapped in her wings, noting the good general condition of the specimen, taking photos and videos to document the status of the animal. After this intervention, a press conference which was attended by all the major newspapers and television channels was called, turning this event into an opportunity for the dissemination of accurate information on such important issues such as the respect of wildlife species and their habitat, while focusing on a species that is now thought to be endemic to the Mediterranean Sea but little known to the public due to its pelagic life. This species listed as Endangered (Serena et al., 2006) is probably on the way to recovery thanks to the measures of protection of the habitat in which it lives.

Press releases and social networks played a key role in the continuous updating of the local population and the mutual exchange of information. The animal became the darling of the swimmers until she decided herself to go back into the pelagic realm.

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A BRIEF GUIDE TO AUTHORS

Updated 2018

This guide is intended for those not accustomed to using a "Guide to Authors", as provided by more formal periodicals. Historically only about 5% of *D&C* authors get this correct ☺. Please help me out folks!

As always, typical Drum & Croaker articles are not peer reviewed and content will not be edited, other than to correct obvious errors, clarify translations, modify incorrect or cumbersome formatting, or delete superfluous material. Other types of contributions (announcements, etc.) may be edited to meet space limitations.

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Submit articles via email as a Microsoft Word document (or a file that can be opened in Word). **My E-mail address is petemohan55@gmail.com.**

All Articles Must Adhere to the Following Basic Format:

- Use Times New Roman 12-point font throughout (except figure and table legends as noted below).
- A4 users please reformat to 8 ½ x 11-inch documents (North American "letter").
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- **Format the title section with the line spacing set on 1.5 lines (not another method) and using centered, boldface font. Only the title should be CAPITALIZED (except italicized *Scientific namesii*).**
- Double-space after your "institution name" to begin the body of your text. It should look like this:

USE OF DUCT TAPE IN THE HUSBANDRY OF *Genus species* AT FISHLAND

Jill Fishhead, Senior Aquarist jfishhead@fishstinking.com

Fishland of South Dakota, 1 Stinking Desert Highway, Badlands, SD, USA

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- Use bullets or numbers to make lists. It is easier to reformat these later if needed.

Short Contributions ("Ichthyological Notes")

These include any articles, observations, or points of interest that are about a page or less in length. A brief bold faced and capitalized title should be centered, the body text should be formatted as above, and **author and affiliation should be placed at the end of the piece** with the left end of each bolded line right of the center of the page. Reformatting that must be done by the editor may reduce a shorter "main" article to a note, or may bump a note up to main article status.

Reviews, abstracts, translations (with proper permissions) and bibliographies are welcome. Humor, apocrypha, and serious technical articles are equally appreciated.