## 10<sup>th</sup> INTERNATIONAL AQUARIUM CONGRESS FUKUSHIMA 2018

Theme: Think about Our Future on the Water Planet Date: 2018. Novenber. 7-10 Venue: Onahama Fish Market

# PROCEEDINGS





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#### Keynote Speech-1 Pollution of the Water Planet

Introduction of Practical Technologies for Reduction of Micro Plastic Pollution by Injection Molded Products Made of Biodegradable Plastic Resin, Poly Lactic Acid (PLA)



Michio Komatsu Master of Monodzukuri (Manufacturing), President of Komatsu & Associates

Marine litter from petroleum-based plastic product causes micro plastic pollution in the world. To reduce its pollution, plant-based biodegradable plastic product is considered as one of the practical solution. By the way of replacing petroleum-based plastic products with PLA, we could reduce the total volume of waste changing to micro plastic. Biodegradation is a chemical process during which microorganisms that are available in the environment convert materials into natural substances such as water, CO<sub>2</sub>, and compost. The process of biodegradation depends on the surrounding environmental conditions, on the material and on the products. According to several estimates, about 80 % of the litters found in the marine environment comes from land-based activities. The source of marine litter is not necessarily limited to human activities along the seashore. Even when disposed of on land, rivers, floods and wind carry the litter to the sea. The original materials of PLA are just starch and lactobacillus. Lactobacillus change starch to lactic acid by fermentation. Lactic acid is changed to PLA thermoplastic resin by chemical synthetic process. PLA is available for injection molding, blow molding or sheet process. The waste of PLA product may bury in the ground, then PLA is biodegraded by microbe. Also in the ocean, PLA is biodegraded by microbe in seawater too. By biodegradation, PLA changes into just CO<sub>2</sub> and H<sub>2</sub>O. By photosynthesis of plant, CO<sub>2</sub> and H<sub>2</sub>O changes into starch again. By composting process, PLA will be biodegraded around 2 weeks to 6 month depends on the product shape. Biodegradation in seawater is so slower than land filling as composting due to smaller number of microbe, or lower temperature than land. But, Biodegradable plastic resin will be composed in seawater finally. PLA had 3 weak points, high material cost, poor heat resistance, up to around 70 °C and bad fluidity by injection molding. Caused by these reasons, PLA products were limited as thin sheet products by vacuum molding. They didn't have enough rigidity so that deforms easily. For promotion of wider adoption of PLA products, I have developed 4 injection molding technologies. The products doesn't deform easily, with high value and quality and possible to match the market cost.1st technology is for heat resistant PLA injection molding method. 2nd technology is for heat resistant PLA heat insulated product by nitrogen super critical micro cellular injection molding method. 3rd technology is thin wall PLA cup CO<sub>2</sub> super critical micro cellular injection molding method for mass production. 4th technology is PLA product containing natural cellulose fiber by nitrogen super critical micro cellular injection molding method. These technologies are expected for application of single-use tableware, cutlery, food package, medical package and fisheries, also durable tableware, toy, agriculture, gardening and civil engineering. In conclusion, injection molding is brand new way to produce PLA application products. PLA products are effective to reduce micro plastics, but biodegrading process should be in compost or landfill. It is necessary to cooperate all field, science, government, engineering, media and consumers for reducing micro plastic in the world.

## Sea Turtles in the Mediterranean: A Dangerous Journey for Their Survival



Flegra Bentivegna Former Director of the Aquarium and the Scientific Museum of the Dohrn Zoological Station of Naples

Mediterranean Sea turtles constantly "sail" in hazardous waters seriously threatening their survival. The Mediterranean is a small sea, very similar to a big salty lake, as well as a serious threat to the survival of marine turtles due to its physical and hydrographic characteristics and the presence of a high number of Countries, really diverse from each other, along its coasts.

Out of the three species of sea turtles populating the Mediterranean (Caretta caretta, Chelonia mydas and Dermochelys coriacea), the first two species evolved local populations and are demographically independent from their Atlantic ancestors, that implies their major vulnerability compared to those in the neighborhood Atlantic. The pressure of human activities, as bycatch, marine pollution, and in particular marine litter, as well as the use of nesting beaches for recreational activities and climate change have severely impacted marine turtles and therefore immediate actions are required to recover both the biological populations and their habitats.

The current conservation efforts cannot be successful whether based only on laws and regulations. They should be mainstreamed in a common shared strategy going well beyond the socio-economic, cultural and ethnic Mediterranean barriers.

Pending the achievments of the said political targets, in order to significantly reduce the mortality of marine turtles, several rescue centers for the cure and rehabilitation of these animals have been set up in strategic points of the Mediterranean.

Further, a number of Aquarium have also included the cure and rehabilitation of sea turtles among their conservation activities for endangered species, to foster the increasing of wild populations and, at the same time, allow people to have a closer look on these endangered species by raising awareness on their crucial role in our environment.

Sea Turtles are charismatic animals attracting the attention of both scientists and generic audience. These species play important roles in the ecosystems in which they live, although much is still unknown on the said roles cause populations currently are seriously depleted.

## Samurai and Falconer



Takahiro Yamana Chief Priest of the Ohkunitama Shinto Shrine

The art of falconry was introduced to Japan from the kingdom of Baekje of Korea at around the first half of the 5th century, during the reign of the 16th emperor Nintoku (current Emperor Akihito is the 125th emperor of Japan.) Since then up until around the 14th century, falconry was occupied by members of the Imperial Family and aristocrats.

The "Takagyou" texts depicting advanced falconry techniques were brought over from China (Tang dynasty,) but in the beginning of the 9th century, the 52nd emperor Saga created his own set of texts named "Shinshuu Takakyo" that took into consideration Japan's natural environment.

The concept of animism has been practiced in Japan since ancient times, with people recognizing that spirits existed within nature and animals and believing in the reincarnation of life. For example, they believed that the souls of the dead ascended to heaven (Takama-ga-hara,) carried by swans and cranes. Falconry was practiced to summon the souls of familiar faces and pray for them to bring happiness to this world.

During New Year events, aristocrats enjoyed performing rituals involving flying their falcons and binding birds of prey with branches of autumn leaves and plums while taking a walk.

These rituals were based on the belief that the spirits descended down to earth from the heavens during the New Year to provide peace and prosperity.

Around the 14th century, the political force of Japan had shifted to the hands of the samurai class, but falconry continued to thrive among shoguns and powerful warlords. Since the 8th century, Japanese have believed in both Shintoism and Buddhism. As "do not kill" is the first precept of Buddhism, falconry was forbidden to engage in. However, a compromise was made at Suwa Shrine (Nagano Prefecture,) a location known for its hunting rituals.

It was believed that the fish and birds that were killed would be able to rest in peace by being eaten by people. The reasoning behind this was that as long as those people kept a compassionate heart and made efforts to live their ideal lives, the fish and bird would eventually be saved.

These kinds of beliefs continued to spread until the mid-19th century. The falcons used for falconry were revered for giving the lives of animals inhabiting the mountains to people, and were not seen as committing the sin of killing.

In the 16th century, Oda Nobunaga and Toyotomi Hideyoshi unified Japan, but these two men also developed falconry on a grand scale by securing skilled falcons and performing rituals that consisted of offering captured game to the Emperor.

Tokugawa Ieyasu established the Edo Bafuku (also known as the Tokugawa Shogunate) during the early 17th century, and as a fan of falcons by birth, he completely monopolized all of the falcons within the country. On top of that, in order to keep falcon nests safe, he protected the forests and claimed the areas surrounding Edo where migratory birds flocked as areas where falconry was practiced.

Ieyasu was deified after his death, and a portrait of a falcon hangs at his mausoleum in Nikko Toshogu Shrine.

However, with the end of the Edo Bafuku (1867,) it brought an end to the practice of traditional falconry.

## Looking Back the Research Activities for Technical Improvement on Aquarium Display



Hiroshi Kabasawa Former Director of Keikyu Aburatsubo Marine Park

The contents of this presentation consist of two subjects: "Mechanical devices and instruments for aquarium display on the sensory biology of fishes and their environmental factors" and "Circadian rhythms in locomotor activity of the hagfishes".

The founding director, late Dr. Suyehiro maintained that a composition of aquarium display should be planned not only to collect rare fishes, but also to develop applications of experimental physiology of fish, i.e., sensitive perception of fish. Since the physiology treated rather endogenous phenomena of living fishes, it was hard to develop the systems to be applied for aquarium display. The sensory responses of fish include being on sight, hearing, smell, taste, and electricity. In the initial step, I had to make up the waterproof transducers both for generating ambient stimuli and for recording the reaction of fish in response to the stimuli. Some fundamental experiments were carried out and the effective devices were redesigned and drafted to familiarize my staffs with the use for educational interests. After the experiments had been repeatedly carried out, some successful results were seasonally exhibited to the public with some failed results. Throughout all the experiments, histological slide preparations were made for additional details in reference to aquarium display.

I have had some chances to collaborate my work with anatomists, medical doctors and an electrical engineer where I had learned valuable things. One of them was another subject in this presentation: "Circadian rhythms in locomotor activity of the hagfishes" that had been conducted at Misaki Marine Biological Station (MMBS), University of Tokyo during 1980-1998. This was my own personal study away from the aquarium exhibition. Using two species of hagfish, Eptatretus burgeri, and Paramyxine atami (=Eptatretus atami), a study of a relationship between the external light-dark conditions and locomotor activity of the animals were conducted using various methods and consequently excellent results were obtained. The hagfish showed a clear locomotor activity rhythm in the dark period entraining to the external 12:00 light and 12:00 dark (12L:12D) cycle. In addition, the animal displayed a distinct free-running rhythm in continuous darkness (DD).

The brain of the hagfish was studied to locate its circadian pacemaker. The suprachiasmatic nucleus (SCN) in the hypothalamus and the pineal gland in vertebrates are possible locations of circadian pacemaker. However, in E. burgeri, one of the most primitive vertebrates, neither a pineal gland nor the SCN has been detected in its brain. The characteristic locomotor activity rhythms were lost in the animal lacking the anterior part of the hypothalamus that includes the preoptic nucleus (PON). Incisions in front of the PON did not affect the rhythm, whereas incisions behind the PON caused the animals to lose their rhythms. Destruction of the PON with a high-frequency lesion generator also caused a loss of rhythms. These findings indicated that a candidate location of the circadian pacemaker in the hagfish might lie in the PON. By looking back on 50-year research work as described above, I was actually aware that modern excessive developments of science and technology would have rapidly estranged the human from wild animals. However, both are "genuine animals" and have coexisted together on the earth. Comparative anatomy and phylogenetic taxonomy tell us that they are all members of vertebrates. The study of the hagfish impressed me that aquariums would be one of the most suitable facilities for a long-term research work especially on ethological activities in captive aquatic animals.

## **Challenge to Diverse Aquarium Education**



Koji Takada Professor of Fukuyama University

I worked in aquariums for nearly 40 years from 1976 to 2015. Since 1980, I have placed "Dissemination of Education" at the center of my work, taking advantage of the curator's qualification. Moreover, after retiring from my aquarium job, I have been studying Museum Education at the aquarium of the University of Fukuyama. It's not an exaggeration to say that most of my work life has been dedicated to aquarium education. It universally recognized that the role of education is extremely important for zoos and aquariums today, and various activities are conducted in many facilities and in the field.

On the other hand, when we look at practical programs, we focus on the dissemination of environmental protection, wildlife conservation, and sustainable fisheries through biological, natural, marine, and environmental education. However, I have been strongly convinced since the beginning of my career in this area that aquariums cannot achieve their educational purposes/goals in such a narrow sphere.

Therefore, I worked on activities to achieve various educational purposes in a broader field. For example, we collaborated with humanities museums, such as history, art, folklore, and museums of ethnology, with social educational institutions such as libraries and community centers, and with kindergartens and the elderly welfare facilities. Educational cooperation with schools utilized information technology called IT and ICT. We also collaborated with business schools such as schools focused in architecture and Art design, with special support schools for children with developmental disabilities, and with local manufactural and agricultural industries. The target of the education, the purpose of the learning, and their achievement goals were very diverse. In school education, we have been attempting to collaborate not only with science-related subjects but also with other subjects such as Japanese Language, Mathematics, Sociology, Physical Education, Music, and Home Economics, in order to learn comprehensively.

Among various creatures, humans are animals that have evolved to demonstrate their "ability to learn" the most. We should consider aquariums as holistic educational institutions useful for all kinds of learning opportunities. As the factors that may threaten the existence of aquariums, such as global environmental problems, issues of wildlife conservation and endangered species, and different values for animal husbandry, are diversified, aquariums must function for all types of learning. In addition, the aquarium should not only be a place for education but also be a public place for humans to improve themselves by gathering and communicating with others.



## Fishery: A Relation Between Human Being and Sea

Hisashi Kurokura Professor Emeritus of the University of Tokyo

When we visit botanical garden, we are sometimes stimulated appetite by flavor of fruit and flower. Living organisms inspire our internal reactions. Most of such reactions are emotional such as beauty, love, fear and appetite. However, some reactions are more rational such as surprise, intelligent curiosity, old memory and so on. Nowadays, many people make contacts with living organisms in zoo, aquarium and botanical garden. When we compare aquarium with zoo and botanical garden, stimulation we get in aquarium is less emotional and more rational than those from zoo and aquarium. It may be because of discontinuation of space. Human being and fish are separated by glass wall in aquarium, and human beings contact with living organisms only by eyes. Touch, smell, and sound cannot be main tool for contact. Generally, physical contact is prohibited in aquarium. Of course, we cannot eat fish in aquarium. Aquarium satisfies intellectual curiosity in morphology, taxonomy, ecology, behavioristics and environmental sciences. However, several people are stimulated their appetite, and people who love angling can feel movement of muscle of fish, when they look aquatic organisms. Human being can superimpose actual scene with their memory and relive their previous experiences. Assimilation of scientific knowledge and aesthesis in our daily life is important function of zoo, botanical garden and aquarium. We should accept people who are stimulated appetite in aquarium. Modern aquarium should not be dragon palace in sea and it must connect scientific knowledge with life of people. Fisheries and aquaculture are important contact point of aquatic organisms and human being in our society. Introduction of actual status and history of fisheries and aquaculture enhance understanding of aquatic organisms and environment including relation with human being. When we go back to river head of relation between human being and aquatic organisms, there exist aquatic organisms as food. Securement of foods was most important work for ancient people, Livestock were domesticated as food at first. It was a sophistication of hunting. It is said that flooding fisheries was one of the origin of fisheries. Fish were left in horseshoe lake after flooding. Catching such fish is called flooding fisheries. Several people started feeding to the fish in horseshoe lake. There are various origins of aquaculture, though this is a typical case of start of aquaculture. Human beings have accumulated knowledges by farming of aquatic organisms. Fisheries have also provided many biological knowledges to us, though knowledge by aquaculture include detailed information obtained by direct observation by rearing such as metabolism and maturation physiology. Development of fisheries and aquaculture technology means changes of the relation between aquatic environment and ecosystem and human society. Human society have been changed by the development of fisheries and technology.

People in Taichi forms institutional whaling group called "Kuzira-gumi" with development of net whaling method in Edo era in Japan. Development of largescale set net enabled community operation fisheries. Sometimes development of technology provided tragedies to our society. Trolling and purse sein was introduced to Japan in Meiji era. New technologies often made serious social conflict in Japan. Displays of history of fisheries and aquaculture deepen our understanding of aquatic ecosystem and its relation to human society.



## Healthy Mountains Make Healthy Oceans

Masaru Asaishi Former Professor of Tokyo City University

The mountains and the ocean are connected. Natural dams can be found in forests, keeping a constant amount of river water and controlling floodwaters. According to Kesennuma-based oyster fisherman Shigeatsu Hatakeyama, important movements are being conducted in order to increase the number of fish and shellfish by supplying nutrients to rivers and oceans that can increase the amount of phytoplankton and seaweed.

Here I will be explaining how the environmental aquarium initiative, which was born from the design processes of the Tropical Dream Center, Nagasaki Bio Park and Tama Zoological Park Butterfly Garden, came to fruition at Aquamarine Fukushima.

Tropical Dream Center was built to appear as ruins inside Ocean Expo Park. The subtropical climate provides an environment that is suitable for tropical plants to grow, but the site is also heavily affected by winter monsoons, sea breeze and typhoons. In order to prevent these, the enclosed windproof area was constructed with large arc-shaped brick walls, greenhouses and circular buildings to enable visitors to enjoy looking at the beautiful flowers, fruits and trees inside a space that resembles ancient ruins.

Nagasaki Bio Park is an integrated exhibitions style zoo where the animals are released from their cages so that they can roam free so that visitors can witness them in their natural habitats. In one section of the zoo, buildings such as the tropical and arctic areas have been carefully inserted, and by establishing an intimate relationship between the nature of the mountains and these buildings, a simple yet relaxing environment where time flows slowly and visitors can enjoy the changing of the seasons was successfully created. Tama Zoological Park Butterfly Garden was built to resemble a giant butterfly resting its wings inside a giant forest of Sawtooth oak and Konara oak. Using the difference in elevation of the valleys, the large greenhouse is a dynamic space that is integrated with the sea of trees that expands outside. Visitors can enjoy walking along the sloped path and observing various insects living in their natural habitats, including the butterflies flying from one flower to another from the top of the trees.

With the construction of manatee caves, ponds where aquatic insects can be observed and fish such as pirarucu and arowana can swim around, I started to want to build an aquarium that closely resembled the global environment that could connect the terrestrial environment with the underwater world. Using an aquarium competition held in Fukushima Prefecture as an opportunity, we decided to create an ecosystem that would connect the terrestrial surface where the sun shines, the wind blows, flowers bloom and lush greenery grows, with the underwater world at the water's edge. And all of this was to be gently enclosed in a coating of glass. This is how our vision for a unique and innovative aquarium came to become a reality. On March 11, 2011, the tsunami caused almost all of the fish to die, and the rest of the sea creatures were evacuated to other aquariums. In response to the many voices of support that "restoring the aquarium would bring joy to people," the aquarium was re-opened four months later. The spotted seal that helped bring back children to the aquarium was given the name "Kibou," which is the Japanese word for "hope."

#### Aquariums: Economic Engines for Our Communities but How Do We Help the Planet also?



Suzanne M. Gendron Executive Director of Zoological Operations and Conservation in Ocean Park Hong Kong

Zoos and aquariums have traditionally been animal focused living collections, museum-like even in our terminology, such as curating our collections. Today, a number of zoological facilities are no longer animal only but have added rides, such as roller coasters, carousels and zip lines, as well as catering services, and even schools to their campuses. When the Hong Kong Jockey Club first conceived of Ocean Park in 1975, it was to be a place of education and entertainment for the people of Hong Kong with a focus on oceanic animals. A saltwater aquarium, a whale and dolphin performance pool and a sea lion and seal exhibit were the main attractions at the new Park when it opened in 1977 with 1.85 million visitors. In 1984, after six years of deficit financial results, the Park added roller coasters, flume rides and a new gate, Water World, thus becoming one of the first hybrid zoological parks. Visitor numbers began to stabilise and a surplus was realised for the first year since opening. With the Park on firmer ground financially, the Hong Kong Jockey Club donated the Park to the Hong Kong Government in 1987. It became a not-for-profit, statutory body under the Hong Kong Government but was independently managed and financed under the leadership of a Chief Executive that answered to a Hong Kong Government appointed Board. Another economic challenge arrived with the Asian economic crisis in 1997. The Hong Kong dollar was pegged to the USA dollar, thus making Hong Kong a very expensive city for Asians to visit. Ocean Park visitor numbers fell from 3.7 million visitors to 2.8 million visitors after the Handover in 1997, resulting in deficits 5 of the next 6 years until after Hong Kong recovered from the Severe Acute Respiratory Syndrome (SARS) outbreak in the second half of 2003. The announcement in 1999 that Disneyland would build a park in Hong Kong further raised concerns that Ocean Park would not survive the economic downturns, SARS and Disneyland's arrival in Hong Kong. The Hong Kong Government appointed Dr. Allan Zeman, well respected entrepreneur, to chair Ocean Park's Board. He hired Mr. Tom Mehrmann, formerly of Knott's Berry Farm, Six Flags Animal Kingdom and Warner Bros. Movie World Madrid as the Chief Executive to lead the team to develop a strategy to counter Disney's arrival and transform Ocean Park into a global zoological theme park. Visitor numbers recovered from a low of 2.8 million in 1998-1999, to 7.7 million in 2012/2013 after a period of rapid expansion and growth within the Park. This talk will share the variety of economic models under which aquariums are run and share the strategy and tactics that took Ocean Park from a Park with 2.8 million visitors and a deficit of HK\$80.5 million (US\$10.3 million) in the fiscal year of 1998/1999 to the visitor attendance of 7.7 million and surplus of HK\$127.3 million in fiscal year of 2012/2013. Lesson learned and future trends will also be explored.

## Forty Years of Ingenuity at the Sunshine Aquarium

Tsuneo Arahata Former Director of Sunshine Aquarium, Japan

ABSTRACT: Sunshine aquarium opened in 1978. It attracted attention by being installed in a skyscraper 40 m above the ground.

40 years have passed since the opening, but during that time, we have tried and errored in order to continue the aquarium, and have changed in various ways.

I would like to talk about various ideas that the aquarium has had.

#### **INTRODUCTION**

A major feature of the Sunshine Aquarium is that it was installed on the roof of a skyscraper 40 meters above the ground in 1978. (Fig.1.)

It was a revolutionary idea. It was seen by the world with surprising eyes, but even more surprisingly, though, 40 years have passed since this establishment. I do not see other examples in the world.

At the time of the installation, many people thought the idea was absolutely impossible. But Horike believed in his idea and as the first director of the Sunshine Aquarium he gathered the building technology along with the equipment and materials needed.



Fig. 1. Sunshine City complex

## **FULL TEXT**

Initially, even though searching everywhere, we could not find a large acrylic tank especially one with a strong enough reinforcement frame with a 15 meters frontage. (Fig.2.)

Also, with Acrylics, we made a special "Diamond Cut" and "Wine Glass" shaped tank that would be good for living things. (Fig.3.)



Fig.2.

Fig.3.

The urban environment without the surroundings, securing the seawater was more difficult than collecting living things.

The problem of securing seawater was solved by systematization of the seawater supply and development of filtration equipment to purify the water.

The seawater is collected off Hachijojima from the ballast of the ship and brought to the port of Tokyo Bay. (Fig.4.) We transfer 24 tons from the ship to the tank truck, and land transport it to the aquarium.

The seawater is transferred to a basement water reservoir. This underground reservoir is waterproofed prevent groundwater to contamination and can store 350 tons.

The seawater in the underground reservoir is transferred to an elevated tank on the roof 60 meters above the ground using a huge pump. This elevated tank is 10 tons in size.

From the elevated storage tank, it is a system that can be supplied by a natural waterfall through pipes to each tank. (Fig.5)



The piping material used in the aquarium is lightweight and rust-free. Polyvinyl chloride is used.

The use of a Filter was indispensable in the high-rise aquarium where we can not use sea water abundantly. А pressure type rapid filter demonstrates great power in a narrow aquarium.

The advantage of having a fast filtration speed and a small facility area is that it matched the highrise aquarium.

When we first opened the aquarium, the visitors just stared and seemed bored. So we released and displayed:

An inventory of all the animals in the Tank with pictures.

We described the special behavior of each fish.

And we started performances by divers feeding fish.

We showed the different reactions of the fish like pulling, popping and swimming through a ring with conditioning lights. (Fig.6.7.)

We started using a female guide to introduce fish habits and ecology. These features were popular.





Fig.6. Popping

Fig.7. Through a ring

The show using sea lions was released for the purpose of introducing the characteristics and habits of animals while showing various tricks.

The animals that appeared were South American Sea Lion (*Otaria flavescens*), and California Sea Lion (*Zalophus californianus*). (Fig.8.)

There are two ways to organize the show, and it can be divided into a story show introducing it in a comical theater style, and a sports show introducing various actions of the sea lions. (Fig.9.)



Fig.8.

Fig.9.

Both of them introduced the high level of exercise and ecology functions of sea lions, and the visitors were surprised.

The performance by a diver is a fish feeding show.

It is a show with a female diver in a bathing suit feeding the fish in a tank that displays fish living in the temperate zone, centering on the White Trevally (*Pseudocaranx dente*). (Fig.10.)

This show introduces fish while showing the fishes unique movements and cute feeding scenes. (Fig.11.)



Fig.10.



Fig.11.

The creatures on display are collected from all over the world. In anticipation of the times, I always tried to exhibit precious collections. I will introduce some of exhibited organisms.

#### 5 kinds of Garden Eel

(Heteroconger hassi, Heteroconger polyzona, Gorgasia japonica, Gorgasia taiannensis, Gorgasia preclara).





H.hassi

H.polyzona





G.japonica

G.preclara

6 kinds of Lungfish

Australian Lungfish (*Neoceratodus forsteri*), South American Lungfish (*Lepidosiren paradoxa*), African Lungfish (*Protopterus aethiopicus, Protopterus annectens, Protopterus dolloi, Protopterus amphibious*).





N.forsteri

P.aethiopicus



P.dolloi



P.amphibious

Sunfish (Mola mola), Sea Angel (Clione limacina), Sea Otter (Enhydra Lutris), Weedy Sea-Dragon (Phyllopteryx taeniolatus), Mexican Salamander (Ambystoma mexicanum).



M.mola





E.lutris



P.taeniolatus

C.limacina

#### And so on.

However, as the times have progressed, organisms around the world can be seen in any aquarium. Creatures that could only be seen in specific places became general exhibits. Just to display only the creature, the management of the aquarium had to become more creative.

After that the exhibition method shifted to an ecological exhibition aiming at simultaneously displaying various creatures and the environment where they live.

A tank which reproduced the natural environment which was cut out of one frame of natural scenery was made. (Fig.12.13.14.)



Fig.12. World's three largest reefs.



Fig.13. Amazon basin

Fig.14. Mangrove

The creatures that played the leading role in aquariums reproducing the natural environments were not only fish, but also included small mammals, Family of Aves, Family of Reptilia, Family of Amphibia, and so on.

34 years since the opening of the building, The Sunshine Aquarium will be renewed in full.

The reason for the renewal was that we felt that the facilities are getting old and we asked ideas for changes from customers for a new exhibition.

A new attempt was an idea that mainstreamed a "behavior exhibition". Always being able to see actions that living things do not in normal reality really show.

For example, The Longheaded Eagle Ray (Aetobatus narutobiei) is a scene that explores baits by digging in the sand at the bottom of the ocean. (Fig.15.16.)



Fig.15.

Fig.16.

Next is a scene where large fish interrupt and attack the herd of Japanese Sardines!(Sardinops melanostictus). (Fig.17.)

Next is a special tank that allows you to observe the California Sea Lion from directly beneath and realize the wonder of its swimming ability. (Fig.18.)



Fig.18.

In addition, a special exhibition hall held at the venue next to the main building was variously devised. I will introduce two examples.

Collaboration with artists.

It is a contrasting exhibition of numerous works which have Goldfish (Carassius auratus auratus) made with acrylics as the main character alongside genuine living Goldfish.

The contrast between the goldfish created with acrylics that captured the instant and the lively goldfish with fluid movement was an interesting project. (Fig.19.20.)



Fig.19.

Fig.20.

Collaboration with the author.

An interesting and curious introduction from an amateur's eye by the author. And, as seen from curators, there are introductions that are serious and uninteresting. Visitors were able to enjoy the commentary on exhibited organisms in two ways. (Fig.21.22.)



 Ended
 Image: State S

Fig.21.

SUMMARY

When thinking about long-term management, the attitude that always introduces new things is necessary for the operation of the aquarium. While having the difficulty of the aquarium on the higher floor, we have used much devised ingenuity.



Sunshine Lagoon



Underwater Performance

How to respond to customer's needs and how to express it is largely due to the results of curators and the keepers thoughts and efforts.

It is a great driving force for curators and caretakers to develop a workable environment that will exert their power as a mediator between customers and exhibited organisms as well as growing the aquarium as an attractive facility.

In the future Sunshine Aquarium will continue to develop and provide "Discoveries that will inspire you "as an aquarium closer to the living things.

We wait to welcome you at the Sunshine Aquarium.



Penguins in the Sky

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## **Ctenophore Culture at the Monterey Bay Aquarium**

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**ABSTRACT:** The Monterey Bay Aquarium has been displaying and culturing gelatinous zooplankton since the late 1980's. Successes in many areas of jellyfish husbandry have led to the development of numerous temporary exhibits, as well the installation of a permanent jellyfish gallery in 1996. Since that time newly discovered culture techniques have greatly expanded the capacity for sustainable collections, as well the diverse array of species exhibited. While a few species of comb jellies (ctenophores) have been displayed opportunistically and irregularly, the only successes in culture has been limited to cnidarians, both hydrozoans and scyphozoans. Ctenophores, however have proven to be outstanding display animals and have the capacity to dazzle aquarium visitors with stunning displays of movement and kaleidoscope color patterns. Until recently, the Monterey Bay Aquarium's ability to culture comb jellies has remained unreachable. After decades of effort, recent breakthroughs in husbandry techniques have led to the successful culture and display of five different species of ctenophores, including the most recent success with the spotted comb jelly, *Leucothea pulchra*. The purpose of this presentation is to share the elements of collaboration, the steps in trial and error, and the key technical components that led to the successful culture and subsequent display of five species of comb jelly.

#### The Beginning of Jellyfish Culture at MBA

The exhibition and culturing of jellyfish has been an important part of the history of the Monterey Bay Aquarium (MBA). MBA's jelly program started with a moon jelly exhibit in the late 1980's after receiving polyps and husbandry advice from Mr. Yoshitaka Abe, who was at the time a curator at the Ueno Zoo. Success with moon jellies led the husbandry staff to experiment with the numerous jellyfish species which are local to Monterey Bay. To accommodate the fragility of these jellies, MBA designers worked with Dr. William Hamner of UCLA to adapt his planktonkreisel design for use in an aquarium setting. In addition, figuring out how to culture jellies was essential to creating long-term exhibitions. While jellies can be collected from the wild for short-term displays, many species only appear seasonally and in small numbers, and they tend not to live very long. Therefore, laboratory culturing is necessary to maintain well-stocked exhibits of numerous species for longer periods of time. The new kreisel tanks and focused research efforts led to many breakthroughs in jelly husbandry, including the first polyp culture of the Purple Stripe jelly, Chrysaora colorata, and its subsequent reclassification from the genus Pelagia to Chrysaora (Gershwin & Collins 2002).

There was some concern within MBA that jellies would be not be popular with the aquarium visitors, but numerous successful showings in temporary exhibitions demonstrated that the jellies were popular and enhanced the guest experience. The husbandry staff's expanding knowledge of jelly husbandry along with the new popularity of jellies led to the first permanent jellyfish gallery in the United States. In 1996, MBA opened its new Outer Bay Wing, which featured the Drifters Gallery, a representation of gelatinous life in Monterey Bay's pelagic waters. The Drifters Gallery has remained largely unchanged in the past few decades, yet it remains one of the most popular areas with aquarium visitors. The sustained popularity of jellyfish has led MBA to feature jellies from around the world in the temporary exhibitions Planet of the Jellies (1992-1993), Jellies: Living Art (2002-2008), and The Jellies Experience (2011-2015), and over 40 species of jellies have been cultured and exhibited at MBA over the years.

Some of the most popular iellies at MBA are the beautiful and delicate ctenophores. Known commonly as comb jellies, they are not related to cnidarian jellyfish as they lack stinging cells in favor of sticky colloblast cells on their tentacles (Pang & Martindale 2008b). They are prolific predators that can eat up to ten times their body weight per day (Reeve et al. 1978), and they are the largest animal that uses ciliary action for locomotion (Tamm 2014). These cilia, the combs, can create a hypnotizing prismatic rainbow effect visitors which leaves aquarium in awe. Ctenophores are possibly the oldest metazoan group on the planet (Dunn et al. 2008), and they are simultaneous hermaphrodites, meaning they can produce egg and sperm at the same time (Baker & Nevertheless, maintaining Reeve 1974). а ctenophore exhibition has always been challenging. Knowledge of how to culture pelagic ctenophores is limited to a few universities and aquariums working with Mnemiopsis leidvi, as well as the Kamo Aquarium and the Kujukushima Aquarium which have both succeeded in culturing Bolinopsis mikado and Beroe cucumis. In addition, a few institutions

have had success culturing benthic ctenophores. To our knowledge, no other ctenophores are being cultured, and no protocols for the culturing of successive generations of ctenophores are available in the literature. Therefore, it has always been necessary either to collect or buy the animals for exhibition, and that was only possible when they would appear seasonally. In order to maintain a ctenophore exhibit year-round, the development of reliable laboratory culturing protocols was necessary.

#### Mnemiopsis leidyi culture

In 2014, jelly aquarists at the Monterey Bay Aquarium developed a protocol for culturing successive generations of the warm water lobate comb jelly, *Mnemiopsis leidyi*, for display in the temporary exhibition, "The Jellies Experience." Aquarists had attempted to culture *M. leidyi* in previous years but were met with limited success. Ultimately, collaboration with other institutions and the development of new husbandry techniques were key to success.

M. leidyi is a warm water, cosmopolitan lobate ctenophore whose life cycle includes a pelagic juvenile stage commonly referred to as a cydippid (Fig. 1). These jellies can be collected from the wild and immediately spawned utilizing photoperiod as a cue, resulting in the production of thousands of fertilized embryos (Baker & Reeve 1974, Pang & Martindale 2008b). The fertilized embryos quickly hatch and form swarms of tiny, free-swimming cydippids. Because of this species' rapid development and translucent appearance, M. leidyi has been used extensively in embryology studies (Pang & Martindale 2008a). Consequently there exist numerous protocols describing how to spawn wild-caught M. leidvi in laboratory conditions (Baker & Reeve 1974, Pang & Martindale 2008b). The process of growing cydippid larvae up to the adult form for display in public aquaria, however, has been poorly studied and documented. Protocols for raising *M. leidyi* to adulthood are not available in the literature.



Fig. 1. Adult *Mnemiopsis leidyi* pictured on the left. On the right, the juvenile form of *M. leidyi* referred to as a cydippid.

Under natural conditions, wild *M. leidyi* are triggered to spawn five to eight hours after nightfall

(Baker & Reeve 1974, Pang & Martindale 2008b), so culture attempts always involve placing robust individuals in complete darkness for at least eight hours. Various techniques have been tried, and different setups included dishes in water baths, indirect flow setups, large cylinders with artificial lighting, and small standalone tanks. Various temperature and salinity parameters were tried in each of these set-ups. Despite many efforts, these methods resulted in limited success. The fertilized embryos were very few and very weak, generally not living past seven days. However, through collaboration with the University of Miami's professor Bill Browne, the aquarist team learned that one of the most important aspects of ctenophore culturing is broodstock nutrition. Dr. Browne advised that feeding the parent generation larval fish daily caused them to spawn hundreds of healthy embryos that were strong and grew fast, similar to what has been observed in freshly collected M. leidyi from the wild. Previously, the adults at MBA had only been fed Artemia salina nauplii, which did not provide them with adequate nutrition for spawning.

With a new understanding of the importance of broodstock nutrition, the jelly culture team went forward with a simple, straightforward culturing method. To spawn M. leidyi, one to four mature, well-fed adults were placed into ten-liter tanks with lids to limit evaporation. The tanks were filled 60% full with filtered seawater and dechlorinated fresh water mixed to a salinity of 28 ppt at 24°C. These water parameters were selected to mimic the conditions in which the adults were originally collected. The tanks were placed in total darkness for at least five hours, and if the spawning attempts were successful, tiny, sparkly eggs could be observed in the water column. The cydippid larvae began to hatch 24 hours after fertilization, and were observed immediately capturing and consuming prey items. The cydippids were fed rotifers daily, and sometimes once to twice-a-day depending on the speed of consumption.

The young cydippid larvae were too delicate to transfer in the early stages, so salinity was maintained and relative ammonia levels in the tank were reduced by adding additional 28 ppt water plus microalgae to enrich any remaining live foods. Four days after hatching, the cydippids were robust enough to endure a gentle transfer. They had doubled in size, needed more room to grow and needed an environment with reduced ammonia levels. To achieve this, the population was split into two identical ten-liter tanks. Using a glass dish, the delicate cydippids were transferred very slowly in order not to damage their delicate bodies. After the population had been split, a 20% water change was performed every other day until the cydippids were large enough to be moved to a more traditional jelly kreisel tank (two to three weeks).

Because of the knowledge gained by collaboration with Dr. Browne, larval fish were offered to the juvenile M. leidvi as soon as they had formed their lobes. This provided them with the nutrition and energy needed to spawn the next generation. Another benefit of using larval fish as a food item was the rapid tissue regeneration observed in individuals that had been damaged. By culturing both zebrafish (Danio rerio) and topsmelt (Atherinops affinis), the aquarists were able to give the growing ctenophores access to a high calorie diet which supported the production of hundreds of adult comb jellies over three generations. M. leidyi is bioluminescent and will readily luminesce when disturbed (Freeman & Reynolds 1973). This happens only when larval fish are fed daily, which demonstrates the importance of this food item to the overall health of ctenophores, including their ability to reproduce.

#### **Culturing of Northeast Pacific Ctenophores**

After successfully culturing Mnemiopsis leidyi, the aquarists were eager to try these new techniques on ctenophores local to Monterey Bay and the northeast Pacific Ocean. The next success came from working with the lobate ctenophore Bolinopsis infundibulum. B. infundibulum resembles M. leidyi, but grows larger and is much more fragile. For seawater conditions favorable to B. infundibulum, seawater was kept at full salinity (34 ppt) and the temperature was kept at 10°C. Since the parent generation was spawned immediately after collection from Monterey Bay, they were able to produce a large, robust F1 generation without needing supplemental feedings of larval fish. However, in order to culture the F2 generation and beyond, larval fish feedings were necessary for successful spawning and embryo development.

Concerns about the delicate nature of this species led to several new tank designs that allowed for a more gentle exchange of seawater. A cylindrical tank with a 20 micron bottom screen suspended in a larger tank was created to house the delicate cydippid larvae of *B. infundibulum*. Flow from a spray bar would indirectly contact the outside of the screen, allowing for passive seawater exchange (Fig. 2). This improvement helped to maintain good water quality without creating outward pressure on the screen, eliminating the need for frequent water changes and transfers, and further advancing the concept of a gentle environment.

*B. infundibulum* developed similarly to *M. leidyi*, but more slowly due to the colder seawater

temperature. When their lobes developed, the aquarists began offering fish fry and live mysid shrimp (*Mysidopsis bahia*). After two months, the adult *B. infundibulum* were transferred to a flow-through pseudokreisel, and *Artemia salina* nauplii were incorporated into their diet. These first generation *B. infundibulum* lasted from June 2015 until September 2017 and grew to over 8 cm in length. The MBA Jelly Team has since grown second generation *B. infundibulum* to adulthood.



Fig. 2. A cylindrical ctenophore rearing cylinder inside of a pseudokreisel. Seawater flow (red arrows) from a spray bar allows for passive seawater exchange through a 20 micron screen at the bottom of the cylinder.

#### **Double Cylinder Design**

The cylinder concept worked very well for Bolinopsis infundibulum, and the clear acrylic cylinder made observations much easier. To improve upon this design, a new, taller double cylinder system was invented, allowing for more uninterrupted vertical swimming. Instead of a cylinder within another larger tank, previously described for the *B. infundibulum* culture, the outer tank of this new double cylinder was reduced to a cylinder slightly larger than the inner one. Seawater flowed into the outer cylinder only, creating a passive water exchange through the 20 micron screen at the bottom of the inner cylinder. The seawater then overflowed over the edge of the outer cylinder and ran down the side onto a wet table. Rigid, perforated material, elevated the inner cylinder, allowing seawater to flow freely around the fine screen. An acrylic plate was glued to the bottom of the outer cylinder, creating a water-tight bottom (Fig. 3).

This new cylinder concept was utilized to culture *Pleurobrachia bachei*, a ctenophore local to the northeast Pacific Ocean (Fig. 3). In order to

spawn P. bachei, mature individuals were collected from Monterey Bay and identified by creamycolored eggs and sperm, developing beneath the comb rows. Mature P. bachei were placed into the double cylinder at 12.5°C and shrouded in complete darkness for approximately 16 hours. The next morning, within one hour of exposure to light, spawning occurred. The adult P. bachei were allowed to spawn for several hours and were then removed from the cylinder, leaving hundreds of fertilized embryos dispersed through the inner cylinder. P. bachei cydippids are approximately 100 microns along the oral-aboral axis after hatching, five times smaller than Mnemiopsis leidyi and B. infundibulum larvae. Therefore, a very small food item, the nauplii of the copepod Parvocalanus crassirostris (40-70 microns), were essential to the larvae's survival in their early stages of development. As they grew, rotifers and adult copepods became an essential component of the diet as well. After one month, the P. bachei had fully developed and were transferred into a flow-through kreisel or pseudokreisel where Artemia salina nauplii were incorporated into their diets. The MBA Jelly Team has cultured two generations of P. bachei using these methods and have since applied identical protocols to Hormiphora californensis, reaching F3 generation.



Fig. 3. Diagram of the double cylinder (left), and two adult *Pleurobrachia bachei* spawning inside the double cylinder (right).

#### The Spotted Comb Jelly, Leucothea pulchra

Leucothea pulchra is one of the most visibly striking ctenophore species with its hypnotic, undulating auricles and flexible orange papille that pepper its exterior (Fig. 4). L. pulchra has been displayed at the Monterey Bay Aquarium opportunistically, whenever aquarists were fortunate enough to collect them. With frequent collections, L. pulchra has been exhibited for stretches of up to six months at a time, but it was necessary to replace the animals monthly and consequently required much effort. The jelly aquarists were thrilled at the prospect of being able to start a reliable L. pulchra culture and share this enthralling species year round with guests, but first they had to scale up their double cylinder rearing system to house such a large ctenophore.

A two meter tall, 30 cm wide double cylinder system was constructed and filled with 17°C filtered seawater. In December 2017, four 20 cm long L. pulchra were collected from Monterey Bay and placed into this larger cylinder. Similar light deprivation methods were used, resulting in the production of hundreds of fertilized embryos the following day. When the cydippid larvae hatched, they were 500 microns along the oral-aboral axis. By 12 days post-hatch, tentacles were starting to retract, lobes were forming, and the unique, orange papillae were beginning to appear. By February 20th, 2018, the first cultured L. pulchra generation in the world was displayed in MBA's Drifters gallery. As of the time of this writing, over eight months since introduction onto exhibit, these animals remain on display and have grown to an impressive 15 cm in length. The jelly aquarists are currently growing the F2 generation using identical methods.



Fig. 4. Adult Spotted comb jelly, Leucothea pulchra.

## The Bloody Belly Comb Jelly, Lampocteis cruentiventer

Quality food items, uniquely designed tanks and team collaboration have proven to be crucial components of ctenophore culturing. Currently, the jelly aquarists are working with *Lampocteis cruentiventer* (the Bloody Belly comb jelly), a brilliantly red, lobate ctenophore found approximately 300 to 3000 meters deep in the Monterey Submarine Canyon in Monterey Bay and first described by scientists at the Monterey Bay Aquarium Research Institute (Harbison *et al.* 2001). Efforts continue to find variations on epipelagic ctenophore culturing protocols that can be applied to deep sea species of ctenophores. Traditional photoperiod cues have not proven to induce spawning in this species, likely an aspect of this species' deep sea life history. Also likely related to this species' living in "the deep" is what appears to be the acceptance of a wide range of food types. As with larval fish providing *Bolinopsis* infundibulum and Mnemiopsis leidyi with the nutritional energy to spawn in a laboratory setting, current thinking is that a species of gelatinous zooplankton, cultured in house, might meet a similar requirement for L. cruentiventer. Because so much energy has gone into ctenophore culture at the Monterey Bay Aquarium in the last four years, there are many species that are now available as food items. Hormiphora californensis has proven to be a preferred food item for L. cruentiventer and observations of L. cruentiventer's ability to regenerate lost tissue as well their increased longevity in captivity suggests that nutritional requirements are being met. L. cruentiventer inhabits the oxygen minimum zone (OMZ) where pH and oxygen are lower than the sunlit surface waters (Harbison et al. 2001). Current research and development efforts are being directed at developing L. cruentiventer life support systems that mimic these OMZ parameters. Though these efforts are in their infancy, OMZ conditions seem to benefit the longevity and gonad development of L. cruentiventer.

#### Conclusions

Every new success in the exploration of ctenophore culture has opened a door to future discovery: from the basic understanding of broodstock nutrition for Mnemiopsis leidvi and Bolinopsis infundibulum leading all the way to Hormiphora californensis being cultured as a food item for the elusive deep sea ctenophore, Lampocteis cruentiventer. Ongoing efforts will continue with the aim of expanding the list of cultured ctenophore species and evolving the necessary techniques and technologies. Not only are ctenophores exciting display organisms, inspiring conservation of the oceans for aquarium guests, but they are also important model organisms for future biomedical research and animal phylogeny studies. The breakthroughs in MBA ctenophore culture would not have been possible without collaboration with Dr. Bill Browne at the University of Miami and the scientists at the Monterey Bay Aquarium Research Institute These collaborations are rooted in the common goal of furthering our understanding of ctenophores and

their ecosystems, and the sharing of our individual contributions has led to our collective success in ctenophore culturing.

#### References

- BAKER, L.D., REEVE M.R.1974, Laboratory Culture of the Lobate Ctenophore *Mnemiopsis mccradyi* with Notes on Feeding and Fecundity, *Marine Biology*. **26**: 57-62
- BAKER, L.D., WALTER, M.A., IKEDA, T. 1978, Laboratory Studies of Ingestion and Food Utilization in Lobate and Tentaculate Ctenophores, *Limnology and Oceanography*. 23(4): 740-751
- DUNN, C.D., HEJNOL, A., MATUS, D.Q., PANG, K., BROWNE, W.E., SMITH, S.A., SEAVER, E., ROUSE, G.W., OBST, M., EDGECOMBE, G.D., SØRENSEN, M.V., HADDOCK S.H.D., SCHMIDT-RHAESA, A., OKUSU, A., KRISTENSEN, R.M., WHEELER, W.C., MARTINDALE, M.Q., GIRIBET, G. 2008, Broad phylogenomic sampling improves resolution of the animals tree of life, *Nature*. 452: 745-749.
- FREEMAN, G., REYNOLDS, G.T. 1973. The development of bioluminescence in the ctenophore Mnemiopsis leidyi, *Developmental Biology*. **31**(1): 61-100
- GERSHWIN, L., COLLINS, A. 2002, A preliminary phylogeny of Pelagiidae (Cnidaria, Scyphozoa), with new observations of Chrysaora colorata comb. nov., *Journal of Natural History*. **36**: 127–148
- HARBISON, G.R, MATSUMOTO, G.I., ROBISON B.H. 2001, *Lampocteis cruentiventer* gen. nov., sp. nov.: A new mesopelagic lobate ctenophore, representing the type of a new family (Class Tentaculata, Order Lobata, Family Lampoctenidae, fam. nov.), *Bulletin of Marine Science*. **68**(2):299-311
- PANG, K., MARTINDALE, M.Q. 2008a, Comb Jellies (Ctenophora): A Model for Basal Metazoan Evolution and Development, *Cold Spring Harb Protoc*; doi:10.1101/pdb.emo106
- PANG, K., MARTINDALE, M.Q. 2008b, *Mnemiopsis leidyi* Spawning and Embryo Collection, *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot5085

POWELL, D. C., EARLE, S. 2001, A Fascination for Fish: Adventures of an Underwater Pioneer. Berkeley: University of California Press

RASKOFF, K., SOMMER, F.A., HAMNER, W.M.,

CROSS, K.M. 2003, Collection and Culture Techniques for Gelatinous Zooplankton, *The Biological bulletin*. **204**: 68-80

TAMM, S.L. 2014, Cilia and the life of ctenophores, *Invertebrate Biology*. **133**(1):1-46

## The Greatest Fish Story of the Aquamarine Fukushima



Yoshitaka Abe Aquamarine Fukushima

Fig.1. Iron Coelacanth, worked by Atsushi HIBI welcome you to the Aquamarine Fukushima.

## INTRODUSTION TO THE EVOLUTIONARY STORY

My personal history for the aquarium started 1964 to the present. The story traced back to the Ueno Zoo Aquarium in the Ueno Zoological Gardens, the oldest zoo in Japan, managed by the Tokyo Metropolitan Government, where a new aquarium was opened to public in 1964 to share the exhibits of creatures of lower branches of the phylogenetic tree in the zoo.

1 engaged the new aquarium project before opening for the preparation. My job was to correct and maintain for the creatures on the lower branches of the phylogenetic tree in the 4 stories building, such as jellyfish.

There are so many tanks, pools and cages to cover the collections as shown the phylogenetic drawing on the wall. The creatures tanks on the branch of tanks and cages are numbered No number means no collection like the branch of the Coelacanth. This coelacanth branch has been weighing on my mind since then.



Fig.2. Ueno Zoo Aquarium (1964-1992), 4 story "Phylogenetic Tree".



Fig.3. Phylogenetic Tree in the Ueno Zoo Aquarium.

Next stage was the Tokyo Sea Life Park challenging to realize the collection of sea fishes from the Seven Seas, including tuna migration within aquarium tank. Open to public in 1989. Present author engaged collecting fish from the Seven Seas including Comoro Islands, Coelacanth habitat, and tuna husbandry.



Fig.4. Tokyo Sea Life Park (1989-), 100m diameter, 2 story building.



Fig.5. School of tuna realized. 2000ton, round tank.

Then present author engaged the Project of Aquamarine Fukushima, Marine Science Museum (AMF here after) to build aquarium at the 2<sup>nd</sup> wharf of the Onahama Port. Under the waterfront developing project in the Port, Fukushima Prefecture opened 2000. AMF, conceptual phrase, "Pondering through the seas, the future of humankind and the earth" is well reflected the strategy of the environmental aquarium, enlightening present environmental issues and pondering the ancient environment.



Fig.6. Aquamarine Fukushima, Marine Science Museum.(2000)

Prologue Started with the Evolution and Extinction. The story will connect the greatest fish story with us Aquamarine Fukushima.

These are one of the backgrounds why AMF has been focusing on the Coelacanth Project. Because Coelacanth is not merely for the interests of Menageries age but those for the concept of conservation of such precious creatures of the water planet.

Dr. Teruya Uyeno paleontologist of the National Museum was the advisor of the Prologues to started with the evolution. Now specimens of two species of coelacanth are in the galleries. Through working for three aquariums, the coelacanth is always located at the center of my fish story. The strategy of the environmental aquarium, enlightening present environmental issues and pondering the ancient environment.



Fig.7. Prologue themed Evolution and Extinction.

## Coelacanth survey is placed as the In-situ Conservation Abroad

Aquamarine Fukushima, Marine Science Museum (AMF here after) prologues "Evolution and Extinction" and fossil and living fossil are arranged. Exhibit of two species of Coelacanth, *Latimeria charmnae* and *L. menadoensis* will be sending the message above. Since WAZA, World Association Zoos and Aquariums, has stated that standard zoos and aquariums should have at least one of the In-situ Conservation Programs abroad. AMF has placed the Coelacanth Survey as the insitu conservation project abroad and established the cooperative relation with the Indonesian Institute of Sciences LIPI, and also exchanged MOU with



Fig.8. Established cooperative research relation with LIPI Indonesian Institute of Sciences in 2004/11.MOU Exchanged. Dr. Suharsono Director of LIPI(right), Dr. Kasim Moosa, LIPI-RCO(center) and Y, ABE.

SAIAB, South African Institute for Aquatic Biodiversity, and Comoro National Museum. I agree the message of Dr. Tony Ribbink, Director of SAIAB, "Conservation of the coral reef is finally well connected with those of the Coelacanth, since rich coral reef biodiversity supply food for the Coelacanth.



Fig.9. Established MOU with SAIAB, South African Institute for Aquatic Biodiversity. 2006/4 MOU exchanged.

#### **Prologue of AMF Evolution and Extinction**

Life emerged about 3.8 billion years ago, and the world has seen many species briefly flourished only ebb and disappear. Evolution does not necessary mean progress. Actually, evolution can also be seen as the history of extinction. Fossils and so-called living fossils like coelacanth have a message for humans, the only creatures with a civilization. Evolution and extinction is the theme of the prologue of the aquarium. AMF shows the fossils and living fossils through the windows of evolution.

#### AMF steps to Fathom the Mystery

Temporary exhibits named, "The Coelacanth, Fathom the Mystery" was held in AMF in 2001 following the Domestic Symposium. AMF has held and concerned the two international symposiums on the Coelacanth. AMF international symposiums was held in February 2002 in Onahama Japan, and Marathon Symposium in Florida was held in December 2002. The domestic committee meetings held in April 2003. Through these symposiums and meetings, two proceedings have been published from Aquamarine Fukushima. Following is the my fish story of events on the evolution and coelacanth concerned.

Aquarists are always challenging to develop new stars from the non-charismatic species. The greeneye family is one of the themes. Tapetum lucida apparatus in eyeball is common characteristics with these fishes to reflect the light of dawn at the depth of continental shelf. Iwaki City designated the symbol fish of the City as greeneye, Chlorophatahlmus spp in 2001 and consigned AMF to make biological survey on the fish. This is rather tough species to keep alive more than tuna Evolution and Extinction. This will be a good simulation for the coelacanth stewardship.



Fig.10. Remotely Operated Vehicle ROV has forced the project.

AMF is conducting to make survey the greeneye off shore Iwaki with the specially ordered Remote Operated Vehicle, ROV, with pull down underwater camera, operation unit with TV monitor has already introduced to AMF. These could be operated max 300m deep. This is the smallest, right weight and high quality, portable ROV. Total weight of vehicle is 38 kg.

AMF project committee has already established the following sub-committees; Technical Survey, Government and Public Relation, Evolutionary survey and Conservation Awareness Survey, Educational Survey, Science-Population Size Survey, Symposium.

AMF desire is to develop the greeneye project

toward the proposal. We could not be satisfied with the stuffing or iron coelacanth or movie any more. Aquarium group has developed techniques making survey, capturing, transporting and stewardship on the so many difficult fish same level as coelacanth. Present author hopes the dream breeding this phylogenetic milestone in captivity comes true in near future. AMF is one of the most appropriate aquariums to conduct survey on the coelacanth. Coelacanth should be one of the characters of aquarium in the future.

# Zoogeography of Coelacanth, story behind the scene.

Present author discussed on the distribution of coelacanth in the zoogeographical viewpoint in the previous symposium (Abe 2002). For the Zoogeographical treatment of tropical marine fishes, the term Indo-western Pacific has been advocated by a number of authors including Ekman (1953), Cohen (1960,1973), Talbot (1970) and others. They mostly agree that the other three regional areas for tropical fish distribution are West Africa, the West Indies (Caribbean) and the Eastern Pacific (Panamanian). The fish fauna of the Indian Ocean and Western Pacific Ocean face each other through a transitional zone ranging from southern coast of Sumatra southward to the north eastern coast of Australia. It appears that tropical fish fauna of the Indian Ocean resulted from the dispersal and differentiation of the pacific species. The number of species constantly decreases as we proceed in a westerly direction.



Fig.11. Coelacanth survived in the Indo-western Pacific.

Summarizing my past zoogeographical study in the waters of the Arabian Gulf (Kuronuma and Abe 1972, 1986) and Dr. Mochizuki's hypothesis mentioned at the last symposium (Mochizuki 2002), the distribution of coelacanth will be centered in the Western Pacific Region as the pattern shown by many other tropical marine fishes in the Indowestern Pacific. The discovery of Indonesian coelacanth was made by Dr. Mark Ardman, American Biologist.

Theme of AMF is the Oyashio cold current from north and Kuroshio warm current from the Indo-Western Pacific link with the greatest fish story with us AMF.

## CLOSING, WITH THE TV CM, GRUMBLINGS OF COELACANTH ON OUR EARTH

Raja Laut Grumbling for the peaceful world.



Three hundred Million years ago Series: Father of Raja Laut; "OH! It passed 3 hundreds million years since started Coelacanth!" Son; "Shall we evolve soon!"

Father; "You are green! Its important don't change now"

#### ACKNOWLEDGEMENT

At that time named JLB Smith Institute of Ichthyology, Director Dr. Paul Skeleton kindly lent a juvenile specimen for us and Dr. Tim Andrews came to visit for the preparation of the temporary exhibits on coelacanth held at AMF in the summer of 2001, to whom, present author would like to express deep appreciation. The exhibits started in June and continued through September in 2001. I am remembering the International symposium had scheduled September 24, but September 11 terrorist's attacks caused the postponement until next February 16, 2003. Finally, I would like to express my appreciation the SAIAB's staff for their effort accomplished the conference, in the stage of initiation. Everything of the Greeneye Project of AMF has started with the Exhibition in 2001.

Many people engaged the projects of the greatest fish story since 1964 to whom we have to deeply appreciate.



Fig.12. Exhibit of the Greatest Fish Story in 2001.

Year	Month	Activities			
1964	MAR	Ueno Zoo Aquarium opened to public to commemorate 80 <sup>th</sup> anniversary of Ueno Zoo.			
1982	MAR-MAY	Special exhibit of Evolutional Tree, "You are now climbing the evolutional tree" in			
		Ueno Zoo Aquarium.			
1989	OCT	Tokyo Sea Life Park opened to public.			
1992	JUN 30	Ueno Zoo Aquarium closed			
2000	JUL 15	Aquamarine Fukushima open to public			
	JUL	C7 Established 7 years long term Program			
2001	MAR	Domestic Symposium. Established "Greeneye Research Project"			
	JUL	Exhibit, "Coelacanth, Fathom the Mysteries" at AMF			
2002	FEB 16	AMF 1st Coelacanth International Symposium (Proceedings published)			
	FEB 17	Project Committee was held.			
	DEC 04-07	Marathon, Florida International Symposium			
2003	APR 21	Domestic symposium II(Proceedings published including the above symposium)			
	DEC African Coelacanth, Conservation Ecosystem Symposium, East London. SA				
2004	NOV	Started Cooperative Research with Indonesian Institute of Sciences, LIPI			
2005	APR	Survey with ROV, in Manado, Sulawesi Island with ROV and Deep Sea Diving			
2006	MAY	Succeeded Filming Indonesian Coelacanth in in off Buol, Sulawesi			
	DEC	Tracing Coelacanth offshore Buol, Sulawesi, Indonesia			
2007	MAY	Survey offshore Manado, Sulawesi, Indonesia			
	OCT	Survey off Tanga, Tanzania			
	NOV 02	Int. Coelacanth Symposium			
2008	DEC	Survey Trisei Isl. Manado, Sulawesi Isl. Indonesia			
2009	OCT	Juvenile Coelacanth found off Manado, Sulawesi, Indonesia			
2010	NOV	Survey off Papua, Indonesia			
2011	MAR	Research stopped because of the disasters			
2012	MAY	Survey North Sulawesi, Two specimens filmed, dissected in Indonesia. Found the			
		garbage bag in the stomach.			
2013	JUN	Survey Northern Sulawesi, one specimen filmed.			
2014	AUG	Program to establish Coelacanth Research Station started in Sulawesi, Lolak/Bitung.			
2015	MAY	Filmed Coelacanth off Lolak/Bitung.			
2016	NOV 02-03	3rd International Coelacanth Symposium, "Fathom the Mysteries" held.			
2017	AUG	Morphological and anatomical joint studies on both species Indonesian and African was			
		conducted.			
2018	NOV 05-10	10th IAC was held in Onahama Fisheries Port, Fukushima			

## Table1. the Greatest Fish Story

## LITERATURE CITED

Abe, Y. 2002. *In* proceedings. The Greatest Fish Story. The Coelacanth, Fathom the Mystery. Aquamarine Symposium. Proceedings. Feb.16, 2002. Iwaki City, Fukushima Prefecture, Japan.

Cohen, D. M. 1960. Geographical distribution of fishes. Encyclopedia Americana Vol.II: 275-

277.

- Cohen, D. M. 1973. Zoogeography of fishes of the Indian Ocean. In: The Biology of the Indian Ocean. Springer-Verlag Berlin: 451-463, Fig. 1.
- Ekman, S. 1953. Zoogeography of the Sea. Sidgwick& Jackson, Ltd., London: I-xiv, 1-417, Figs. 1- 121.
- Kuronuma, K and Y. Abe 1972. Fishes of Kuwait. Kuwait Institute for Scientific Research. Sci. Res., Kuwait: I-xiv, 1-123, Figs 1-37, Pls.1-20

Kuronuma, K and Y. Abe 1986. Fishes of the

Arabian Gulf, Kuwait Institute for Scientific Research. Sci. Res., Kuwait:i-xii, 1-356, Plates 1-30.

- Mochizuki, K. 2002. *In* proceedings. The Greatest Fish Story. The Coelacanth, Fathom the Mystery.
- Aquamarine Symposium Feb.16, 2002. Iwaki City, Fukushima Prefecture, Japan. Figs. 1-19, Pls. 1-56.
- Talbot, F. H. 1970. The South East Asian area as a center of marine speciation: an ecological analysis of causes. Rep. Aust. Akd. Sci., 12:43-50.

## Preliminary Study on eDNA of the Coelacanth's Habitat Around Deep Sea Conservation Areas of North Sulawesi, Indonesia

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**ABSTRACT:** Indonesian marine waters have lots of resources, especially rocky fishes including ornamental fishes. However, the utilization levels of these resources are difficult to be assessed due to the limitation of technology and budget. The new technology called Environmental Deoxyribo Nucleic Acid (eDNA) approaches is a promising one that is expected to overcome those issues because of their efficiency in getting results and cost effectiveness. Here, we report the results of our preliminary researches on deep sea eDNA. We collected from 10 stations ranging in 110-200m depths from two areas in North Sulawesi where many coelacanths have been discovered by the Green Eye Project on ecological and distribution studies of Indonesian coelacanths, using Nansen bottle water sampler (1500cc). The collected waters were filtered using Power Water Sterivex DNA Isolation Kits and preserved with the DNAiso Reagent, then were transported to Center for Strategy Research Project, University of the Ryukyus, Okinawa, Japan, where eDNA analyses were conducted. Our preliminary results revealed that the concentrations of eDNA are good, indicating eDNA was successfully extracted. Furthermore, 40 species were detection by using a high through put Illumina MiSeq platform for sequencing analyses, even though we couldn't have detected any coelacanth information.

Key words: deep-sea, environmental DNA, and nansen water sampler

#### INTRODUCTION

Indonesia as a country has contains of the highest marine biodiversity on the planet laid between  $6^0$  E to  $10^0$  S and from  $95^0$  E to  $142^0$  E comprises about 18.110 islands with coastlines of about 108.920 km. About 78 % of the Indonesia waters cover territory with shallow seas in the western and eastern parts, the Sunda and Sahul plates separated by the deep Banda Sea is part and center of the Coral Triangle of the earth, which covers only about 2 percent of the global ocean but comprises 76 percent of all known coral species. Although some of the donator from all of the world are giving attention to protect the region's critical biological diversity and marine-dependent livelihoods (Anonymous 2004).

Indonesia has a strategic role as a world maritime shaft in the global supply chain system to connect the Asia-Pacific area with Australia, since it is located between the Asian Continent and the Australia Continent, and also between the Pacific Ocean and the Indian Ocean. With those potentials, God blesses Indonesia with immense marine resources including the biggest marine biodiversity, to control those marine resources abundance; the lots of the budget and times are needed.

A new technology approach can be overcoming of this problem were available can be implemented at around Indonesian marine waters by using the environmental DNA approach. Environmental DNA (eDNA) in aquatic environments refers to genetic material found in the water column. In the case of multicellular organisms, eDNA originates from various sources, such as metabolic waste, damaged tissue or sloughed skin cells (Kelly et al., 2014) (Ficetolla et al., 2008) Ficetola *et al.*<sup>[2]</sup> was the first study demonstrating the use of eDNA for detecting an aquatic vertebrate species (invasive American bullfrog) from controlled environments and natural wetland, published in 2008. However, this technology is still rare implementation at marine water especially for deep-sea water.



Fig. 1. Map of collection sites of Deep-sea water sampler collected from two areas at Lolak Waters and Manado Bay North of Sulawesi, Indonesia.

Here we would like to predict the coelacanth (*Latimeria menadoensis*) and the others marine fisheries resources existence around the discovered coelacanth sites since years 2006-2015, and also is a potential fishing location by traditional fishermen of the both areas explained above by using the new and sophisticated technology of eDNA. If can be implementation of this technology around Indonesian marine waters, then the research activities of the marine biodiversity abundance will become more efficient and effective in relationship with the budget and times.

#### METHODOLOGY

Deep-sea water sampling was collected from 10 sites ranging from 110m-200m in depth (table 1.) at front side of the International Coelacanth Research Center and Museum Base at Lolak Waters and Manado Bay North of Sulawesi using Nansen Bottle Sampler (1500 cc) as shown in Fig. 2. The positions were follows the discovered of coelacanth by Green Eye Project on 2007-2015, (Masamitsu I. *et al.*)

The collected waters were filtered using Power Water Sterivex DNA Isolation Kits (Fig.2) and preserved with the DNAiso Reagent and kept in a deep freezer -25<sup>o</sup> C at Faculty of Fisheries and Marine Science, Sam Ratulangi University, until they were transported to Center for Strategy Research Project, University of the Ryukyus, Okinawa, where eDNA analyses were conducted following MiFish protocol at (Miya M. *et al.*2018). Therefore, e-DNA extraction was conducted at Center for Strategy Research Project at University of the Ryukyus Okinawa Japan by using Power Water Sterivex DNA Isolation Kit Samples by followed its protocol as follow (Anonymous, 1993).



Fig. 2. Research activities in the field collections and at Laboratory of Ryukyu University.

We conducted electrophoresis for each part  $1^{st}$ -PCR and  $2^{nd}$ -PCR to amplify the

Intense Signal of MiFish eDNA by Using Universal Primers MiFish-U-F/R, then it was used

for MiSeq sequencing process. All the sequence was analyzed for sequence identity at taxonomic assignment processes.

#### **RESULTS AND DISCUSSIONS**

The results show us a good quality of DNA concentration of water samples after extraction. The eDNA water sampler was collected from those two areas, Manado Bay and Lolak waters, Sulawesi sea of Indonesia, were each collections site has five stations as shown in Table. 1. And we become more exiting cause the electrophoresis of each part 1<sup>st</sup>-PCR and 2<sup>nd</sup>-PCR amplification products show as the sign of the target sequence bp (Fig. 3). As positive control the river samples as exhibited intense signal of MiFish eDNA amplification and negative controls (DW) showed no clear bands. Suggestions that no contamination cleared and feasible to be continued to next of the genome work.

Some number of the species has been analyses at The Center the Strategic research Projects of University of the Ryukyus.

Table 1. Results of collections site, satellite positions, sea's depth and the number of fish's species was detected at Lolak Waters and Manado Bay North of Sulawesi.

Stations	Positions		Sea's Depth	Species
	(Deglees)		(Wieter)	(INUIIDEI)
	Latitude (N)	Longitude (E)		
1	0.9298	124.0351	115	8
2	0.9338	124.0066	150	6
3	0.9339	123.9961	196	14
4	0.9217	124.9889	170	2
5	0.9128	123.9869	110	11
6	1.4699	124.8199	95	3
7	1.4707	124.8179	120	2
8	1.4686	124.8153	135	8
9	1.4659	124.8112	120	6
10	1.4661	124.8156	90	4

As shown in table 1. were 10 sampled sites of two areas are indicated of column one, the second column is satellite positions at latitude of north and longitude of east in degrees, the third column is seas` depth were ranged from 95-196 meters measured by videos eco-sounder with its Global Positioning System (GPS) and the fifth column are the detected fish's species.



Fig. 3. The 1<sup>st</sup>-PCR and 2<sup>nd</sup>-PCR Shows Intense Signal of MiFish eDNA by Using Universal Primers MiFish-U-F/R.
Then finally it can be explained (Table. 2), from all 10 collection sites we found that, the number of fish species have been obtained for taxonomic assignment analysis and the results of species was detection by deposit analysis of eDNA water sampler, based on fish base database, GBIF, Mito fish, and NCBI as shown in Table 4. By using a high through put Illumina MiSeq platform for sequencing analyses, we detected eDNA from 40 fish's species fishes. Even though we couldn't get any information about coelacanth.

Table 2. The results of fishes' detection at Manado Bay and Lolak Waters, Sulawesi Sea of Indonesia by using eDNA

Number	Family	Species Scientific Name	Swimming Layers (M)	Locations
1	Hemiscyllidae	Chiloscyllium plagiosum	50	Lolak
2	Dasyatidae	Neotrygon kuhlii	170	Lolak
3	Anguillidae	Anguilla celebesensis	10	Sariouw,Manado
4		Anguilla japonica (%1)	10	Sariouw
				Sariouw, Lolak,
5		Anguilla marmorata	10	Manado
6	Engraulidae	Encrasicholina punctifer	35	Lolak,Manado
7	Chanidae	Chanos Chanos	50	Lolak
8	Sternoptychidae	Maurolicus sp.	400	Lolak, Manado
9	Myctophidae	Benthosema pterotum	500	Manado
10		Diaphus regain	500	Lolak
11		Myctophum orientale	400	Manado
12	Mugilidae	Chelon affinis	20	Lolak
13	0	Chelon macrolepis (※1)	10	Manado
14		Mugil cephalus	120	Manado
15	Serranidae	Odontanthias borbonius	300	Manado
16	Symphysanodontidae	Symphysanodon katayamai	183	Lolak, Manado
17	Menidae	Mene maculate	200	Manado
18	Carangidae	Caranx latus (%2)	140	Manado
19		Caranx sexfasciatus	146	Lolak, Manado
20		Decapterus akaadsi	170	Manado
21		Decapterus macarellus	200	Lolak
22		Decapterus macrosoma	214	Manado
23		Selar crumenophthalmus	170	Lolak
24	Mullidae	Upeneus subvittatus	100	Manado
25	Pomacanthidae	Apolemichthys trimaculatus	60	Manado
26		Pomacanthus imperator	100	Lolak
27		Oreochromis niloticus	5	Lolak
28		Kuhlia marginata	5	Lolak
29		Kali indica	500	Lolak
30		Oxyeleotris marmorata	10	Sariouw
31		Sicyopterus japonicus (%1)	5	Sariouw
32		Sicyopterus lagocephalus	5	Sariouw
33		Euthynnus alletteratus(%2)	150	Sariouw
34		Katsuwonus pelamis	260	Lolak
35		Rastrelliger kanagurta	90	Lolak, Manado
36		Thunnus albacares	250	Lolak, Manado
37		Thunnus maccoyii	500	Manado
38		Paramonacanthus japonicus	46	Lolak
39		Lagocephalus gloveri	450	Manado
40		Cyclichthys orbicularis	170	Manado

Table 4. Taxonomic assignment analysis and the results of species detection by Deposit analysis of eDNA water sampler from Manado Bay and Lolak Waters C01-01-Tanjung0mpu01-KP60-1 SLUniv processed

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Furthermore, this bioinformatics data was compared to the fish's distributions and ecological as reported from the Green Eye project 2007-2015 (Masamitsu I. *et al.*) where the site also known as fishing grounds of those target fishes. Here we can have explained that, the results of collections sites, satellite positions, sea's depth and the number of fish's species was detected at Lolak Waters and Manado Bay North of Sulawesi, explained that the collection site at Lolak Waters relatively higher fisheries resources existence abundance.



Fig.4. The relationships between the collection sites number and sea's depth as shown at Fig. 4-a by radar chart and Collections Sites Seas' Depth with balloons pattern analyses (4-b)

As shown at Fig. 4. The relationships between the collection sites number and sea's depth by radar chart approach and the number of the fishes' species detected was analyzed and perform by using the balloons pattern for easer to understand.



Fig. 5. Relationships between Detected Fishes`Species and Swimming layers of each.

More interesting 40 fishes' species were detected by environmental DNA tools in this study. We found that 8 species are the deep-sea fishes' species or 20 % from the total species detections as shown in Fig. 5. According to the former researches results have been done by Green eye Project team in collaborating among Aquamarine Fukushima, Sam Ratulangi University and Indonesian Institution of Science Republic of Indonesia under umbrella of International Coelacanth Research Center and Marine Museum Indonesia by using the sophisticated equipment of remotely operating vehicles (ROV) during periods surveyed 2006-2016 depths, ranged of 150-225 in meters were encountered swimming layers of the

coelacanth (*Latimeria menadoensis*) around Indonesia seas.

The mostly species were detection are the shallower swimming layers, coral fishes and pelagic species (Fig.6). Only about 7 deep-sea fish species were detected from eDNA water sampler, caused by some reasons the water samples were pickup by Nansen water sampler just limited to the seas` depth ranges around 200 meters.



Fig. 6. Relationships between the Sample Sites, Fishes` Swimming Layers and the number of the detected aquatic creatures by spider chart pattern.



Fig. 7. The relationships between the collections and Fishes` Species Swimming Layers.

Furthermore, we comparing among whole DNA data samples, Lolak sea and Manado Bay in Fig. 7, explained that the percentage of the fish's species were detected by the environmental DNA tools and perform by the lunar eclipse pattern. From this figure show that the mostly the fishes were detected are living at shallower swimming layers. About 90 % of the 40 fish's species are living at shallower sea waters or less than 300 meters in depth and around 10 % of the fish's species are living at deep-sea or more than 300 meters in depth. In Fig. 7-b the fish's species were detected at Lolak waters, in this figure show that only 8 % of the fish's species were detected are at swimming layers categorized of deep-sea and the others of them are more than 90 % are living at shallower and at coral And Fig. 7-c is at reefs. Manado Bay with 12 % at deep-sea were detected and clear based on the PCR results. During our surveyed at the fields by pick up the deep-sea waters at two areas consisted by 10 stations around the discovery of coelacanth and also became the fishing grounds by local fisherman from long years ago, unfortunately we have not yet got any DNA of the coelacanth due to this organism are vey rare in the sea, or because they living in the sea bed cave.

# CONCLUSSIONS AND REMARKS

Based on our results analysis to developed universal primer MiFish in a metabarcoding approach to fish eDNA we confirmed that the Lolak Waters are having relatively higher the fisheries resources existence abundance comparing of that on Manado bay. In implementation of the deep-sea environmental DNA research, the un-contamination aspect during fields work is absolutely necessary, therefore for effectiveness and efficiencies research of the marine fisheries resource's existence abundance point of view, the environmental DNA technology approach is suitable to be applied. Finally, this marine environmental DNA technology could be get fruitful in the near future if it could be implemented to Indonesian marine and freshwater due to Indonesia country have very wider territorial in the view of point forecasting the fisheries resources existence abundance.

#### ACKNOWLEDGEMENT

We would like to thank first to Japanese Government through the Ministry of Education, Culture, Sports, Science and Technology (MEXT) which gave opportunity to us for doing this project due to the very rare moment, to the Dean of Faculty of Fisheries and Marine Science, Sam Ratulangi University, who giving support to the team during surveyed and gave us the letter of the material transferred agreement (MTA) of deep-sea water samples and brought to Japan. Then to the Tropical Biosphere Research Center and The Center for Strategic Research Project University of the Ryukyus which have been funding and very eager to support this project and allowed us to conducted the eDNA laboratory works. View, the environmental DNA technology approach is suitable to be applied. Finally, this marine environmental DNA technology could be get fruitful in the near future if it could be implemented to Indonesian marine and freshwater due to Indonesia country have very wider territorial in the view of point forecasting the fisheries resources existence abundance.

#### REFERENCES

ANONIMOUS. 1993 DNA Isolation Kit Sample (For isolation of genomic DNA from Sterivex TM filter units, Millipore catalog# SVGPL10RC), (Catalog No. 14600-S) USA

- FICETOLA GF., MIAUD C., POMPANON F., TABERLET P. 2008 Species detection using environmental DNA from water samples. *Biol. Lett.* 4, 423–425. (doi:10.1098/ rsbl.2008.0118)
- MASAMITSU I., et. al., 2016 Biological Study of the Indonesia Coelacanth.
- KELLY RP., PORT JA., YAMAHARA KM.,

MARTONE RG., LOWELL N., THOMSEN PF., MACH ME., BENNET M., PRAHLER E., CALDWELL MR. 2014 Harnessing DNA to improve environmental management. *Science* **344**, 1455–1456. (doi:10.1126/science. 1251156) *Fish Research.* **83**: 115-123

MIYA M *et al.* 2015 MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *R. Soc. open sci.* **2**: 150088.http://dx.doi.org/10.1098/rsos.150088

# Aquariums in Manga - Possibility of Aquarium Education

Yuji Kurihara Kyoto National Museum, Japan

**ABSTRACT:** Today, Manga is the culture of representing Japan and its quality and quantity are world's highest and appreciated led by a youth in the foreign countries. Museums, zoos, aquariums also become the stage or the subject of Manga in a variety ways.Considering the necessity of aquarium education including species conservation, environmental conservation or SDGs and human resources development of keeper and experts in aquarium, we can't ignore the power of Manga which is the medium read by many citizens because of its high expressive power. This presentation introduces these works and think about our future on the water planet by Manga.

#### **INTRODUCTION**

Currently, the Special Exhibition "Swords of Kyoto" is showing at the Kyoto National Museum. In the past, exhibitions about swords did not draw huge crowds – they would typically pull in experts, as well as the more curious museum-goers. But, every day, we have suddenly found ourselves welcoming many female visitors. So much so that they have been forming a long line, to get in. This queue of women was so noticeable that our security staff have had to manage the unwelcome interest of certain, let us say; 'lonely men' that appeared - not to see the exhibition, but to pester the waiting visitors. In Japan, online gaming is extremely popular, and certain games are aimed at female audiences. One such game is called "Token Ranbu", and in this game legendary swords are depicted as handsome young men. So, we believe this to be the reason why we are seeing a sharp rise in female visitors coming to museums to see Japan's historic sword masterpieces, not just in Kyoto, but all over the country. Currently, this collaborative exhibition is proving to be very popular.

Manga is one of the cultural representations of Japan - and many believe it to be the world's best animation. The quality and quantity of manga is appreciated - largely with young people at the vanguard - in every country. The course of study specifies the utilisation of manga, and comics that are used for school textbooks and examinations. Local government utilize Manga to introduce their history. Many examples are utilizing Manga as guidebook or introduction. Central government utilize Manga to introduce their task. Museums or historical site utilize Manga for display. Manga Museums and Memorial Museums to Manga Artists have been established in many places. There is also a movement to set up a Manga Museum Network in Japan. Some Manga Museums are contributing to regional revitalization. Recently, Manga exhibitions have been held at more than 100 museums or cultural facilities, every year in Japan. Many manga exhibitions are held abroad, as well. Manga museums have even come to be established abroad.

# MUSEUMS BOTH FEATURE AS A THEME IN, AND PROVIDE A PLATTFORM FOR MANGA

I would like to introduce some examples of the relationship between Manga and museum.

- Fujihiko Hosono "Gallery Fake"
- The main character called Reiji Fujita was the curator of Metropolitan Museum, but he is learned in every field, and now in the black market. It introduces the backstage life of the museum and the work of the curators.
- Eishi Ai, Kei Satomi "ZERO The Man of the Creation"

The main character, Zero is deeply knowledgeable, with superior sensory powers and memory, and completely reproduces everything that exists in the world.

- Kazuo Koike, Seisaku Kano "Auction House" The main character, Ryu Sogen is a world-famous art expert, and confronts the "dark side" of the art word to exact revenge.
- Motohiro Kato "C.M.B. Cause list of Shinra Museum"

The main character has extensive wide knowledge and solves many difficult cases.

- Sachiko Aoki "ZOO KEEPER" It is a fiction, but it prompts some interesting ideas. The main character has eyes which can perceive temperature and she finds, treats and captures her cases.
- Masato Fujisaki "Wildlife"

The main character can understand the heartbeat and respiratory sounds of animals, and can distinguish a case with perfect pitch.

In these Museum Manga, the main character is an exceptionally talented expert. It is a little bit old fashioned, but it shows some of Manga's charms.

There is a lot of Museum Manga, but time is limited. I'll quickly introduce some of them.

- Jiro Okazaki's "National Museum Story"
- Kazuhiro Fujita "Black Museum Springald"
- Mariko Iwadate "Art museum still on August"
- Mami Kashiwabara "At the mercy of the space"

Recently, comic essays that introduce the backstage workings of museums, showing the curators and museum staff, is increasing. For example;

- Izuma Kuroda "People in the museum"
- Yuri Ono "Working in the art museum"
- Fuki Inoue "You of glasses and the museum"
- Mitsuko Usae "A Museum Girl"

These are very interesting and educational for beginners or persons who would like to work at a museum.

## AQUARIUMS BOTH FEATURE AS A THEME IN, AND PROVIDE A OLATTFORM FOR MANGA

Aquariums and Zoos also become the platform for, or the subject of Manga in a variety of ways. Koichi limori "My zoo diary" is the first Manga featuring a zoo theme. The basis of the main character is Toshio Nishiyama, who was a keeper at Ueno Zoo, and former director of Tobu Zoo. This is documentary manga.

Now, there is a lot of Aquarium Manga, just as with the aforementioned Museum Manga. These Manga may be divided into four broad types.

(1) The personification of fish or marine animals; many works for children like "Finding Nemo" and "Zootopia". In Japan, "Jungle Emperor Leo"by Osamu Tezuka is most famous. For example;

- Kiriko Kubo "Rice in the bucket".
- Ryoko Sugihara "Natural Aquarium"
- Watanabe Denki Inc. "Aquarium for Life"
- Nakayoshi Yoshida"Asahi! Let's go to the Zoo"
- Kohei Horikoshi "Twilight Zoo"
- "Kemono Friends"

(2) Documentary records of existing aquariums; for example, Okinawa Churaumi Aqarium or Asahikawa City Asahiyama Zoo. For example;

- Kenji Yoshida, Fujio Fukamitsu "Okinawa Churaumi Aquarium Story"
- Yumin Mori, Kei Honjyo "ASAHIYAMA ZOO STORY"
- Katsumi Tatsuzawa"BENJIE"(DeNA 2018)

(3) Fiction set in the aquarium; especially the sort of stories where the aquarium staff are the heroes of the tale, are most popular, and sometimes the main character has exceptional abilities, I mentioned before. For example;

- Midori Takanashi "A Sea Animal" the main character can relate to Sea Animals.
- Jyotaro Mokumiya, Aoi Amagi "Aquarium Girl"
- Masumi Sudo "Aquarium"
- Masumi Sudo "NANANABANI Garden"
- Maomi Ito "Aquarium of the Little Mermaid"
- Kiyomi Sugishita "Deep Sea Aquarium MagMell"
- Wataru Ishi "Suizoku Company!"
- Yu Abiko "Welcome to a Happy Aquarium"
- Akiyo Kurosawa "Zoo at all"
- panpanya "Ashizuri Aquarium"
- Nae Serizawa "Desires Aquarium"
- Makoto Ogyu "The tropical fish longs for snow"

(4) The essay style of Manga that introduces the backstage work of museums, or the work of the keepers, trainers and staffs at aquariums. For example;

- Tomokichi Hidaka "I will work in an aquarium"
- Qtaro Hanamizawa "Animal Illustrated Book"
- Saku Yamaura"Animal trail"
- Kikka "I'm working in Zoo"
- Satoshi Miyakoshi "The casual day at the Zoo"

#### **CONCLUSION0**

A When the Manga of fishing made a hit in the 1970s, a fishing boom came in Japan. Also, when the Manga of the veterinarian made a hit in the 1990s, a competition rate of the veterinarian department rose at a stretch.

The influence of Manga is significant as much as that. The weekly Manga magazine records sales more than 2 million copies, and there may not be the hand which does not utilize this.

Considering the necessity for aquariums to educate – on the subjects of species conservation, environmental conservation or SDGs and human resources development for the staff and specialists at the aquarium - we cannot ignore the power of Manga, which is a medium read by many citizens for its high expressive power and ability to influence. It would be great that if every country could publish the Manga that featured the theme of aquariums and environment.

# The Primorsky Aquarium: Modern Trends in Its Educational and Awareness-Raising Activities

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**ABSTRACT:** The Primorsky Aquarium is a branch of the National Scientific Center of Marine Biology (Far Eastern Branch of the Russian Academy of Sciences). The Aquarium started educational activities right after its opening in October, 2016. By now, two educational projects, *Educational Environment* and *Growing up at the Aquarium*, have been carried out at the Primorsky Aquarium. The main goal of the *Educational Environment* project is to reinforce and deepen the knowledge obtained by pupils at school. Special emphasis is placed on school children's self-directed work with data given on information displays at the Aquarium exhibits. The *Growing up at the Aquarium* project is designed to develop a cognitive interest in the science of marine biology in children aged five and older through knowing the natural environment at the Aquarium. Both projects are aimed at bringing up kind hearted and environmentally educated people who will take good care of our planet.



Fig. 1. Main building of the Primorsky Aquarium.

The Primorsky Aquarium is a branch of the National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences. The Aquarium was open to general public on September 3rd, 2016 (Fig. 1). Its significant feature is that this research and education center is included into the Russian Academy of Sciences, and its main goal is to create and display collections of marine and freshwater inhabitants for research and educational purposes.

The first educational project, *Lesson at the Aquarium*, was launched right after the opening of the Aquarium in October, 2016. Wednesday has been chosen for the work with organized groups of pupils, and on this weekday admission for other visitors is limited. The *Lesson at the Aquarium* has the same core idea as the Lesson at the Museum project does. In Russia museum lessons are extremely popular since they are intended to reinforce and deepen the knowledge of pupils about

some topics, with museum exhibits, or tank dwellers (if classes are conducted at the Aquarium exhibits), serving not only as a visual aid but also a source of information for study purposes. Such lessons are an effective tool for socializing children, and they will help to 'vitalize' the educational process, captivate pupils interest, broaden their minds and enhance their cognitive abilities.

The integral part of the Aquarium is an educational block consisting of lecture rooms, learning laboratories, a movie theatre and a library equipped with modern equipment. A conference hall, classrooms and exhibit collections facilitate the implementation of unique educational programs on marine biology and ecology. The Aquarium serves as a teaching ground for preschool and school children and university students providing them with an opportunity to participate in scientific research process.

The concept of the Lesson at the Aquarium

project implies a single class or a series of classes held in the framework of a particular school subject on the basis of the Aquarium exhibits for the purpose of in-depth study of the subject. The lesson necessarily corresponds to particular themes from the school curriculum and includes additional research and creative tasks beyond the curriculum (Fig. 2).



Fig. 2. School children performing a task on their own.

On the basis of the Federal State Educational Standards a total of 13 lessons have been developed for children of primary, secondary and upper secondary schools on such subjects as Science, Biology and Geography: "Coral Reef Fishes", "What is a Fish?", "Life in Cold Water", "Save the Tropical Rainforest", "Four Homes for Fish ", "Tropical Rain Forest Community", "Life in a Water Drop", "Research Methods in Biology", "Emerald Green Belt of Earth", "They are So Different, Interesting and Sometimes Dangerous", "Far Eastern Seas", "Explore the Invisible World", "Unwind the Spiral of Evolution".

Distinctive features of the project classes are as follows:

•Classes are held at one or, rarely, two of the exhibits;

•Each lesson has its scenario reflecting the specifics of the exhibit and a developed worksheet;

•During the classes a special emphasis is made on self-directed work of pupils;

•Children have an opportunity to work with educational films (the 'Evolution of Life in the Ocean' and 'Rivers and Lakes' exhibits), microscopes (the 'Microworld' exhibit), a touch pool and interactive displays ('The Sea of Japan' exhibit);

•Pupils are enabled to perform research in the learning laboratories.

Being conducted at the Aquarium exhibits, the classes give children a chance to see live marine and freshwater animals in conditions most similar to the ones of their natural habitats. The content of the Aquarium exhibits corresponds to the themes of the lessons and includes topics on nature protection and commercial fishery. To investigate microorganisms and cellular texture of different organisms, the Aquarium has learning laboratories with modern microscopy equipment.

The Aquarium classes are to be attended by an organized group of school children accompanied by their teacher and one parent. The classes are provided free of charge by specialists of the Environmental Education Center of the Aquarium. If pupils want, they can reinforce the obtained knowledge at workshops or laboratory practical on the studied topic. The following structure of a lesson is considered the most efficient: a short lecture acquainting children with the exhibit, questions on the topic and individual work by pupils performing the worksheet tasks. At the end of the lesson children discuss their results and fill in a feedback form. On average, over 400 schoolchildren take lessons at the Aquarium exhibits every Wednesday (Fig. 3). During the implementation of the project almost 20,000 children have taken part in it.



Fig. 3. A secondary school pupil doing self-directed learning beside the information display.

To date, the *Lesson at the Aquarium* has developed into the *Educational Environment* project and gained larger audience. On Wednesdays the Aquarium is visited by groups of preschoolers for whom our specialists hold entertaining excursions (Fig. 4).

In March, 2018, the *Educational Environment* project was joined by organized groups of children with disabilities. Among them there are visually and hearing impaired children and children with autism and Down syndrome. Before their visits qualified specialists conduct a long-term training, with a visiting program being developed. In addition to the educational component, the project gives an excellent opportunity to socialize these children.



Fig. 4. Children aged 5+ during an entertaining excursion at the Aquarium exhibit.

The Aquarium is developing cooperation with secondary and higher educational institutions of Vladivostok and Primorsky krai. While young and school children take classes held by the Aquarium specialists, university students specializing in such fields as Biology, Ecology, Ichthyology, Architecture and Engineering are given lectures by their teachers at the Aquarium exhibits (Figs 5, 6).



Fig. 5. Students reinforcing the studied material with the help of information displays.



Fig. 6. Foreign students having classes in the Aquarium's lecture room after their visit to the exhibits.

The Russian language classes conducted at the Aquarium are extremely popular among foreign students studying Biology in various universities of Vladivostok. The significant feature of this language program is its applicability: students study the specialty language being surrounded by aquatic inhabitants. Such interactive lectures contribute to a better assimilation of both vocabulary and biological knowledge.

The second large scale project of the Primorsky Aquarium, *Growing up at the Aquarium*, began its work in October 2018. Its main idea is to raise humanity in children aged 5–15 through the creation of an educational space which is focused on the manifestation of such qualities such as kindness, empathy, compassion, responsibility, and helps to develop in children personal responsibility and cognitive interest in the science of marine biology.

The *Growing up at the Aquarium* project objectives are as follows:

- •To acquaint children with science as a way of knowing the world;
- •To generate conditions for cultivating environmental literacy among children aged 5–15; •To create conditions for raising children's awareness about the unicity of aquatic inhabitants and forming careful attitude to them;
- •To provide necessary conditions for nurturing in children aged 5–15 the best human qualities, team working skills and personal responsibility in interaction with Nature on the basis of their cognitive interest in marine biology;
- •To produce an emotive environment for children with disabilities;
- •To implement programs, the content of which is based on the child's cognitive interest in marine biology in the context of its continuous study.

The project implies the continuous study of marine biology and consists of 4 learning stages, each of them is designed for children of particular age and will be held from October to May. At each stage children get information from various fields of marine biology.

The first of them is "Belyok" ('belyok' is the Russian name for a newborn pup of the Baikal seal), an environmental biology studio for children aged 5 to 7, which started its work 3 years ago. Here children get acquainted with such professions as biologist-divers, marine mammal trainers, marine biologists, paleontologists, ichthyologists that are related to providing the aquatic dwellers of the Aquarium with comfortable living conditions (Fig. 7).

One of the main goals of the kids' studio is to instill a positive attitude to the environment in children and lead them to understanding of intrinsic value of any living being. Since most studio alumni want to know more about the aquatic world and its inhabitants, they pass to the new learning stages of the *Growing up at the Aquarium* project.



Fig. 7. Preschoolers getting acquainted with the profession of ichthyology in the Research Building for Adaptation of the Primorsky Aquarium.

second "Young The learning stage, ichthyologist", is aimed at children aged 7-8. During the classes children will "dive" into the world of aquarium fish to become familiar with their behaviors. The pupils will learn how to produce conditions favorable to certain fish species and collectively choose plants and fishes to add to their common fish tank which will be later placed at one of the Aquarium's exhibits. This stage is designed to plunge a pupil into research activity and involve him into the process of creating a habitat comfortable for his/her pet fish. A child who knows special aspects of keeping of aquatic inhabitants will never do any harm to them and learn what 'taking care of someone' means.

The following stage, a "Man and Sea" art studio, is designed for children at the age of 9–10. Its purpose is to cultivate empathy in a child. Pupils will learn about the harmony of the surrounding world and see the beauty of marine inhabitants. With the help of collaborative and individual art projects children will understand how to tell people about the problems of the World Ocean, and what each individual can do to protect it. The participants will express their feelings through creative art works and share their experiences with peers within scheduled meetings (Fig. 8).

"Aqua Laboratory", the fourth stage of the project, invites school children aged 11 and older and is intended to develop personal responsibility and team working skills in children. The classes will be held in the Aquarium's learning laboratories equipped with research microscopes. Children will study microscopic organisms constituting plankton and investigate a complex life cycle of marine invertebrates and specifics of their anatomy (Figs 9, 10).



Fig. 8. Children depicting the world of aquatic animals.



Fig. 9. The world in a water drop can be seen using a light microscope.



Fig. 10. Modern research equipment used in educational projects of the Primorsky Aquarium.

One of the "Aqua Laboratory" sections is dedicated to the evolution of life in the ocean. Using the paleontological collection and exhibits of the Aquarium, pupils will trace how anthropods, mollusks and chordates have been changing in the course of millions of years. The stage also includes paleontological excursions to the Zhitkov Peninsula of Russky Island where Triassic layers crop out. A separate section of the program deals with dinosaurs and ichthyosaurs, and the final part of the stage is dedicated to man and his place on Earth. The *Growing up at the Aquarium* classes are provided free of charge by specialists of the Environmental Education Center of the Primorsky Aquarium and take place several times a week.

The project is designed to bring up small biologists, starting with children of preschool age.

And even if in future the project alumni choose some other professions, not connected with natural sciences, they will turn into environmentally educated people taking good care of our planet.

The website of the Primorsky Aquarium is www.primocean.ru.

# **Charting a Course for Sustainable Fisheries**

Julie Packard Monterey Bay Aquarium, USA

**ABSTRACT:** Over a quarter century has passed since the international aquarium community gathered in Monaco at the first IAC meeting to share ideas and best practices. Over time, our missions have evolved as the world has changed, toward more focus on our role in solving the growing environmental crisis that surrounds us. But, are we doing enough? Freshwater aquatic systems are a basic requirement for human survival, from drinking water to food security. And, healthy ocean and aquatic ecosystems are critical to enabling life on Earth to exist. Their future will determine our future... and, in fact, our very survival. The ocean is our lungs, our pantry, our playground, a massive driver of global commerce and a storehouse for innovation to meet human needs. But, we know now that these aquatic systems are changing at a dangerous pace.

Our global community of aquariums has a massive untapped opportunity to turn this tide. We have done a good job of helping millions of visitors know more and care more about the ocean and aquatic life, but we haven't done nearly enough to guide them to take the next step. Fortunately, we have created an amazing array of effective models for ocean and aquatic conservation, from marine protected areas to fisheries governance reform to consumer movements for sustainable seafood. And, we now have a road map in the new UN Sustainable Development Goals which include specific targets for freshwater and life in the sea.

I am confident we can turn the tide -- by investing in people and ideas to demonstrate solutions, nurturing hope and aspiration, and showing our audiences how they can engage to make change. The collective action of everyone in this room -- whatever we decide to do at our institutions in the next few years -- will help shape the future for humanity on this planet.

# **INTRODUCTION**

The global aquarium community has done a fantastic job in providing experiences that are engaging, educational and fun for our visitors. And since we first gathered as an international community in Monaco 58 years ago, we've been talking about the urgency of taking our missions to the next level, to help people not only know more and care more about the aquatic world, but also to do more on its behalf. Today I'm happy to say that the vast majority of us have the word conservation in our mission statements. But are we doing enough to make good on that promise?

Healthy ocean and aquatic ecosystems are critical to life on Earth. Their future will determine our future. The ocean is our lungs, our pantry, our playground, a vast driver of economic security and a storehouse for innovation to meet human needs. But, we know now that the aquatic systems that provide these services are being degraded at a dangerous pace.

# THE CHALLENGE WE FACE

The ocean today is changing before our eyes, becoming more impoverished in biodiversity and more unpredictable in the services it provides to humanity. Scientists agree on the overall causes of this decline: Unsustainable fishing; land-based pollution including plastic pollution; and climate change. Global climate change is clearly the biggest threat to healthy ocean ecosystems. Already greenhouse gas emissions are causing big changes in ocean circulation and ecosystem composition. Pollution from land-based sources – plastic, nutrients, and chemicals – is a second major concern. Unsustainable fishing is the third big impact area; how much seafood can we extract from the sea and still expect it to function and to feed the millions who depend on it for sustenance and livelihoods?

World catch of wild fish peaked in the mid-1980's and has remained level. Since that time, aquaculture has escalated to meet the growing global demand, expanding at a nearly exponential pace to meet the needs of a growing population. While many forms of aquaculture have undesirable environmental impacts, done in a sustainable way, aquaculture will be key to enabling food and economic security to millions of people. The good news is that aquaculture practices are rapidly improving and on their way to sustainability. In contrast, we have a long way to go to achieve sustainable wild fisheries.

Humans have looked to the sea for sustenance for thousands of years. What has changed is how we fish and the scale at which we do it. Global wild fishery production has peaked, with over 4 million vessels catching 80 million metric tons in 2016. Of the large scale commercial and managed fisheries for which we have data, 33% are overfished, 60% are fully fished, and only 7% are considered underexploited (FAO 2018). The global average rate of overfishing has grown steadily over the past 30 years and in many regions shows no sign of slowing until effective management action is taken. For many fisheries, such as tunas, overfishing is occurring at a much higher rate. In addition to the basic harvest of seafood, our increasingly effective fishing methods are wreaking havoc on ocean life including many valuable food species, and it is estimated that 9-15% of global fish catch is thrown away as wasted non-target species (Gustavsson et al. 2011). Fishery management is generally very poor in most countries, characterized by a lack of science to inform policy along with ineffective regulations and enforcement. Many countries lack sufficient data capability or governance to address the crisis in their waters, not to mention the crisis in international waters governed by ineffective treaty organizations. Illegal, unregulated and unreported (IUU) fishing is rampant. Solving the IUU fishing problem and achieving traceability of sustainable and legal seafood is exacerbated by the highly complex nature of the global seafood supply chain. Finally, today's industrial scale seafood harvest technology and even small scale fishery methods are causing rampant degradation of ocean ecosystems, from bottom trawling to coral reef dynamiting.

#### FISHERIES PROBLEMS ARE SOLVABLE

Unlike many of the intractable environmental problems humanity is trying to turn around, the good news about fisheries is that we have the solutions. We know what to do. We know what a successful model looks like, the ingredients for success and we've seen time and time again how severely depleted fisheries can recover to provide food and economic security once again. Attention is finally escalating to action on the global stage at a growing number of international conferences on the subject, building on the remarkable progress made so far by business and non-governmental organizations (NGO) leaders. New revelations about the scale of IUU fishing and the dark secrets behind the human rights abuses in the seafood industry have brought a new lens and a new urgency that has captured the public's attention.

The U.S. aquarium community has been a huge driver of this success through our collaborative work to raise public awareness of sustainable seafood through Seafood Watch. Together, we have helped build a global sustainable seafood movement, built on the premise of leveraging the buying power of the U.S. market which imports over 80% of the seafood Americans eat.

Seafood Watch assesses seafood sustainability across a range of dimensions consistently applied, based on published data from governments and the scientific literature. Our team has rated nearly all globally traded and domestic seafood products consumed in the U.S., however information is still sparse at the global scale. Third party certification programs like the Marine Stewardship Council and Aquaculture Stewardship Council are making progress but to date, only 7% of global seafood is certified and many fisheries lack the data to even being to assess their status.

Seafood Watch is based on the following theory of change. Consumers create market demand; businesses commit to sustainable sourcing; producers improve production practices; and governments improve regulations to lock in effective management long term.

In the early years, Monterey Bay Aquarium's Seafood Watch pocket guide and underlying seafood sustainability rankings were focused on building consumer awareness. We also enlisted the help of celebrity chefs to talk about the issues and their own commitment to sustainable seafood, broadening our audience and our media reach.

As media attention and business interest grew, we turned to a higher impact strategy to enlist sustainable seafood commitments from the large retail and food service corporations in the U.S., in turn leveraging their buying power to demand change among producers. As interest grew, a robust community of collaborating NGOs evolved to meet the need. Along with the U.S. aquarium partners, these groups coordinate their strategies and work collaboratively through the Conservation Alliance for Seafood Solutions. Today, thanks to everyone's collective efforts, nearly all of the big grocery retailers and food service companies in the U.S. have made time-bound commitments to source only sustainable seafood that meets Seafood Watch guidelines.

We now have moved to the next big challenge: meeting the growing demand for sustainable seafood products. This has required a shift in strategy. We now are focusing on working directly with seafood producers to meet sustainable standards required by their buyers. This means advising them on what aspects of their fishing and aquaculture practices need to improve to meet an acceptable Seafood Watch rating. Seafood Watch sustainability criteria and assessment methods have become the most widely accepted sustainability standard for seafood worldwide, and our team of staff and global contractors have produced assessment reports for thousands of global fisheries, with more to come. Beyond big business commitments, Seafood Watch standards are increasingly used as the basis for regional and country-wide initiatives for sustainable seafood, from Brazil to Southeast Asia.

## SEAFOOD SOLUTIONS AT SCALE

Today we are working with our NGO partners to effect change on a regional and global scale. An example of this is the Southeast Asia Sustainable Fisheries and Aquaculture Initiative. As part of this initiative, at the recent Our Ocean Conference in Bali last week I was excited to announce our partnership with two of the largest seafood companies in the world – Thai Union and Minh Phu Seafood - to improve practices of their thousands of shrimp farms in Viet Nam and fishery and aquaculture farms across Southeast Asia. Thai Union committed \$28 million to this effort, which is unprecedented. Minh Phu committed to bringing 20,000 shrimp farms in the Mekong Delta up to a Seafood Watch green rating by 2025 year. This will give these farmers access to the U.S. market and reduce the damaging impacts of current shrimp farming practices, from water pollution to clearing of mangrove forests that sequester carbon and protect communities from devastating tropic storms.

As we move forward, we are continuing to refine and improve our definition of sustainability. Seafood Watch and NGO Liberty Asia recently created a human rights risk tool to help businesses identify the labor practice risks associated with certain countries, regions and fisheries. This tool will give them guidance on where to delve deeper to demand higher standards where needed or refrain from doing business with bad actors.

At the broader scale, the Monterey Bay Aquarium's Seafood Watch program is part of our overarching model for conservation success that goes far beyond the markets-based work. We focus on linking the power of four strategies for impact: audience engagement, science, markets and policy. Voluntary business commitments are a start, but to lock in long term effective management, robust science-based policy approaches must be adopted by governments.

# A CRITICAL TIME FOR AQUARIUMS TO STEP UP

Achieving impact at a global scale is not something we can do alone and the success of the sustainable seafood movement in the U.S. has required a massive amount of collaboration among players across sectors and across the globe. Achieving victories like the tuna-fishing nations' recent agreement on the need for a plan to replenish severely depleted Pacific Bluefin tuna stocks required experts, advocates and international diplomacy work over a decade. I'm proud of the role of the aquarium community in these successes. And, I know we can do more. Toward this end, we have recently launched the Aquarium Conservation Partnership, a collaboration of 22 U.S. aquariums to expand our collective conservation impact.

Along with advocating for strong ocean protection policies, the Partnership has adopted plastic pollution as its primary focal area. To start, last year all member aquariums pledged to immediately eliminate all plastic straws from our food service and takeaway bags from our gift shops, and significantly reduce or eliminate plastic beverage bottles by 2020. Monterey Bay Aquarium has already phased out our single-use beverage bottles, along with all other single-use plastic in our front-of-the-house food service operations. We are also working with our food and retail service provider to reduce single-use plastic in our gift stores.

We know this isn't easy, which is why we work to champion businesses willing to make incremental change in the right direction. We hope the more committed we are to change, the more new and innovative solution strategies will emerge to solve the problem of ocean plastic pollution for good.

Each of our organizations has assets to bring to the cause of ocean conservation, and the time is ripe to take advantage of the growing global focus on the urgency of turning around the ocean's decline. Your engagement in conservation action in your own country will require its own approach. I offer these U.S. experiences only to inspire each of us to think about what we can bring to the cause. As an example, here in Japan, seafood businesses are starting to make sustainability commitments working with a Japanese organization called Seafood Legacy. In Japan, seafood is a deeply cherished part of Japanese culture, much more so than in the U.S. Seafood Legacy, which is not an advocacy NGO, is making great progress working directly with business on the premise that everyone wants to ensure that the rich Japanese seafood experience will be here for the next generation. Japan also has set a sustainability theme for the 2020 Olympics that includes goals for sustainable seafood.

World leaders have issued a global call to action to all of us who care about the future of the ocean and the people who depend it. For the first time the U.N. Sustainable Development goals have a dedicated goal for the ocean, #14 "Life Below Water." It states a mandate to "conserve and sustainably use, the oceans, seas and marine resources" with specific goals and timeframes for action. We must rally around this opportunity.

This is just the beginning. We have the power to inspire and engage through our exhibits and experiences, to get people to know more and care more about the ocean. It's time now to ask them to do more for the ocean. We need to lead the way, before it's too late. Global leaders are realizing we are running out of time to turn the tide on climate change and ocean health. We have the power to both inspire action and demonstrate success in our own countries and across the world.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- CEDERBERG, C., GUSTAVSSON, J., MEYBECK, A., SONESSON, U., VAN OTTERDIJK, R.,
- C. 2011, Global food losses and food waste Extent, causes and prevention.
- FAO. 2018, The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. License: CC BY-NC-SA 3.0 IGO.

# Aquaria Food Sustainability: Optimising Aquarium Feeding Strategies by Utilising Aquaculture Feeding Practices and Developments

•Tony McEwan<sup>1</sup>, Maryke Musson<sup>2</sup> South African Association of Marine Biological Research<sup>1</sup> Two Ocean Aquarium<sup>2</sup>



# Aquariums

Exhibits hold many species and size range of individuals large.

The types of feeding varies from carnivore to herbivore.





Developed a general balanced aquarium pelleted feed.

### Important features:

- Formulation maintenance (lower protein and fat) pre-mixes (vitamins, colorants, etc.)
- Ingredients what is available locally
- Manufacturing technique floating / sinking
- Pellet size

#### 9

- Assumption: what we have been feeding for the past 50yrs is successful.
- Artificial feed (pellets) tested against what we currently feed.
- Feed experiment duration 4 months.



10

Test was as Scientifically Rigorous as possible. Two common species randomly mixed into two tanks.



11



Length/Weight (fish BMI) Blood at start and end (hematocrit, metabalomics) Statistics – four populations, normality; equal variance; growth slopes; ANOVA; non-linear mixed effects model





# Results:

- 1. Fish healthy no mortalities
- 2. No difference in populations (growth)
- 3. Diets similar
- Assumptions correct;
   a) nutrients similar
   b) food acceptance
   similar
- A. Hematocrit
  - B. Metabalomics

13

# Results:

- Pelleted feed 39% cheaper
- Amount pellets fed 41% less
- Total Cost saving 83%

#### Consequences:

- In 8 months saved 6 tons wet feed out of 22 tons per year
- Happy boss
- Never completely replace wet feeds
- Dubai Aquarium and Underwater Zoo tested artificial feeds and found good health husbandry and cost benefits

#### Consequences:

- Less feed preparation
- · Easier feeding
- Less mess in water
- Testing of more feeds
- Testing of additives that claim improved digestibility





17

15

Take note of Aquaculture feed and other developments.

Increase utilization of artificial feeds:

- Cost savings
- Nutritional control
- Increase sustainability

# Sustainability

- · Use less wet feed
- Feed companies sustainability statements – nutrition, ingredients, operations, community responsibilities

19

## Aquarium Sustainability Program:

- Energy saving lights, pumps, heaters
- Less waste recycling
- Less effect on environment carbon footprint

20



21

18

# Experimental Captive Breeding and Exhibition of Post-Larvae "Shirasu" of the Japanese Anchovy *Engraulis japonicus*

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ABSTRACT: In Japan, variety of fish species are considered valuable food sources. Post-larval stage of Japanese Anchovy Engraulis japonicus is one of common traditional ingredients used in Japanese cuisine known as "Shirasu". In this study, we revisited current captive breeding techniques of the Japanese Anchovy and developed optimized techniques for culturing this species. Initially, live adult fishes caught in Sagami Bay were transported to the Enoshima aquarium. A fiberglass reinforced plastics tank, containing approximately 3500 L seawater was used to hold adult fishes. The lights on the tank were kept on for approximately 16 h/day. The anchovy in the tank received and consumed sufficient amount of food (over 5% of fish weight/day) for stimulating their growth. Under this condition, they matured and spawned eggs after approximately one month. The eggs (axis length: about 1.3 mm) were collected in plankton nets (0.5 mm mesh) and floated in tanks containing 600 – 1400 L sea water until hatching. The hatched larvae (total length: about 3.0 mm) received Brachionus plicatilis, Artemia salina, and pellets as food. They grew to over 30.0 mm TL after 30-40 days and gradually metamorphosed into juveniles. When they were kept under the same conditions as the adults, they grew to over 70.0 mm TL and matured after 5 - 7 months. We displayed the different development stages of the Japanese anchovy larvae, with explanatory information about the species and video footage of the moment of egg hatchings on small monitors at the Enoshima Aquarium from April 16, 2014, to the date. There are, however, some aspects related to cluture of this species that have not yet been completely resolved. For example, maintaining stable culture of the most important larval food, Brachionus plicatilis, is relatively difficult to achieve.

#### **INTRODUCTION**

Japanese anchovies (*Engraulis japonicus*) are distributed in the seas around Japan off the coast of Honshu, and around Taiwan, the Philippines, and Sulawesi Island (Miyachi *et al.*, 2013). "Shirasu" is a common name designating to the larvae of several fish species found in Japanese water, such as anchovy, sardine, ayu, and eel. Larvae harvested in Sagami Bay near the Shonan region of Japan are specifically called "Shonan Shirasu" and are an important traditional Japanese food ingredient (Mitani, 1987). Several studies have been done on their culturing and breeding. However, it is difficult to exhibit live larvae in an aquarium over a long period of time.

In this study, we revisited the current breeding techniques commonly used with Japanese anchovies in captivity then developed more suitable methods for culturing this species. We here present this process and discuss topics emerging from it.

# THE NEW EXHIBITION "SHIRASU SCIENCE"

We started to study breeding techniques of Japanese anchovy at the Enoshima aquarium in February 2013. The first experimental display "Exhibition of live whitebait" was opened in April 2014. A series of displays focusing more on their development ("Shirasu science") opened three years later in May 2017 (Fig. 1A-B, Fig. 4). A large tank displays juvenile and adult anchovies together with some other species of local fish (e.g., *Pleuronichthys japonicus* and *Sillago japonica*).



Fig. 1. The facilities of "Shirasu Science" A: Frontview of exhibition. B: Behind of exhibition tanks (culture tanks for the anchoby larvae). C: Culture tanks of live food, rotifers.

Two smaller tanks display exhibits two different stages of anchovy's larval development. Information labels and looped videos were placed nearby in order to explain the life cycle and ecological context of the anchovies. The exhibition remains active to this day, with the sixth captive bred generation larvae being now on display. Next, we discuss some studies we performed as well as issues relating to the maintenance of the exhibition.

### ADULTS AND EGGS

## Maturing adult fish

In June 2013, live wild adult anchovies that had been captured in Sagami Bay were transported to the Enoshima aquarium. Captured adult anchovies were kept in a fiberglass-reinforced plastic tank containing approximately 3500L of seawater. The lights in the tank were kept on for approximately 16 h/day. The fish were fed and consumed sufficient amount of food (in excess of 5% of fish weight/day) to promote their growth. They matured and began spawning after approximately one month

(Kawaguchi *et al.*, 1990; Yoneda & Kitano, 2012). Larvae were kept in different tank systems from adults till it reaches to juvenile stage. Juveniles matures five to seven month after they moved into the adult fish system. These processes were repeated over generations of captive breeding.

#### Egg development

Eggs (approximate axis length 1.3 mm) (Fig. 2A) were collected in plankton nets (0.5 mm mesh size). Because adult fish spawn at night, the nets were fastened to the drain pipe connecting from the main tank to the filtration tank in the evening and the eggs were recovered in the next morning. The eggs were moved to tanks containing 600-1400L of sea water until hatching. The eggs hatched about 48 hours in water temperature around 20  $^{\circ}$ C (Fig. 2B).

#### Egg toughness

Fertilized eggs are relatively resistant to minor physical shocks and can therefore be safely transported from the collecting net to the tank before hatching. (This transfer is not possible with live larvae.) Fertilized eggs may also be transported over long distances. We also studied relationship between the water temperature and the hatching rate of the eggs (Fig. 3). Eggs hatched at temperatures between 13 °C and 32 °C, however, more than 70% of all eggs hatched between 15 °C and 28 °C. The hatching rate around 20 °C was generally high.

# FROM THE PRE-LARVA TO THE JUVENILE STAGE

#### Culturing at the pre-larva stage

Anchovies at the pre-larva stage are very small, with a total length of approximately 3 mm (Fig. 2B). They consume nutrient from large yolk sack for approximately 48 hours. During this stage, they do not have ability of swimming and rather suspend in the water column. Therefore we used kreisel tank (approx. water volume 20 L - specifically designed to keep jelly fish) to keep pre-larva. In order to grow pre-larva to juvenile stage, however, we need to use much larger tank (approx. water volume 1400 L) for both culture and exhibit purpose (Fig. 1B).

#### Culturing at the post-larva stage

At the initial post-larva stage, total length typically reaches 4 mm TL. Because of their small mouth, they can only eat small zooplankton in the first three weeks after hatching (Imai & Tanaka, 1996). It is therefore necessary to culture small live food sources, such as sea rotifers (*Brachionus plicatilis*), which have typical size of 0.1 mm. Kept density of rotifer at 15 rotifers/cm<sup>3</sup> in the tank was normally sufficient to feed the post-larvae. After consuming rotifers over about two weeks in 20 $\Box$ C environment, the post-larvae grow to 10 mm. At this stage, they can then eat larger organisms, e.g., nauplius of *Artemia* (small strain from Vietnam, approximate size 0.3 mm) fed along with rotifers (Fig. 2C).



Fig. 2. Photographs of several stages of Japanese anchovy.

A: egg (immediately after spawning). B: egg and prelarvae. Black scales indicate 1 mm. C: Post-larva. D: Juvenile. White scales indicate 10 mm.



Fig.3. Relationship between water temperature and hatching rate.

Spots indicate average hatching rate.

Vertical bars indicate standard deviations.

Brackish water is arguably a good medium for larval cultures. Japanese anchovies occasionally enter river streams and larvae has been recorded that caught around the estuary in the wild (Mitani, 1987). We therefore studied their tolerance to salinity. When the water salinity was reduced gradually to a level just above 3 per mil, most fish survived for more than three days. The salinity of water in the tank was reduced further and then kept to approximately 25 per mil (Fig. 4). At this salinity, larvae looked healthy and their live rotifer food was also likely to survive well. The Approximately two months after hatching post-larvae become juveniles (typical larger than 30 mm TL) and could be transferred carefully to the larger tank (Fig. 2D). At this stage, they could eat small feed pellets such as the New Artech K-2 (Chlorella Industry Co.). They were grown in the exhibition tank or mature in the tank at behind the scene.



# **ROTIFER CULTURING**

Culturing large numbers of anchovies in the post-larva stage requires culturing suitable small live food, rotifers as well at our aquarium (Fig. 1C). Live rotifers are very popular in fish and invertebrates breeding facilities, and various studies have focused specifically on its culturing techniques (Ishibashi et al., 2003). We have adopted current culturing practices, such as extensive continuous culturing and batch culturing, on a daily basis. Rotifers are fed with live phytoplankton, Chlorella. When the conditions of the tank water are adequate and with right Chlorella concentration, rotifers breed rapidly. Maintaining stable culture of the most important larval food (Brachionus plicatilis) is relatively difficult to achieve. We culture the rotifers carefully using proven techniques and state-of-theart knowledge (Yamauchi, 1993; Yoshimatsu, 2001; Yoshimura, 2002; Tomoda et al., 2007; Tanaka, 2013).

### CONCLUSION

We succeeded in breeding and exhibiting Japanese anchovies from post-larva stage to adult. The exhibition of this kind is currently only presented at the Enoshima aquarium. Our research has also yielded useful insight into larval culturing. For example, the eggs were found to be relatively durable, whereas the larvae themselves are very fragile and require careful handling. Maintaining a stable culture of live food rotifers is relatively difficult but important nonetheless. We aim to continue improving our breeding and culturing techniques for the "Shirasu". Important new information on the life cycle and biology of Japanese anchovies may also be gathered by this aquarium setup.

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#### REFERENCES

- IMAI, C., TANAKA, S. 1996, Effects of sea water temperature on growth under unfed conditions and marginal feeding conditions for first feeding in Japanese anchovy Engraulis larvae, Journal of National japonicus *Fisheries University.* **45**(2): 39-45. (In Japanese with English abstract)
- ISHIBASHI, Y., OZAWA, M., HIRATA, H., KUMAI, H. 2003, Ontogenic changes in various stress tolerances of larval and juvenile

red sea bream *Pagrus major*. *Nippon Suisan Gakkaishi*. **69**(1): 36-43. (In Japanese with English abstract)

- KAWAGUCHI, K., YAMASHITA, Y., HAYASHI,
  A. 1990, Some aspects of spawning of the reared Japanese anchovy (*Engraulis japonicus* H.) in relation to the photoperiod, water temperature and starvation. *Bulletin of the Japanese Society of fisheries Oceanography*. 54: 364-372.
- MITANI, I. 1988, Food habits of Japanese anchovy in the shirasu fishing ground within Sagami Bay. *Nippon Suisan Gakkaishi*.
  54(11): 1859-1865. (In Japanese with English abstract)
- MIYACHI, S., MAYAHARA, T., TSUSHIMA, K., SASADA, K., KOHNO, E. 2013, Using δ13C and δ15N, the study of migrated population for Japanese anchovy, *Engraulis japonicus*, in Sagami Bay, Japan. *Papers on environmental information science*. **27**: 27-32. (In Japanese with English abstract)
- TANAKA, K. 2013, III-3. Relation between growth of rotifer and nutrition condition under photoirradiation. *Nippon Suisan Gakkaishi*.

**79**(5): 888. (In Japanese with English abstract)

- TOMODA, T., KOISO, M., SHIMA, Y. 2007, Dietary value of marine rotifer *Brachionus plicatilis* after enrichment produced by batch culture and extensive continuous culture methods. *Nippon Suisan Gakkaishi*. **73**(3): 505-507. (In Japanese with English abstract)
- YAMAUCHI, S. 1993, Effect of antibacterial substances on the growth of rotifer *Brachionus plicatillis*. *Nippon Suisan Gakkaishi*. **59**(6): 1001-1006. (In Japanese with English abstract)
- YONEDA, M., KITANO, H. 2012, III-2. Tank experiments: Japanese anchovy *Engraulis japonicus*. *Nippon Suisan Gakkaishi*. **78**(4): 807. (In Japanese with English abstract)
- YOSHIMATSU, T. 2001, Feeds for high density mass culture of rotifer. *Nippon Suisan Gakkaishi*. **67**(6): 1144-1145. (In Japanese with English abstract)
- YOSHIMURA, K. 2002, Techniques for highdensity mass culture of rotifer. *Nippon Suisan Gakkaishi*. **68**(5): 629-632. (In Japanese with English abstract)

# **Frilled Shark Research Project**

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**ABSTRACT:** Marine Science Museum of Tokai University and Aquamarine Fukushima jointly started-up a new Frilled Shark research project in April 2016. This was a follow-up of a study conducted back in 1980s, through which we gained preliminary experience with the shark. In the current project, we gained further experience of culturing both embryos in egg shell and fetuses without egg shell. The previous record of culturing embryos was up to 134 days, but we managed to extend the record to 154 - 361 days in the culture experiments in 2016. In spring 2017, we obtained fetuses with external yolk sacs without egg shell. We conducted culture experiments on these fetuses (144 - 420 mmTL) and managed to culture a fetus as long as 116 days.

# **INTRODUCTION**

In the 1980s, Marine Science Museum of Tokai University (MSM) conducted tentative research on the Frilled Shark. Chlamvdoselachus anguineus (Fig. 1), in Suruga Bay, thereby obtained preliminary knowledge on the captive breeding of the species. The study was suspended until the authors jointly started-up a new Filled Shark Research Project in April 2016. This project was motivated by the launch of the long-term exhibition of Frilled Shark in captive breeding by the two aquariums. In our previous study, a mature adult only survived about 1 week. However, we managed to rear shark embryos in egg capsules retrieved by laparotomy for a certain period of time. As a followup project, our objective this time was to demonstrate successful culturing of the shark embryos and fetuses for a more extended period.



Fig. 1. Frilled Shark, Chlamydoselachus anguineus.

# **ACTIVITIES OF THE PROJECT**

#### **Collection of Frilled Sharks**

Since we started-up the project, we have been actively collecting specimens in collaboration with the local fishery. As a result, we were able to collect in total 20 adult Frilled Sharks (8 Male, 12 Female). All Frilled Sharks were captured as bycatch by the local shrimping and gill nets. They were collected most frequently in April and May during the height of the spring shrimping season.

#### Culturing embryos in egg shell

A mature female shark was collected and brought in to MSM on 17th May 2016. The condition of the shark deteriorated after a few days, therefore, 4 fertilized eggs had been retrieved by laparotomy. Among them, small embryos were observed on the surface of yolk sac of 3 eggs (Fig. 2). Therefore, we conducted risk diversification and temperature matching experiments for culturing embryos in egg shell. The previous record of embryos survival in the 1980s was up to 134 days (Shiobara et al. 1997), but we managed to extend the record to 154 - 361 days.



Fig. 2. Fertilized egg of Filled Shark with embryo.

#### Culturing fetuses without egg shell

In spring 2017, we obtained fetuses with external yolk sacs of different sizes without egg shell for the first time in our project (Fig.3). Since there had been no previous report on the successful culturing of the shark fetuses with external yolk sac, we decided to make further culturing experiments on these fetuses ranging in size from 144 - 420 mm

in length. There were variations in the survival rates, but we managed to culture a fetus as long as 116 days.



Fig. 3. Fetuses of Filled Shark without egg shell. Fetuses with large external yolk sac (A). Fetuses with small external yolk sac (B).

### **Exhibition of fetuses**

We made an exhibition open to public in MSM and Aquamarine Fukushima on 9th June 2017. This became the world's first captive exhibition of the frilled shark fetuses without egg shell. We exhibited the fetus for about 2 months (Fig.4).



Fig. 4. The Filled Shark fetus in an exhibition tank.

#### ACKNOWLEDGEMENT

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### REFERENCES

Shiobara, Y., K. Suzuki and S. Tanaka. 1997, Ecological Note and Rearing on the Frilled Shark from Suruga Bay, Proceedings of the International Aquarium Congress Tokyo: 75-78

# Activity of Reproductive Management Committee for Sand Tiger Sharks

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

# **INTRODUCTION**

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

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

To exchange more reproductive information about C. *taurus*, Reproductive committee for Sand Tiger Sharks (RMCS) in Japan was established with Six aquariums, MARINEWORLD unionnakamichi (MW), Keikyu Aburatsubo marine park (KAM), Ibaraki Prefecture Oarai aquarium (IPO), Science museum Tokai University (TUM) and Yokohama Hakkeijima Sea paradise (YHS), and Shinagawa aquarium (SA) joined from 2018. We introduce the basic data of RMCS, our current study, and future plan in this paper.

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

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

# BASIC PHYSICAL CHARACTERISTICS AND CONDITIONS OF RMCS

## Sex ratio of population.

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

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

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

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

#### Water temperature condition

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

#### Lighting

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

## **REPRODUCTIVE BEHAVIOR**

# Male - Effect of water temperature and photoperiod

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



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

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



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

#### Female

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



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

#### **Reproductive steroid hormone levels**

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



Fig. 4. Free swimming blood sampling.

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

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



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



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

# Relationship between food consumption and breeding status in female.

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

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



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



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

#### In Situ, Resarch

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

## Monitoring program –Photo identification

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

#### Fresh mating scars in female

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



Fig.9. First observation of fresh mating scars.

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#### REFERENCES

- Bass AJ., D'Aubrey JD., Kistnasamy N. 1975, Sharks of the east coast of southern Africa. IV. Yhe families Odontaspididae, Scapanorhynchidae, Isuridae, Cetorhinidae, Alopiidae, Orectolobidae and Rhiniodontidae, *Invest Rep Oceanogr Res Inst Durban.* 39: 1-102
- Branstetter S., Musick JA. 1994, Age and growth estimates for the sand tiger in the North Atrantic Ocean, *Trans Am Fish Soc.* 123: 242-254
- Bansemer CB., Bennett MB. 2009, Reproductive periodicity, localized movements and behavioural segregation of pregnant *Carcharias taurus* at Wolf Rock, southeast Queensland, Australia . *Mar Ecol Prog Ser.* 374:215-227.
- Dicken ML., Booth AJ., Smale MJ. 2006a, Spital and seasonal distribution patterns of the ragged-tooth shark *Carcharias taurus* along the coast of South Africa. *Afr J Mar Sci.* 28(3):603-616
- Dicken ML,,Booth AJ., Smale MJ., Cliff G. 2007, Spital and seasonal distribution of juvenile and adult raggedtooth sharks (*Carcharias Taurus*) tagged off the east coast of South Africa. *Mar Freshw Res.* 58:127-134
- Davidson B., Cliff G. 2011, Liver lipids of female *Carcharias taurus* (spotted raggedtooth) sharks : a comparison between seasons. *Fish Physiol Biochem.* 37:613-618.

- Henningsen AD., Smale M., Garner R., Golden I., Marinr-Osorno R., Kinnunen N. 2004,. Captive breeding and sexual conflict *In :Smith M., Warmolt D., Thony D., Hueter R., (eds) Elasmoblanch husbandry manual. Ohaio Biological Survey, Columbus.*
- Henningsen AD., Murru FL., Rasmussen LEL.,Whitaker BR., Villetta GV. 2008, Serum levels of reproductive sreriod hormones in captive sand tiger sharks, *Carcharias taurus* (Rafinesque), and comments on their relation to sexual conflicts. *Fish Physiol Biochem*. 34:437-446.
- Henningsen AD., Claus E., Littlehare D., Choromanski J., Goedon E., Willson E. 2017,. Reproduction of the sand tiger sharks, *Carcharias taurus*, in aquaria: a framework for a managed breeding program, Chapter 37. *In :Smith M., Warmolt D., Thony D., Hueter R., Murry M., Ezucurra J., (eds) Elasmoblanch husbandry manual II .Ohaio Biological Survey, Columbus.*
- Licifira LO., Menni RC., Escalante AH. 2002, Reproductive ecology and abundance of the sand tiger shark, *Carcharias Taurus*, from the southwest Atlantic. *JCES J Mar Sci*. 59:553-561
- Gilmore RG.,Dodeill JW.,Linley PA. 1983 Reproduction and embryonic development of the sand tiger shark, *Odontaspis Taurus* (Rafinesque). *Fish Bull*. 81(2):201-225.
- Gilmore RG. 1993, Reproductive biology of lamnoid sharks. *Environ BiolFishl*. 38:95-114

- Pollard DA., Lincoln Smith MP. 1996, The biology and conservation status of the grey nurse shark(*Carcharias taurus* Rafinesque 1810) in New South Wales, Australia. *Aquat Conserv Ma Freshw Ecosystr*. 6:1-20
- Pollard D., Smith A. 2009, *Carcharias taurus. The IUCN Red List of Threatened Species*, Retrieved from www.iucnredlist.org/

# **Energy Consumption at the Aquamarine Fukushima**

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**ABSTRACT:** Energy consumption at the Aquamarine Fukushima (AMF) has been monitored since the aquarium opened in 2000. Energy consumption at the AMF decreased after the Great East Japan Earthquake Disaster of 11<sup>th</sup> March 2011, resulting from reduced system infrastructure (e.g., system pumps). Following the Great East Japan Earthquake, it was difficult to replace damaged equipment due to transport stoppages, closure of pump factories, etc. Even though aquarium infrastructure was only partially restored, AMF was reopened on 15<sup>th</sup> July 2011. At this time, only three of six pumps were available for the largest exhibit, the Sea of Kuroshio. The volumetric turnover time for this exhibit rose from one hour to two hours (i.e., it dropped from 24 to 12 turnovers per day). Many other exhibits also were operated at slower turnover rates. An energy reduction ratio of over 20% was recorded by the AMF when operating at this reduced capacity. Complete infrastructure replacement was concluded two years after the AMF re-opened. At this time, the Sea of Kuroshio exhibit had a full complement of six operating pumps. However, only three pumps were used to operate the exhibit from that point forward, as water quality was deemed acceptable using the slower turnover rate, and electricity consumption and energy costs were correspondingly significantly decreased. Reduced energy consumption resulted in a secondary effect of reduced CO<sub>2</sub> emissions. This operating strategy has beneficial implications for the environmental mission of the AMF.

This paper describes variation of energy consumption from 2000, reason of the heat source system renovation, in addition to that energy consumption reduction after the Great Disaster, and perspective of environmental friendly aquarium.

Key Words: Great East Japan Disaster, Reduction of electricity consumption, Reduction of CO<sub>2</sub> emission

# **1 INTRODUCTION**

The primary design concept of building services is reliability and environmentally friendly system. It is heavy duty for building services of aquarium to maintain the life of fishes, so the reliability is very important concept. Now a days global warming is one of the most important issue all of the world. But it was not serious in 1996. So it was effect to reduce the energy consumption not only for ecology but for economy.

Aquarium is a facility that consumes a lot of energy. So it has been big problem for AMF to reduce energy consumption since grand opening. The heat source system equipment must be carried out large scale maintenance after 10 years using. This maintenance cost was very high. Furthermore the natural gas price was very high in Onahama area. Because of these reason, the heat source system was changed from natural gas using system to electric heat pump system. That was very large scale construction, but the cost was near to the large scale maintenance of the heat source system, therefore heat source system renovation was chosen.

After large scale renovation of the heat source system, the energy cost was reduced and the environmental performance was good. But after only one year, the Great Disaster had come to the AMF.

# 2 DESCRIPTION OF HEAT SOURCE SYSTEM

# **2.1 Description of the heat source system from the grand opening**

The concept of the heat source system (before heat source system renovation) was "high reliability". So Co-Generation System (CGS) was chosen (co-generation system is called as Conbined Heat and Power (CHP) in Europe) as the main heat source system equipment. The CGS generates electricity and heat from natural gas. Therefore primary energy efficiency is high. The heat source system was composed of natural gas equipment and the electricity equipment. Natural gas equipment were CGS, Absorption Refrigeration and Heating Machine (RB) and Vacuum Boiler (B). Electricity equipment is Turbo Chiller (TR).

Table 1 shows the heat source equipment specification until heat source system renovation. Fig. 1 shows the schematic diagram of the heat source system. CGS generates the electricity and exhaust heat is collected from heat exchanger for heat source water in the same time. Exhaust heat from CGS is used to heating directly in the winter season. Exhaust heat is sent to the absorption refrigeration machine (AR) and AR makes cold water in the summer season. This is a basic CGS using.

Table1 Heat Source Equipment Specification until 2010

Description	Qty.	Specification	Location				
Turbo Chiller (TR)	1	Heating capacity:527kW	Main Machine Room				
Absorption Refrigeration Machine (AR)	3	Cooling capacity : 334kW	Main Machine Room				
Vacuum Boiler (B)	1	Heating capacity:930kW	Main Machine Room				
Co-Generation System (CGS)	1	Heating capacity:512kW	Main machine Room				
Absorption Refrigeration and Heating	2	Cooling capacity:703kW Heating	Main Machina Boom				
Machine (RB)	2	capacity:780kW					



Fig.1 A schematic diagram of the heat source system until 2010

Electricity is sent from electric power company and generated in AMF. So the reliability of the electricity is higher than normal heat source system. This system is good energy performance and economical condition if energy price is low. But if the natural gas price is higher economical condition becomes disadvantageous.

The heat source system consists of a cooling system, a heating system and a cooling / heating

switching system. A cooling / heating switching system supplies heating water in the winter season, supplies cooling water in the summer season. Cooling and heating is switched by season. A cooling / heating switching system supplies basically for air conditioning system. A heating system and a cooling system supplies for water treatment system and special air conditioning system.

Table2 Heat Source Equipment Specification after renovation

Description	Qty.	Specification	Location
Air cooled heat pump chiller for Cooling (CHP)	1	Total cooling capacity : 288kW	Outdoor Machine Area
Air cooled heat pump for Heating (HHP)	1	Total heating capacity : 215kW	Outdoor Machine Area
Air cooled heat pump chiller for Cooling and Heating 1(CHHP1)	1	Total cooling capacity :710kW Total heating capacity :574kW	Outdoor Machine Area
Air cooled heat pump chiller for Cooling and Heating 2(CHHP2)	1	Total cooling capacity : 355kW Total heating capacity : 287kW	Outdoor Machine Area
Turbo Chiller (TR)	1	Cooling capacity:527kW	Main Machine Room



Fig.2 A schematic diagram of the heat source system after renovation

# **2.2 Description of the heat source system after heat source system renovation**

The concept of the heat source system (after heat source system renovation) was "high Performance" and "simple system". So air cooled heat pump chiller was chosen. CGS and Vacuum Boiler were replaced by Air cooled heat pump (only heating). Absorption refrigeration machine was replaced by Air cooled heat pump chiller (only cooling). But Turbo chiller was not replaced, that chiller became a backup role. Absorption refrigeration and heating machine was replaced by Air cooled heat pump chiller (heating and cooling swiching).

Heat source system became very simple. Table 2 shows the Heat Source Equipment Specification after renovation. The grouping of heat supply is same as before. The composing of cooling system, heating system, cooling and heating switching system were same as before.

#### 2.3 Variation of energy consumption tendency

Fig.3 shows variation of monthly electricity consumption for 10 years. As a result of energy conversion, average consumption increased from 374MWh to 596MWh, peak load increased by 30%. Fig. 4 shows variation of monthly natural gas consumption for 10 years. After large scale renovation, natural gas consumption became 0.

Fig. 5 shows variation of monthly  $CO_2$  emission for 10 years.  $CO_2$  emission of AMF became from 359(T-CO<sub>2</sub>/month) to 285(T-CO<sub>2</sub>/month) after large scale renovation. That was 21% decreased.

# **3 DESCRIPTION OF THE ENERGY CONSUMPTION AFTER GREAT DISASTER**

#### **3.1 Introduction**

After the Great East Japan Earthquake and Tsunami, AMF suffered excessive damage. But AMF was reopened 4 months after the disaster. Many hard work and idea made it possible to reopen.

Main theme is focused on energy consumption in this paper.

#### 3.2 Variation of energy consumption from 2010

The energy conversion was completed after 2009. After one year operation of the new heat source system, the disaster occurred. There were so many difficulties to recover to the original AMF. However AMF reopened only 4 months after the disaster.

If the environmental effect of the building is evaluated the CO<sub>2</sub> emission is used. But almost nuclear power plant were stopped after the disaster, CO<sub>2</sub> emission factor of electricity was increased. So CO<sub>2</sub> emission of post disaster were compared with pre disaster, the result will be effected by variable factor. Therefore primary energy consumption was used for the evaluation of the environmental effect. Fig. 6 shows the variation of monthly electricity consumption after 2010. The electricity consumption disappeared at 11th March, the yearly average of electricity consumption decreased after reopening.

Fig. 7 shows the variation of monthly primary energy consumption after 2010. The average primary energy consumption since the renovation of heat source system until the Great disaster was 6,358[GJ/month]. But the primary energy consumption became



 $_{2000}$   $_{2001}$   $_{2002}$   $_{2003}$   $_{2004}$   $_{2005}$   $_{2006}$   $_{2007}$   $_{2008}$   $_{2009}$   $_{2010}$  Fig.3 Variation of monthly electricity consumption for 10 years



Fig.4 Variation of monthly natural gas consumption for 10 years









4,935[GJ/month] after reopened, the average primary energy consumption decreased 22%. After great disaster the saving energy intention was spread throughout Japan, so it was assumed that these mind affected the reduction of energy consumption. But it was impossible to reduce over 20% of primary energy consumption by only saving energy intention.

Name of exhibit	Qty of pump	Pump	Saving
	in operation	power	electricity
		[kW]	[kW]
Exhibit 1	2→1	2.2	-2.2
Exhibit 2	2→1	3.7	-3.7
Sea of Kuroshio	6→3	30.0	-90.0
Sea of Oyashio	3→2	15.0	-15.0
Brackish fish	2→1	3.7	-3.7
Southern sea	2→1	7.5	-7.5
Sea lion	2→1	5.5	-5.5
Former sea otter	2→1	5.5	-5.5
Total saving	—		-132.4
electricity			
Remarks			

Table 3 Operation pump and saving electricity afterreopening in 2011

When AMF reopened, it was impossible to replace all damaged pump. Because of pump manufacturer, logistics and so on. Therefore the exhibit with multiplex pumps operated with half number of pumps. Table 3 shows the operation pump and saving electricity after reopening in 2011. The total electricity consumption of the pump of Sea of Kuroshio was reduced 90kW. Sea of Kuroshio is the largest exhibit of the AMF. And the electricity consumption of the pump of Sea of Oyashio was deduced 15kW.

This operation was effective for the power saving. But it was difficult to maintain the quality of exhibit water, minimum quality had been kept. All pumps were replaced new equipment in June 2013. But this operation method has been continued until today.

## **4 CONCLUSIONS**

Large scale heat source system renovation succeeded to reduce energy cost and CO2 emission, approximately 30% of energy cost and 21% of CO2 emission was reduced. Hard work of all staffs and volunteers made early reopening after great disaster. The primary energy consumption was 33% reduced after great disaster. Total reduction of primary energy consumption was 32% before large scale heat source system renovation.

Many trial for reopening were good ideas for energy conservation and BCP (Business Continuity Plan).

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## REFERENCES

- Hisashi Wakayama et al. : History of the Aqua Marine Fukushima Equipment during the Lifecycle Part1 Long Term Repair Plan and Maintenance during 18 Years after Grand Opening, SHASE-J Vol.43 No258, Sep 2018
- Hisashi Wakayama et al. : History of the Aqua Marine Fukushima Equipment during the Lifecycle Part2 Large Scale Renovation of Heat Source System in the 10th Year from Grand Opening, SHASE-J Vol.44 No259, Nov 2018
- Hisashi Wakayama et al. : History of the Aqua Marine Fukushima Equipment during the Lifecycle Part3 The Actual Condition of Aqua Marine Fukushima Damage by the Great East
- Japan Earthquake and Tsunami, SHASE-J Vol.46 No261, Nov 2018

# Next Generation Water Treatment System: Ultrafiltration and Ozone-Enhanced Protein Skimmer

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**ABSTRACT:** Conventional aquatic water treatment systems use nitrifying bacteria in biological filters to detoxify ammonium nitrogen, a by-product of fish and invertebrate metabolism. These biological filters require a large space and need several weeks to stabilize after introduction. In addition, the use of biological filters necessitates replacement of 3 - 10% of system water each day to reduce accumulated nitrate nitrogen to acceptable concentrations. ORGANO Corp. and Kitasato University have developed a compact and easy to operate filtration system that reduces ammonia nitrogen using a combination of ultrafiltration and ozoneenhanced foam fractionation. This new system requires a small installation space, removes ammonium nitrogen immediately upon startup, and results in a reduced accumulation of nitrate nitrogen. We conducted a comparative test of conventional and new system. It was performed on a 100 L (approx.) aquarium supporting moon jelly of the species: Aurelia coerulea. The test was conducted with 2 cases. In the first case, acclimated nitrifying bacteria were not introduced. For the conventional system, all moon jellies died at early stage. On the other hand, all moon-jellies grew ideally in the new system. The second case was conducted with the presence of acclimated nitrifying bacteria in the conventional system. From the commencement of operation, both ammonium nitrogen and nitrate nitrogen were maintained at less than 0.1 and 3.0 mg N/L, respectively, with a seawater replacement of <0.1% per day in the new system. The ratio of wet weight to bell diameter of jellyfish showed an ideal growth compared to the conventional system throughout the trial (180days). Space required for the new filtration system is estimated to be half (50%) of that required for a more conventional water treatment system incorporating a biological filter.

# **INTRODUCTION**

In Japan, large-scale aquariums are built in urban and inland areas. These aquariums reuse seawater through water treatment systems[1].

An important role of the aquatic water treatment system is to detoxify ammonium nitrogen. While conventional aquatic water treatment systems use nitrifying bacteria in biological filters to detoxify ammonium nitrogen, nitrate nitrogen as a by-product is produced. High concentration of nitrate nitrogen is toxic to aquatic organisms. To avoid the accumulation of nitrate nitrogen, it is necessary to replace the aquatic water or add a denitrification system[2]. Seawater replacement increases the operation cost, effluent cost and environmental load by discharge of waste water. Adding a de-nitrification system increases the capital cost and requires a large space because of low reaction rate. In addition, the conventional system will need several weeks to remove toxic ammonium nitrogen with stability[3]. It is then possible that toxic ammonium nitrogen can be accumulated at start up.

Ammonia is decomposed to nitrogen by hypobromine[4]. We considered using the bromine in seawater to produce hypobromine by ozone oxidation. Furthermore, we use ultrafiltration for removal of turbidity and bacteria instead of sand filtration. In this paper, we described a compact and easy to operate filtration system that reduces ammonia nitrogen using a combination of ultrafiltration and ozone-enhanced foam fractionation.

#### **EXPERIMENTAL METHODS**

#### Water treatment system

We tried a comparison test between the conventional biological filter system (hereinafter, conventional system) and the ultrafiltration and ozone-enhanced foam fractionation system (hereinafter, new system).

In the conventional system, the flow passed through the nonwoven fabric filter, the activated carbon column, the ceramic filter and then returned to the tank (Fig.1).



Fig.1. Flow diagram of conventional system

On the other hand, in the new system, the flow passed through the safety filter, the ultrafiltration (UF), and the activated carbon column and returned to the tank (Fig.2). The UF concentrate passed through the ozone-enhanced foam fractionation, and then returned to the tank. The operating conditions are summarized in Table 1.

Moreover, we tested two cases. In the first case, the conventional system didn't use acclimated nitrifying bacteria, while it was used in the second case. Rearing animals in those systems were moonjelly, *Aurelia coerulea*. 20 individuals were used in first case, and 10 individuals were used in second case in each system.



Fig. 2. Flow diagram of new system

### **RESULTS AND DISCUSSION**

## a) First Case Growth of Moon Jelly

The ratio of wet weight to bell diameter of jellyfish in the new system showed an ideal growth for 30 days (Fig.3). The exponent of the power relation was 2.78. On the other hand, all moon-jellies died at early stage, about seven days, in the conventional system. The result was not in correlation with the water quality and reasons are yet to be concluded but the fact still remains that the new system showed ideal growth.

# b) Second Case Seawater Quality

Figs. 4 and 5 show the change of ammonium nitrogen and nitrate nitrogen concentration, respectively. At the start-up, the ammonium nitrogen concentration of the new system was lower than the conventional system because the biological filter was not yet effective in detoxifying ammonium nitrogen due to less acclimatization days. After a few weeks, both new and conventional systems detoxified ammonium nitrogen. But for nitrate nitrogen, there was a difference on the behavior of between two systems. While nitrate nitrogen of conventional system increased up to 11 mg/L, that of new system is less than 3 mg/L for 180

days. These results show the frequency of seawater replacement of the new system to control nitrate nitrogen is less than the conventional system.





Fig. 4. Ammonium Nitrogen.



Fig. 5. Nitrate Nitrogen

#### Growth rate of moon jellies

The ratio of wet weight to bell diameter of jellyfish in the new system showed an ideal growth for 180 days (Fig.6). The exponent of the power relation was 2.81. In the conventional system, the

power relation was 2.52 which is less than that of the new system. These results show that the new system provides better aquatic water conditions for moon-jellies.

## c) Installation space

As an example, we have estimated the installation area for  $300 \text{ m}^3$  aquarium unit based on these results. The installation area of the new system would be about half the space of the conventional system. (New system:  $315 \text{ m}^2$ , Conventional system:  $600 \text{ m}^2$ )



Fig.6. Growth rate (Second Case)

Table 1	. O	perating	conditions

Parameters	unit	Conventional	New
Moon-jellies	-	- Aurelia coerulea	
Convetor		Artificial Seawa	
Seawater	-	(Live SeaSalt) a	bout 3.5%
Tank Volume	L	125	110
Flow rate	turn/d	45	7
Density	kg/m <sup>3</sup>	0.1-3.5	0.1-5.5
Recovery	%/d	> 99.9	1
Temperature	°C	23-26	

#### CONCLUSION

The new system which uses a combination of ultrafiltration and ozone-enhanced foam fractionation controls the ammonium and nitrate nitrogen with the seawater replacement of <0.1 % per day. The ratio of wet weight to bell diameter of jellyfish showed an ideal growth for 180 days. Installation space required for the new filtration system is estimated to be half (50%) of that for the conventional system. This new system is expected to be a candidate for the new generation of water treatment system for aquariums.

# REFERENCES

- [1] M. E. Lickey, R. L. Emigh, F. R. Randle. 1970, A recirculating seawater aquarium system for inland laboratories, Marine Biology 7 : 149-152
- [2] So Kawaguchi, Rob King, Rob Meijers, et.al. 2009, An experimental aquarium for observing the schooling behaviour of Antarctic krill (Euphausia superba), Deep-Sea Research II 57: 683-692
- [3] Michael McGee., Charles Cichra. 1988, Principles of Water Recirculation and Filtration in Aquaculture, *Fisheries and Aquatic Sciences*, University of Florida : FA12
- [4] Hiroshi Tsuno. 2016, *Ozone handbook*, Japan Ozone Association : 68-7
## The Mission of Aquariums in Japan ~How We Show the Mission and Share with Citizen~

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**ABSTRACT:** Aquarium has to be a conservation stronghold however people inclines to regard aquarium is a recreational facility, not a conserving facility for nature. Because conservation activity undoubtedly needs to progress with citizen, mission describing about conservation should be share with stakeholder especially citizen. In this paper, what Japanese aquarium's mission says was investigated from their websites. A few Japanese aquariums affiliated JAZA (Japanese Association of Zoos and Aquariums) set the mission on their websites, and moreover the missions insisting about conservation were scanty. 31 out of 89 zoos affiliated JAZA appear their missions (35%) more than aquariums (15 out of 60 / 25%), and besides aquariums describing about conservation in their missions are only 2 (13%). Japanese aquarium collects many creatures from wild for maintaining the displays, the habitat for collected species has to be conserved by aquariums. This is why aquarium has to set mission for conservation and share with citizen to progress in-situ and exsitu conservation activity.

## **INTRODUCTION**

Nowadays that environment has destructed and degraded rapidly in the world, conservation efforts that aquariums and zoos endeavor have become critical. Because aquariums, especially, collect many creatures from wild for maintaining the displays, the habitat for collected species has to be conserved by the aquariums. Understanding and cooperation with citizen is essential for in-situ conservation activity, therefore the function of environmental education is significantly valuable. For ex-situ conservation, enlightenment about the circumstances of wildlife is important, as well as insitu conservation. In this paper, missions appeared websites of all 60 aquariums affiliated JAZA (Japanese Association of Zoos and Aquariums), and of associations of other regions in the world, have been investigated to reveal whether the mission states about conservation education or not. What each aquarium send the mission widely to citizen by website has to make society to perceive "Aquarium as a Stronghold for Conservation".

#### WHAT MISSION IS

Sufi and Lyons (2003) say that mission statement may convey a company's reason for being, outlining where it is headed and planning, how it will get there. Contents of mission statement are quite various and different by companies and organizations. Although many studies have insisted whether mission is exist or not is not associated with company performance, Dermol (2012) found out that value added per employee (VAE), which is one of the most important performance measures, has been associated with mission existence. Many aquariums, same as zoos, museums and botanical gardens, emphasize their missions for conservation because these institutions exhibit wildlife and have a special connection with nature (Miller *et al.* 2003).

#### **METHODS**

Missions for associations of zoos and aquariums in the world (JAZA, AZA, BIAZA, EAZA, PAAZA, SEAZA and ZAA) were investigated in order to compare what their missions mention.

Missions for all 60 Japanese aquariums and 89 zoos affiliated JAZA were also investigated from their websites between March and May 2017. We classified these missions into 4 categories which are "Conservation", "Education", "Research" and "Recreation" and extracted which categories the missions mention. Mission on website was investigated because publication on the websites represents what the aquarium inclines to send to stakeholder, especially to citizen.

#### RESULTS

#### Mission of zoos and aquariums associations

Missions of 7 associations of zoos and aquariums are below.

#### JAZA (Japan)

Contribute to improvement in culture, promotion of scientific technology and conservation for nature, and then contribute to fruition for symbiotic world between human and nature.

#### AZA (North America)

The Association of Zoos and Aquariums helps its members and the animals in their care thrive by providing services advancing animal welfare, public engagement and the conservation of wildlife.

#### **BIAZA (UK and Ireland)**

We lead and support our members to inspire people to help conserve the natural world, to participate in effective cooperative conservation programmes, to deliver the highest quality environmental education, training and research, to achieve the highest standards of animal care and welfare in zoos, aquariums and in the wild.

### EAZA (Europe)

To facilitate co-operation within the European zoo and aquarium community with the aim of furthering its professional quality in keeping animals and presenting them for the education of the public, and of contributing to scientific research and to the conservation of global biodiversity.

## PAAZA (Africa)

To guide and accredit all African Zoos and Aquaria to become effective and credible centers of animal welfare, conservation, education and research.

#### SEAZA (Southeast Asia)

To enhance the capabilities of Southeast Asian zoos to conserve wildlife. To provide education and recreation for the public. To improve the standards, status, and public images of zoos in South East Asia.

#### ZAA (Australasia)

To lead Australasian zoos and aquariums to work together to save wildlife.

#### Mission of Japanese zoos and aquariums

Number of aquariums appearing mission on their websites is 15 out of 60 facilities (25%) although zoos appear 31 out of 89 facilities (35%) (Fig. 1).



Fig. 1. Number of JAZA affiliated aquariums and zoos appearing mission on website.

In the result of that missions of all aquariums

and zoos affiliated JAZA in March to May 2017, which have been investigated from their websites, have been classified 4 JAZA's objectives ("Conservation", "Education", Research" and "Recreation"), zoos mention much more about "Conservation", "Education", "Research" and "Recreation" than aquariums. Only 2 out of 15 aquariums (13%) mentions about "Conservation", 5 (33%) mentions about "Education", 1 (7%) mention about "Research" and 2 (13%) mentions about "Recreation" on their websites, although 7 (23%), 18 (58%), 3 (10%) and 9 out of 31 zoos (29%) mention respectively (Fig. 2).





Fig. 2. Number of that JAZA affiliated aquariums and zoos appearing mission on website described about conservation, education, research and recreation in the mission.

#### DISSCUSSIONS

#### **Different missions between regions**

Table 1. Category of contents in mission of associations of zoos and aquariums in the world.

	conservation	education	research	recreation
JAZA	$\checkmark$		√	
AZA	$\checkmark$			
BIAZA	$\checkmark$	$\checkmark$	$\checkmark$	
EAZA	$\checkmark$	$\checkmark$	$\checkmark$	
PAAZA	$\checkmark$	$\checkmark$	$\checkmark$	
SEAZA	$\checkmark$	$\checkmark$		$\checkmark$
ZAA	$\checkmark$			

All associations investigated in this paper insist on "Conservation" by their missions (Table 1). It is meant zoos and aquariums exist for conservation. JAZA aims "Conservation" as a method in order to attain the symbiosis between human and nature, and AZA set "Conservation" for thriving the members and animals although other associations insist "Conservation" as one of their aims for existence. Japanese may have different thought or sense toward conservation from other countries. If so, aquariums and zoos in Japan should originally consider how to establish mission and to share with stakeholder such as citizen.

#### Function of mission for aquariums in Japan

Aquariums showing mission on their websites are scanty. These websites often describe news, administrative information and access, and mission statement is hardly appeared on first page of website. Moreover some aquariums describe mission as just atmosphere which seemingly intends people to come to the aquarium. Mission should be philosophy which is reason for being as aquarium.

Missions of aquariums and zoos mentioning about "Education" are relatively plenty. It represents aquariums and zoos consider they should be educational facility, and education about animal welfare or the status quo of wildlife is definitely profitable for conservation. However how does citizen recognize the function for aquarium? It probably is for recreational and enjoyable facility which is one of the aims. Aquariums, however, collect many creatures from wild for maintaining the displays, the habitat for collected species has to be conserved by aquariums. This is why aquarium has to set mission for conservation and share with citizen to progress in-situ and ex-situ conservation activity.

#### CONCLUSION

Japanese aquariums are still not enough setting mission toward conservation. They also need to drive on society forward conservation activity (or at least consent to the thought for conservation activity) because aquarium should be conservation base. In order to progress conservation efforts, support from citizen is undoubtedly significant. Aquariums desperately need to forward to attain the goal with society and must exist as a conservation stronghold.

#### REFERENCES

BRIAN, M., WILLIAM, C., RICHARD, P, R., CHRIS, W., DAVID W., DEVRA, K.,

- STEVEN, M., ALAN, R., BETH, A., MICHAEL, H. 2004, Evaluating the Conservation Mission of Zoos, Aquariums, Botanical Gardens, and Natural History Museums, *Conservation Biology*. 18(1): 1-8
- BRITISH AND IRISH ASSOCIATION OF ZOOS AND AQUARIUMS (https://biaza.org.uk/ourvision), 2018.10.24
- EUROPEAN ASSOCIATION OF ZOOS AND AQUARIA (https://www.eaza.net/about-us/), 2018.10.24
- JAPANESE ASSOCIATION OF ZOOS AND AQUARIUMS (http://www.jaza.jp/jaza\_pdf/library\_jaza/di29 /teikan20180101.pdf), 2018.11.5
- PAN-AFRICAN ASSOCIATION OF ZOOS AND AQUARIA (http://zoosafrica.com/), 2018.10.24
- SOUTH EAST ASIAN ZOOS ASSOCIATION (http://seaza.net/history-objectives), 2018.10.24
- TAHIR, S., HOWARD L. 2003, Mission statements exposed, International Journal of Contemporary Hospitality Management. 15(5): 255-262
- THE ASSOCIATION OF ZOOS AND AQUARIUMS (https://www.aza.org/), 2018.10.24
- VALERIJ, D. 2012, Relationship between mission statement and company performance. *Scientific Annals of the Alexandru Ioan Cuza University of Iasi Economic Sciences*, **59**(1): 321-336
- ZOO AND AQUARIUM ASSOCIATION (https://www.zooaquarium.org.au/wpcontent/uploads/2018/07/1\_2\_Constitution.pd f), 2018.10.24

## **Reproductive Parameters of Captive Sea Turtles in Okinawa Churaumi Aquarium**

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ABSTRACT: Okinawa Churaumi Aquarium, located on Okinawajima Island, Japan, has had a facility for sea turtle reproduction, rescue, and rehabilitation since 1994. The facility has an outdoor holding tank (16.8  $m \times 10.5 m \times 2 m$ ) with an open-water system and a sandy nesting area (115 m2). The water temperature of the tank ranges between 20 °C and 30 °C across a 12-month period, which is similar to the waters near Okinawajima Island. The turtles are fed at approximately 1-day intervals, with a diet that includes fish, squid, cabbage, and Chinese cabbage in quantities equivalent to 2% of their body weight. Loggerhead turtles, green turtles, hawksbill turtles, and a black turtle have produced 76, 100, 38, and 5 clutches in captivity since 1995, 1999, 2012, and 2017, respectively. For loggerhead turtles, the mean  $\pm$  SD of the number of clutches per year, clutch size, and emergence rate was  $3.9 \pm 1.1$  clutches (range: 2–6 clutches),  $113.0 \pm 19.7$  eggs (57–146 eggs), and  $10.4 \pm 21.1\%$  (0–89.9%), respectively. The corresponding values in green turtles were  $4.8 \pm 2.2$  clutches  $(1-8 \text{ clutches}), 91.9 \pm 32.4 \text{ eggs} (14-155 \text{ eggs}), \text{ and } 35.1 \pm 30.3\% (0-96.8\%), \text{ and those in hawksbill turtles}$ were  $2.6 \pm 1.2$  clutches (1–5 clutches),  $127.6 \pm 41.9$  eggs (46–194 eggs), and  $12.9 \pm 21.4\%$  (0–69.6%). A black turtle produced 5 clutches,  $45.2 \pm 13.8$  eggs (29–67 eggs), with an emergence rate of  $12.1 \pm 4.2\%$  (7.3– 13.8%). We suggest that the similarity of the environment at the facility to that in the wild may underlie the breeding success of these four sea turtle species. Further studies are required to extend our knowledge of the reproductive biology and ecology of sea turtles and improve the emergence rate.

## **INTRODUCTION**

The loggerhead (Caretta caretta), green (Chelonia mydas), black (Chelonia agassizii), hawksbill (Eretmochelys imbricata), olive ridley (Lepidochelys olivacea), and leatherback turtle (Dermochelys coriacea) are distributed around Okinawajima Island, Japan (Hirate and Kawazu, 2017a; 2017b; 2017c; Kino and Kawazu, 2014; Kino et al. 2015; Yoshikawa et al. 2016). These sea turtle species are listed as critically endangered in the International Union for Conservation of Nature (IUCN) Red List for threatened species due to the loss of their nesting grounds and their accidental capture in fishing gear (Wallace et al. 2011). The protection and restoration of sea turtle nesting grounds and improvement in fishing gear to reduce bycatch are clearly essential for the conservation of sea turtles (Wallace et al. 2011). In addition to conservation in the wild, captive breeding programs should also be actively implemented to assist with the recovery of these endangered species (Owens and Blanvillain, 2013).

Okinawa Churaumi Aquarium, located on Okinawajima Island, Japan, has had a facility for sea turtle reproduction, rescue, and rehabilitation since 1994. Loggerhead (Kawazu *et al.* 2015a; Kawazu *et al.* 2016; Teruya *et al.* 1997), green (Yanagisawa, 2012), hawksbill (Kawazu *et al.* 2015c), and black turtles (Kawazu *et al.* 2018) have already successfully bred at Okinawa Churaumi Aquarium since 1995, 1999, 2012, and 2017, respectively. The captive bred turtles are tagged and head-started after 1 year of captive rearing to survey their migration. Such captive breeding programs provide important insights into developing pioneering research approaches (Owens and Blanvillain, 2013). For example, captive breeding studies have yielded biological reproductive information on sexual maturation (Kawazu *et al.* 2015b), mating function (Kawazu *et al.* 2014b;



Fig. 1 Photographs of a sea turtle tank (A) and the artificial sandy beach (B) for nesting and hatching in Umigame-kan at the Okinawa Churaumi Aquarium.

2014c; Kawazu *et al.* 2015c; 2015d; Manire *et al.* 2008), and the reproductive cycle (e.g., spermatogenesis and vitellogenesis) of sea turtles (Kawazu *et al.* 2014b; Kawazu *et al.* 2015c). In addition, several techniques used for the captive husbandry of sea turtles (blood sampling and ultrasonographic diagnoses) have been used to study the reproduction and conservation of sea turtles in the wild (Owens and Blanvillain, 2013).

To develop captive breeding techniques, we recorded reproductive parameters, including the number of clutches per year, clutch size, internesting interval, and emergence success rate, in captive loggerhead, green, hawksbill, and a black turtle at Okinawa Churaumi Aquarium during 1995–2017. Our purpose was to clarify the captive breeding technique by comparing between captive and wild reproductive parameters.

#### MATERIALS AND METHODS

#### Captive animals and holding tank

All sea turtles were rescued form around Okinawa Island, Japan and transferred to holding tanks at the Okinawa Churaumi Aquarium, Motobu-cho, Okinawa Prefecture, Japan during 1994 and 2017. The turtles used in the captive breeding program were maintained in an outdoor holding tank ( $16.8 \times 10.5 \times 2.0$  m) that consisted of an open-water system with a sandy nesting ground (115 m2) (Fig. 1). The water temperature of the tank was measured daily for 1 year and ranged between 20 °C and 30 °C, similar to that of the sea surface around Okinawa Island. The turtles were fed a diet that included fish, squid, cabbage, and Chinese cabbage in quantities equivalent to 1–2% of their body weight, at 24-h intervals.

#### Husbandry for breeding

During autumn-winter, vitellogenesis of the adult females was confirmed using blood metabolite measurements, including the triglyceride, total protein, and calcium, because these blood metabolite concentrations increase during winterspring and are stored for follicular development (Kawazu et al. 2015a). One milliliter of blood was sampled from the jugular vein (either the left or right side of the neck) using a 70-mm 20-gauge needle (Terumo Inc., Japan) and a 10-mL syringe (Terumo Inc.) and then stored in heparin vacutainers (Fujifilm Inc., Japan), after which plasma was collected using a centrifugation (speed: 6000 rpm, time: 5 min). Plasma triglyceride, total protein, and calcium were measured from the plasma samples using a biochemistry autoanalyzer for animals (Fuji-drycem 7000 V; Fujifilm Inc., Japan).

During spring, the ovaries of the adult females

were observed to identify the presence of vitellogenic follicles and follicular development using ultrasonography with a 3.5-MHz probe (SSD-900, Aloka Inc., Japan) and a 5-MHz probe (Mini-dock P04341-04, Sonosite Inc., USA) at 1-year intervals. The turtles were placed in dorsal recumbency under a water depth of 200 mm, and the probes were positioned in the inguinal region of both the right and left rear flippers.

Although loggerhead, green, and black turtle females mated with males in the holding tank with a nesting ground, hawksbills females were unreceptive to males and prevented penile insertion into their cloaca by covering their cloaca with their rear flippers. Thus, hawksbill vitellogenic females were transferred separately from the holding tank to the pairing (mating) tank ( $5 \times 5 \times 1$  m, indoor, open water system) with one male, after which we observed presence of calcified eggs using ultrasonography. Females were returned to the holding tank with a nesting ground. The hawksbill males were housed separately to the females throughout all periods, except during the mating procedures.

## **Record of reproductive parameters**

The turtles of all species laid eggs on the nesting ground attached to the holding tank approximately 1 month after mating with the males in the holding tank (loggerhead, green, and black females) and the paring tank (hawksbill females). The date of nesting of each turtle was recorded; thus, the number of clutches per year and internesting interval were calculated. After nesting, the clutch size was recorded as the number of normal eggs in each clutch (i.e., eggs containing both albumen and volk: Miller, 1999). Moreover, emergence success rates (calculated as the ratio of the number of all hatchlings to all oviposited eggs) were recorded. These reproductive parameters for the four sea turtle species were presented as mean  $\pm$  SD and compared with wild values in previous studies (Hirth, 1980; Marquez, 1990; Miller et al. 1997). Data on the reproductive parameters for black turtles were taken from Kawazu et al. (2018).

### RESULTS

#### Loggerhead turtles

Three captive loggerhead turtles produced 76 clutches (9371 eggs) since 1995. The mean  $\pm$  SD of the number of clutches per year, internesting intervals, clutch size, and emergence rate were 3.9  $\pm$  1.1 clutches (2–6 clutches, n = 19), 13.4  $\pm$  2.3 days (9–19 days, n = 53), 113.0  $\pm$  19.7 eggs (57–146 eggs, n = 84), and 10.4  $\pm$  21.1% (0–89.9%, n = 81), respectively (Table 1). Comparison between captive

## **Green turtles**

Three captive green turtles produced 100 clutches (9906 eggs) since 1999. The mean  $\pm$  SD of the number of clutches per year, internesting intervals, clutch size, and emergence rate were 4.8  $\pm$  2.2 clutches (1–8 clutches, n = 18), 12.9  $\pm$  2.1 days (10–19 days, n = 51), 91.9  $\pm$  32.4 eggs (14–155 eggs,

n = 72), and  $35.1 \pm 30.3\%$  (0–96.8%, n = 71), respectively (Table 1). Comparison between captive and wild reproductive parameters indicated that captive green turtles breed in captivity similarly to wild green turtles, with the exception of emergence success rate (Table 1).

Table 1. Reproductive parameters of captive sea turnes ofcu in Okmawa Churaunn Aquantu	Table	1.	Reproductive	parameters of c	aptive sea	turtles bred in	Okinawa	Churaumi Aq	uarium
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Turtles		Clutch frequency (Clutches/season)	Clutch size Internesting (egg) interval (day)		Emergence success rate (%)
	Captive	$3.9 \pm 1.1$	113.0 ± 19.7	13.4 ±2.3	10.4 ±21.1
Loggarhand		(n = 19, 2-6)	(n = 83, 57 - 146)	(n = 53, 9-19)	(n = 81, 0 - 89.9)
Loggernead	Wild	3.5-4.5 *1,2	112–114 *1,2	13-17 *1	77.8 *2
	Captive	$4.8 \pm 2.2$	$91.9 \pm 32.4$	$12.9 \pm 2.1$	35.1 ±30.3
Green	cupure	(n = 18, 1-8)	(n = 72, 14 - 155)	(n = 51, 10 - 19)	(n = 71, 0-96.8)
Gitten	Wild	1.8-4.5 *1.2	104-138 *1,2	10-17 *1	69–86 * <sup>2</sup>
	Captive	$2.6 \pm 1.2$	$127.6 \pm 41.9$	$16.2 \pm 4.1$	$12.9 \pm 21.4$
Hawkshill	cupure	(n = 11, 1-5)	(n = 30, 46 - 194)	(n = 17, 13-28)	(n = 30, 0-69.6)
Hawkson	Wild	1.8-4.0 *1,2	130-182 *1,2	11-28 *1	72-78 *2
	Captive	5	$45.2 \pm 13.8$	$11.8 \pm 3.1$	$12.1 \pm 4.2$
Black		-	(n = 5, 29-67)	(n = 4, 9 - 16)	(n = 3, 7.3 - 13.8)
DIACK	Wild	1.0-8.0 *3	38-152 *3	5-25 *3	40-70 *3

\*1 Miller *et al.* (1997), \*2 Hirth (1980), \*3 Marquez (1990)

Information on the reproductive parameters for black turtles was cited from Kawazu et al. (2018).

#### Hawksbill turtles

Two captive hawksbill turtles produced 38 clutches (3828 eggs) since 2012. The mean  $\pm$  SD of the number of clutches per year, internesting intervals, clutch size, and emergence rate were 2.6  $\pm$  1.2 clutches (1–5 clutches, n = 11), 16.2  $\pm$  4.1 days (13–28 days, n = 17), 127.6  $\pm$  41.9 eggs (46–194 eggs, n = 30), and 12.9  $\pm$  21.4% (0–69.6%, n = 30), respectively (Table 1). Comparison between captive and wild reproductive parameters indicated that captive hawksbill turtles breed in captivity similarly to wild hawksbill turtles, with the exception of emergence success rate (Table 1).

#### **Black turtle**

One captive black turtle produced 5 clutches (226 eggs) in 2017. The mean  $\pm$  SD of the number of clutches per year, internesting intervals, clutch size, egg size, and emergence rate were 5 clutches, 11.8  $\pm$  3.1 days (9–16 days, n = 4), 45.2  $\pm$  13.8 eggs (29–67 eggs, n = 5), and 23.6  $\pm$  13.8% (0–33.3%, n = 2), respectively (Table 1). Comparison between captive and wild reproductive parameters indicated that the captive black turtle bred in captivity similarly to wild black turtles, with the exception of

emergence success rate (Table 1). All results for the black turtle have already been reported by Kawazu *et al.* (2018).

#### DISCUSSION

This study demonstrated that four sea turtle species successfully bred at Okinawa Churaumi Aquarium, in which light and temperature conditions are maintained similar to natural conditions around Okinawajima Island. To the best of our knowledge, Okinawa Churaumi Aquarium has had the most reproductive success for sea turtle species of the world's aquariums and facilities to date. These data for reproductive success provide information of reproductive parameters in captive sea turtles.

A low hatching rate in captive sea turtles is common in green (Wood and Wood, 1980), hawksbill (Kobayashi *et al.* 2010), and Kemp's ridley turtles (Wood and Wood, 1988). Similarly, in Okinawa Churaumi Aquarium, the emergence success rate in the four captive sea turtles was low (11.2% for loggerhead turtles, 33.0% for green turtles, 22.3% for hawksbill turtles, and 23.6% for black turtles). In Okinawa Churaumi Aquarium, the emergence success rate of loggerhead turtle nests that were rescued and transferred to artificial sandy beaches from the wild were high (69%), and similar to those of wild turtles (Kawazu personal com.). Therefore, we suggest that the low emergence success rate could be caused by the egg condition during the egg formation process (i.e., vitellogenesis, mating, and fertility).

Wood and Wood (1980) reported that the hatching rate of captive green turtles improves with the length of time that the female is mounted. If females are mounted for 100–199 min, the hatching success is 30% or less, but this increases if females are mounted for more than 400 min (Wood and Wood, 1980). During mating, ejaculated sperms progress into oviducts, and are stored in the upper portion of each oviduct where they fertilize ovulated eggs (Gist and Jones, 1989; Owens, 1980). Thus, we believe that fertility is affected by the dosage of semen injection. Polyspermic fertilization is a characteristic of some birds and reptiles with large eggs (Mizushima et al. 2014). Further study is required to confirm this hypothesis, which is needed to establish artificial insemination techniques in sea turtles. Semen collection (Kawazu et al. 2014b: Kawazu et al. 2015d), ovulation induction (Kawazu et al. 2014c), and oviposition induction techniques (Kawazu et al. 2014a) have already been developed in sea turtles. However, some problems regarding further development and success of the technique for artificial insemination of sea turtles also need to be solved.

The low emergence success rate might possibly be affected by the condition of the female turtle in captivity. The triglycerides and proteins ingested by females are utilized for follicular development (Kawazu *et al.* 2015c; 2016). Craven *et al.* (2008) reported that yolk fatty acids profiles were different between captive and wild green turtles and were influenced by the diet of the female. Thus, egg condition, involving fertility and embryogenesis, might be affected by diet.

Souza et al. (2018) reported that hatching success in loggerhead and green turtles is affected by egg copper (Cu) and zinc (Zn) concentration. Also, the low hatching rate of leatherback turtles might be caused by a lack of selenium in females (Perrault et al. 2011). In avian eggs, which have the same consistency of calcium, albumen, and yolk as sea turtles, the increase in egg selenium concentration via diet are associated with better antioxidant protection during embryonic development and post-hatching, which leads to high hatching rate and low motility post-hatching (Surai, 2002). Also, Surai (2002) reported that selenium has a sustaining effect on sperm function in male avians, so that a lack of selenium might explain low fertility rates. Further studies are required to assess the relationship between diet, including the contents of fatty acids and trace elements, and fertility and hatching rate, which will contribute to the conservation of wild sea turtles and improve our knowledge of reproductive biology.

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#### REFERENCES

- CRAVEN, K. S., PARSONS, J., TAYLOR, S. A., BELCHER, C. N., OWENS, D. W. 2008, The influence of diet on fatty acids in the egg yolk of green sea turtles, *Chelonia mydas. Journal* of Comparative Physiology B 178: 495-500
- GIST, D. H., JONES, J. M. 1989, Sperm storage within the oviduct of turtles. *Journal of Morphology* 199: 379-384
- HIRATE, K., KAWAZU, I. 2017a, Green turtles. pp. 199–202. *In*: NATURE CONSERVATION DIVISION DEPARTMENT OF ENVIRONMENTAL AFFAIRS OKINAWA PREFECTURAL GOVERNMENT (ed.), *Threatened Wildlife in Okinawa, Third Edition (Animals) Red Data Okinawa*, Nature Conservation Division, Department of Environmental Affairs, Okinawa Prefectural Government, Okinawa
- HIRATE, K., KAWAZU, I. 2017b, Hawksbill turtles. pp. 182–183. *In*: NATURE CONSERVATION DIVISION DEPARTMENT OF ENVIRONMENTAL AFFAIRS OKINAWA PREFECTURAL GOVERNMENT (ed.), *Threatened Wildlife in Okinawa, Third Edition (Animals) Red Data Okinawa*, Nature Conservation Division, Department of Environmental Affairs, Okinawa Prefectural Government, Okinawa
- HIRATE, K., KAWAZU, I. 2017c, Loggerhead turtles. pp. 188–190. *In*: NATURE CONSERVATION DIVISION DEPARTMENT OF ENVIRONMENTAL AFFAIRS OKINAWA PREFECTURAL GOVERNMENT (ed.), *Threatened Wildlife in Okinawa, Third Edition (Animals) Red Data Okinawa*, Nature Conservation Division, Department of Environmental Affairs, Okinawa Prefectural Government, Okinawa

HIRTH, H. F. 1980. Some aspects of the nesting behavior and reproductive biology of sea turtles. *American Zoologist* 20: 507-523

KAWAZU, I., KINO, M., MAEDA K. 2015a, Relationship between the water temperature experienced by captive loggerhead turtles (*Caretta caretta*) and eggshell formation. *Herpetological Review* 46: 364-368

KAWAZU, I., KINO, M., MAEDA, K., TERUYA H. 2015b., Age and body size of captive hawksbill turtles at the onset of follicular development. *Zoo Biology* 34: 178-182

KAWAZU, I., KINO, M., MAEDA, K., YAMAGUCHI, Y., SAWAMUKAI, Y. 2014a, Induction of oviposition by the administration of oxytocin in hawksbill turtles. *Zoological Science* 31: 831-835

KAWAZU, I., KINO, M., YANAGISAWA, M., MAEDA, K., NAKADA, K., YAMAGUCHI, Y., SAWAMUKAI, Y. 2015c, Signals of vitellogenesis and estrus in female hawksbill turtles. *Zoological Science* 32: 114-118

KAWAZU, I., MAEDA, K., FUKADA, S.,
OMATA, M., KOBUCHI, T., MAKABE, M.
2018. Breeding success of captive black turtles in an aquarium. *Current Herpetology* 37: 180-186

KAWAZU, I., MAEDA, K., KINO, M., KOYAGO, M., SAWAMUKAI, Y. 2015d, Optimal intervals for semen collection by electro-ejaculation in hawksbill turtles. *Journal of Japanese Association of Zoos and Aquariums* 56: 9-14

KAWAZU, I., MAEDA, K., KOYAGO, M., NAKADA, K., SAWAMUKAI, Y. 2014b., Semen evaluation of captive hawksbill turtles. *Chelonian Conservation and Biology* 13: 271-278

KAWAZU, I., NAKADA, K. MAEDA, K., SAWAMUKAI, Y. 2016, Daily changes in the blood levels of two steroids and other biochemicals related to vitellogenesis and eggshell formation during internesting intervals in a captive female loggerhead turtle. *Current Herpetology* 35: 14-21

KAWAZU, I., SUZUKI, M., MAEDA, K., KINO, M., KOYAGO, M., MORIYOSHI, M., NAKADA, K., SAWAMUKAI, Y. 2014c, Ovulation induction with follicle-stimulating hormone administration in hawksbill turtles *Eretmochelys imbricata. Current Herpetology* 33: 88-93

KINO, M., KAWAZU, I. 2014, A stranding report of a young juvenile Olive ridley turtle (*Lepidochelys olivacea*) at Yagajijima Island, Okinawa. *Umigame Newsletter of Japan* 100: 7–11

KINO, M., MAEDA, K., KAWAZU, I. 2015, Gastrointestinal contents of a Black turtle (*Chelonia mydas agassizii*) stranded at Okinawa Island, Japan. Umigame Newsletter of Japan 101: 8-11

KOBAYASHI, M., OKUZAWA, K. SOYANO, K., YOSEDA, K. 2010, Reproductive ecology of the hawksbill turtle *Eretmochelys imbricata* in captivity. *Nippon Suisan Gakkaishi* 76: 1056-1065

MANIRE, C. A., BYRD, L., THERRIEN, C. L., MARTIN, K. 2008, Mating-induced ovulation in loggerhead sea turtles, *Caretta caretta*. Zoo *Biology* 27: 213-225

MÁRQUEZ, M. R. 1990, FAO species catalogue Vol.11: Sea turtles of the world. An annotated and illustrated catalogue of sea turtle species known to date. *FAO Fisheries Synopsis* No. 125, FAO, Rome

MILLER, J. D. 1997, Reproduction in Sea Turtles. pp. 51–81. *In*: LUTZ, P. L., MUSICK, J. A. (eds.), *The Biology of Sea Turtles*, CRC Press, Boca Raton, Florida

MILLER, J. D. 1999, Determining clutch size and hatching success. pp 124–129. *In*: ECKERT, K. L., BJORNDAL, K. A., ABREU-GROBOIS, F. A., DONNELLY, M. (eds.), *Research and Management Techniques for the Conservation of Sea Turtles*, IUCN/SSC Marine Turtle Specialist Group, Washington D. C.

MIZUSHIMA, S., HIYAMA., G., SHIBA, K., INABA, K., DOHRA, H., ONO, T., SHIMADA, K., SASANAMI, T. 2014. The birth of quail chicks after intracytoplasmic sperm injection. *Development* 141: 3799– 3806

OWENS, D. W. 1980. The comparative reproductive physiology of sea turtles.

American Zoologist 20: 549-563

- OWENS, D. W., BLANVILLAIN, B. 2013, Captive reproduction of sea turtles: An important success story. pp. 23–40. In: SATO, K. (ed.), Proceedings of the International Symposium on Reproduction of Marine Life, Birth of New Life! Investigating the Mysteries of Reproduction, Okinawa Churashima Foundation, Motobu, Okinawa
- PERRAULT, J., WYNEKEN, J., THOMPSON, L. J., JOHNSON, C., MILLER, D. L. 2011, Why are hatching and emergence success low?
  Mercury and selenium concentrations in nesting leatherback sea turtles (*Dermochelys coriacea*) and their young in Florida. *Marine Pollution Bulletin* 62: 1671-1682
- SOUZA, N. L. N., CARNEIRO, M. T. W. D., PIMENTEL, E. F., FROSSARD, A., FREIRE, J. B., ENDRINGER, D. C., JÚNIOR, P. D. F. 2018, Trace elements influence the hatching success and emergence of *Caretta caretta* and *Chelonia mydas. Journal of Trace Elements in Medicine and Biology* 50: 117-122
- SURAI, P. F. 2002. Selenium in poultry nutrition 2. Reproduction, egg and meat quality and practical applications. *World's Poultry Science Journal* 58: 431-450
- TERUYA, H., KAMEI, Y., UCHIDA, S., ADACHI, K. 1997, New sea turtle tank with nesting-ground and its effect. pp. 113–118. In: CONGRESS CENTRAL OFFICE OF IAC '96 (ed.), Proceedings of the Fourth International Aquarium Congress Tokyo, Congress Central Office of IAC '96, Tokyo

- WALLACE, B. P., DIMATTEO, A. D., BOLTEN, A. B., CHALOUPKA, M. Y., HUTCHINSON, B. J., ABREU-GROBOIS, F. A., MORTIMER, J. A., SEMINOFF, J. A., AMOROCHO, D., BJORNDAL, K. A., BOURJEA, J., BOWEN, B. W., DUEÑAS, R. B., CASALE, P., CHOUDHURY, B. C., COSTA, A., DUTTON, P. H., FALLABRINO, A., FINKBEINER, E. M., GIRARD, A., GIRONDOT, M., HAMANN, M., HURLEY, B. J., LÓPEZ-MENDILAHARSU, M., MARCOVALDI, M. A., MUSICK, J. A., NEL, R., PILCHER, N. J., TROËNG, S., WITHERINGTON, B, MAST, R. B. 2011, Global conservation priorities for marine turtles. PLOS ONE 6: e24510
- WOOD, J. R., WOOD, F. E. 1980, Reproductive biology of captive green sea turtles *Chelonia mydas*. *American Zoologist* 20: 499-505
- WOOD, J. R. WOOD, F. E. 1988, Captive reproduction of Kemp's ridley *Lepidochelys kempi. Herpetological Journal* 1: 247-249
- YANAGISAWA, M. 2012. Reproductive physiology, morphology, and physiology of reproductive organs. pp. 141–163. *In:* Kamezaki, N. (ed.). *Natural History of Sea Turtles in Japan*, University of Tokyo Press, Tokyo
- YOSHIKAWA, N., KAMEZAKI, N., KAWAZU, I., HIRAI, S., TAGUCHI, S. 2016, Stock origin of the leatherback turtles (*Dermochelys coriacea*) found in the vicinity of Japan revealed by mtDNA Haplotypes. *Current Herpetology* 35: 115-121

# Effects of Energy Intake and Water Temperature on the Body Shape of Whale Sharks at the Okinawa Churaumi Aquarium

Rui Matsumoto, Kiyomi Murakumo, Rina Furuyama and Shohei Matsuzaki Okinawa Churaumi Aquarium

ABSTRACT: The Okinawa Churaumi Aquarium exhibit includes a male (total length (TL), 8.7 m) and a female (TL, 8.0 m) whale shark, in addition to several other marine attractions. The male has been successfully maintained for over 23 years, which is a world record. The tank housing the whale sharks is 35  $m \times 27 m \times 10 m$  in dimension and has an open-water system (7500 m<sup>3</sup>) without water temperature control. The water temperature in the tank ranges from 21 to 30°C, similar to that of the sea water near the aquarium. Typically, the whale sharks were fed Euphausia superba, E. pacifica, Engraulis japonicas larvae, Sergia lucens, Scomber japonicas, and Marsupenaeus japonicas. Average energy intake, water temperature, TL, and girth (G) were monitored monthly, between March 2017 and March 2018 for the male, and between December 2015 and March 2018 for the female. Their body shapes were expressed as ratios of G to TL (G/TL). The mean (± standard deviation) energy intake per day of the male and female was observed to be  $11,483 \pm 6,644$  kcal and  $12,638 \pm 5,798$  kcal, respectively. Interestingly, G/TL in the male decreased (-0.007) during periods of high temperature, in August 2017 (29.2 °C), even though its energy intake remained high (18,800 kcal). However, G/TL in the male remained unchanged in February 2018 (21.3 °C), despite lower energy intake (13,400 kcal). Similarly, G/TL in the female decreased (-0.004) in August 2017 (29.2 °C), even though it consumed over 16,600 kcal. Contrastingly, this value increased slightly in February 2018 (21.3 °C), despite lower energy intake (8,500 kcal). This suggests that the changes in body shape were caused by varying metabolic rates of the ectothermic whale sharks, which were in turn determined by the relationship between water temperature and food consumption.

## INTRODUCTION

The whale shark, Rhincodon typus, is the world's largest fish. Being migratory in nature, it has a circumglobal distribution, ranging from the tropical to temperate regions, excluding the Mediterranean Sea (Compagno et al. 2005). This species has been afforded significant management and conservation attention. It is listed as Endangered in the IUCN's (International Union for Conservation of Nature) Red List of Threatened Species, and is also included in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna). However, there is sparse information on its life history traits, viz. food consumption, habitat requirements, growth patterns, sexual maturation, and reproduction, largely because of the challenges of long-term observation of individuals, either in the field or in captivity.

Whale sharks are a planktivorous, migratory species; the filtering apparatus on their pharyngeal arches enable them to ingest small organisms (Motta et al. 2010). They aggregate seasonally to feed on abundant food resources such as fish spawn, zooplankton, small fishes, and crab eggs. These aggregations occur around the world, including in the Ningaloo Reef (Taylor 1996) and Christmas Island (Meekan et al. 2009) in Australia, Belize (Heyman et al. 2001), the Atlantic (de la Parra-Venegas et al. 2011), the Pacific coast of Mexico (Eckert and Stewart 2001), Qatar (Robinson et al. 2013), Seychelles (Rowat and Gore 2007), Madagascar (Jonahson and Harding 2007), the Maldives (Anderson and Ahmed 1993), Djibouti (Rowat et al. 2007), and Mozambique (Speed et al. 2008). However, information on whale shark food consumption patterns and energy requirement is lacking.

The Okinawa Churaumi Aquarium has accomplished the long-term husbandry of whale sharks since 1980. Two individuals of this species a male and a female—form part of the main exhibit, and are maintained in a 7,500 m3 tank. The male has been maintained for over 23 years and measured 871 cm in total length (TL) in December 2017. The female has been maintained for 10 years, and measured 804 cm in TL in December 2017. Their food consumption, changes in body shape, growth rates, and other physical attributes are monitored, and depend on their health conditions.

Here, we examined the relationships between body shape, including girth, and food consumption, including energy intake. The study will help to more accurately determine the daily feeding amounts of captive whale sharks. Furthermore, the results can provide valuable estimation of food consumption at the global aggregation sites.

## MATERIALS AND METHODS

#### **Rearing of the animals**

The whale sharks examined in this study were both caught incidentally, by set-nets in Yomitan, Okinawa Prefecture. The male was caught on 11th March 1995, measuring 460 cm in TL at the time of capture. The female was caught on 2nd April 2008, and measured 500 cm in TL. The two individuals have been maintained for over 23 years and over 10 years, respectively, as of October 2018, in the Kuroshio tank (measuring  $35 \times 27 \times 10$  m in dimension, with a volume of  $7.500 \text{ m}^3$ ). The Kuroshio tank contains seawater, supplied at a rate of 2,000 m<sup>3</sup>/h, and has no water temperature control. The seawater is obtained from a location 300 m off the coast, from a depth of 20 m. It is filtered and circulated at a rate of 12 turnovers per day. Water temperature in the tank ranges from an average of  $23.7 \pm 1.2$ °C in the winter (i.e. June to September) to  $28.7 \pm 1.2^{\circ}$ C in the summer (i.e. December to February).

#### **Body shape measurements**

The TL of each whale shark was calculated using the pre-first dorsal length (PD1), defined by Compagno (1984) as the length between the snouttip and the origin of the first dorsal fin, based on the following equation (Matsumoto et al. 2017):

#### Log TL cm = 0.964 Log PD1 + 0.443

The PD1 was measured directly in water every month. Girth (G) measurements were also taken directly every month at the posterior margins of the pectoral fins of the free-swimming individuals. Body shape was expressed as the ratio of G to TL (G/TL).

#### **Food consumption**

Calorie consumption or energy intake of the whale sharks in captivity were calculated based on each food item consumed and expressed in kcal/100 g food ingested. The caloric values were determined from the total amounts of protein, lipid, and carbohydrate contained in each food, as per the Atwater system. The total amounts of protein, lipid, and carbohydrate in the food were analyzed using the macro-Kjeldahl method, acid digestion method, and direct ashing method, respectively (and were carried out by the Incorporated Foundation Okinawa Prefecture Environment Science Center). The two captive whale sharks were fed on Euphausia superba (660 kcal/kg), E. pacifica (620 kcal/kg), Engraulis japonica (690 kcal/kg), Sergia lucens (700 kcal/kg), Scomber japonicus (1270 kcal/kg), and Marsupenaeus japonicus (970 kcal/kg). The amount of food consumed was recorded daily.



Fig. 1. Change in average daily energy intake and body shape (G/TL) of the male (A) and female (B) whale sharks. Bars and lines indicate the values of energy intake and G/TL, respectively.

#### RESULTS

The body shapes (G/TL) of the male (mean  $\pm$ SD:  $0.41 \pm 0.01$ ) and the female ( $0.41 \pm 0.02$ ) were similar throughout the monitoring periods (Fig. 1). During this time, the daily energy intake of the male and female were observed to be  $11.483 \pm 6.644$  kcal and  $12,638 \pm 5,798$  kcal, respectively. There were periods when the sharks did not feed at all (or consumed too little), such as in March, September, and October 2017, for the male shark (energy intake: 307 kcal, 5,443 kcal, and 3,607 kcal, respectively); and in December 2015, between September and November 2017, and in January 2018, for the female shark (5,320 kcal, 5,443 kcal, and 3,607 kcal, respectively) (Fig. 2). The female's girth was not measured between February 2016 and March 2017 because she intensely disliked being touched by the divers.

G/TL in the male gradually decreased, from 0.418 to 0.387, with a concurrent decrease in energy intake in the period between June (13,912 kcal) and October (3,607 kcal) 2017. Subsequently, this value increased to 0.404 in February 2018 (13,517 kcal). In the female, G/TL also increased between December 2015 (0.403) and June 2016 (0.447), with an increase in energy intake, from 5,320 kcal to

20,948 kcal, during this time. Conversely, when energy intake of the female decreased between June (20,948 kcal) and October (10,165 kcal) 2016, and between May (16,956 kcal) and November 2017 (2,324 kcal), there was a corresponding reduction in G/TL, from 0.447 to 0.410, and from 0.421 to 0.357, respectively.

#### DISCUSSION

Monitoring of the relationship between body shape and caloric intake can only be carried out accurately in a captive environment. There are few reports on food consumption in relation to whale shark husbandry. Schreiber and Coco (2017) described long-term changes in energy intake and the amount of food consumed by this species in the Georgia Aquarium. Dietary rations were set weekly to be within 3% to 5% of the whale sharks' body mass (BM). The daily food amounts were changed from 7,000 kcal to 40,000 kcal over 10 years, between 2006 and 2016. Changes to body shape were not described in their study. Motta et al. (2010) estimated the daily energy intake of whale sharks in the field to be 3,566 kcal for a 443 cm TL whale shark, and 6.717 kcal for a 622 cm TL whale shark. according to their estimated filtering abilities.



Fig. 2. Changes in daily energy intake and water temperature in the male (A) and female (B) whale sharks. The box indicates the 25th and 75th percentiles, the whiskers indicate the minimum and maximum values, and the line within the box indicates the median value of daily energy intake. Doted lines show water temperature.

However, they advised treating these caloric estimates with caution because of possible errors, including an underestimation of the number of feeding hours per day, and failure to account for sub-surface feeding, which could contribute to the feeding hours. The results of our study are more consistent with the results of whale shark energy consumption monitoring in the Georgia Aquarium than with those obtained from field observations. Therefore, we suggest that ex situ research be conducted to clarify the optimal energy intake of whale sharks.

We monitored changes in relationship between energy intake and G/TL, and water temperature. G/TL in the male decreased (-0.010) during periods of high temperature, between June (G/TL: 0.416; 26.4°C) and August 2017 (G/TL: 0.406; 29.2°C), even though its energy intake was  $14,982 \pm 3,968$ kcal (mean  $\pm$  SD). However, its G/TL increased slightly (+0.002) in the low temperature season. from January (G/TL: 0.402; 22.0 °C) to February 2018 (G/TL: 0.404; 21.3 °C), despite its lower energy intake (13,484 ± 2,889 kcal). Similarly, G/TL in the female decreased (-0.013) from June (G/TL: 0.422; 26.4°C) to August (G/TL: 0.409; 29.2°C), even though it consumed  $13,583 \pm 4,846$ kcal. Contrastingly, this value increased slightly (+0.004) between January (G/TL: 0.361; 22.0°C) and February (G/TL: 0.365; 21.3°C), even after lower energy intake  $(8,252 \pm 6,131 \text{ kcal})$ . This suggests that changes in whale shark body shape (G/TL) were caused by changes in the metabolic rates of these ectothermic animals, which were in turn determined by the relationship between the water temperature and food consumption (i.e., energy intake). The present study has demonstrated that girth measurements provide an effective method of monitoring such relationships. Further studies are needed to calculate and estimate suitable food amounts for whale sharks, in captivity as well as in the field.

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#### REFERENCES

- COMPAGNO, L. J. V., DANDO, M., FOWLER, S. 2005, A field guide to sharks of the world. Harper Collins Publishing Ltd., London, 368 pp.
- MOTTA, P.J., MASLANKA, M., HUETER, R.E., DAVIS, R.L., PARRAD, R., MULVANY, S. L., HABEGGER, M. L., STROTHER, J. A., MARA, K. R., GARDINER, J. M., TYMINSK, J. P., ZEIGLER, L. D. 2010, Feeding anatomy, filter-feeding rate, and diet of whale sharks *Rhincodon typus* during surface ram filter feeding off the Yucatan Peninsula, Mexico. *Zoology*. **113**: 199-212
- TAYLOR, J. G. 1996, Seasonal occurrence, distribution and movements of the whale shark, *Rhincodon typus*, at Ningaloo Reef, Western Australia. *Mar Freshwater Res.* **47**:637–642
- MEEKAN, M. G., JARMAN, S. N., MCLEAN, C., SCHULTZ, M. B. 2009, DNA evidence of whale sharks (*Rhincodon typus*) feeding on red crab (*Gecarcoidea natalis*) larvae at Christmas Island, Australia. *Fish Res.* 60: 607–609
- HEYMAN, W. D., GRAHAM, R. T., KJERFVE, B., JOHANNES, R. E. 2001, Whale sharks *Rhincodon typus* aggregate to feed on fish spawn in Belize. *Mar Ecol Prog Ser.* **215**: 275– 282
- VENEGAS, R. P., HUETER, R., CANO, J. G., TYMINSKI, J., REMOLINA, J. G., MASLANKA, M., ORMOS, A., WEIGT, L., CARLSON, B., DOVE, A. 2011, An Unprecedented Aggregation of Whale Sharks, *Rhincodon typus*, in Mexican Coastal Waters of the Caribbean Sea. *PLoS ONE*. 6(4): e18994
- ECKERT, S. A., STEWART, B. S. 2001, Telemetry and satellite tracking of whale sharks, *Rhincodon typus*, in the Sea of Cortez, Mexico, and the north Pacific Ocean. *Environ Biol Fish*. **60**: 299-308.
- ROBINSON, D. P., JAIDAH, M. Y., JABADO, R. W., LEE-BROOKS, K., EL-DIN, N. M. N., MALKI, A. A. A., ELMEER, K., MCCORMICK, P. A., Henderson, A. C., Pierce, S. J. 2013, Whale sharks, *Rhincodon typus*, aggregate around offshore platforms in Qatari waters of the Arabian Gulf to feed on fish spawn. *PLoS ONE*. 8(3): e58255

- ROWAT, D., GORE, M. 2007, Regional scale horizontal and local scale vertical movements of whale sharks in the Indian Ocean off Seychelles. *Fish Res.* **84**(1): 32–40
- JONAHSON, M., HARDING, S. 2007, Occurrence of whale sharks (*Rhincodon typus*) in Madagascar. *Fish Res.* **84**(1): 132–135
- ANDERSON, R. C., AHMED, H. 1993, The shark fisheries of the Maldives: A review, Report to Ministry of Fisheries and Agriculture, Republic of Maldives and Food and Agriculture Organization of the United Nations. 76 pp.
- ROWAT, D., MEEKAN, M. G., ENGELHARDT, U., PARDIGON, B., VELY, M. 2007, Aggregations of juvenile whale sharks (*Rhincodon typus*) in the Gulf of Tadjoura, Djibouti. *Environ Biol Fish.* 80: 465–472
- SPEED, C. W., MEEKAN, M. G., ROWAT, D., PIERCE, S. J., MARSHALL, A. D., BRADSHAW, C. J. A. 2008, Scarring patterns and relative mortality rates of Indian Ocean whale sharks. *J Fish Biol.* **72**(6): 1488–1503

- COMPAGNO, L. J. V. 1984, FAO Species Catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1 -Hexanchiformes to Lamniformes. FAO Fish Synop. **125**(4/1): 1–249.
- MATSUMOTO, R., TODA, M., MATSUMOTO, Y., UEDA, K., NAKAZATO, M., SATO, K., UCHIDA, S. 2017, Notes on husbandry of whale sharks, *Rhincodon typus*, in Aquaria. pp. 15–22. In: SMITH, M., WARMOLTS, D., THONEY, D., HUETER, R., MURRAY, M., EZCURRA, J. (eds.) Elasmobranch Husbandry Manual II. Ohio Biological Survey, Ohio
- SCHREIBER, C., COCO, C. 2017, Husbandry of Whale Sharks. pp. 87–98. In: SMITH, M., WARMOLTS, D., THONEY, D., HUETER, R., MURRAY, M., EZCURRA, J. (eds.) Elasmobranch Husbandry Manual II. Ohio Biological Survey, Ohio

# Surveillance of Epidemic Serotype of *Miamiensis avidus* Causing Scuticociliatosis in Japanese Aquariums

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**ABSTRACT:** cuticociliatosis is one of the most important parasitic diseases in aquaculture farms as well as aquariums. The causative agent of the disease is *Miamiensis avidus*, and there are at least three serotypes of this ciliate. Vaccines against either of two serotypes have provided no cross-protection in vaccinated fish challenged with heterologous serotypes. Therefore, in this study, we surveyed the epidemic serotypes of this parasite in Japanese aquariums for the development and use of effective vaccines. Diseased 52 fishes (26 kinds) were collected from different aquariums. *M. avidus* was detected from all fishes by species-specific PCR. After culturing the ciliates, we identified the serotype by serotype-specific PCR. From the results, 46, 0, and 1 ciliate isolates were identified as serotype I, II, and III, respectively, whereas 5 isolates were found to be of an unknown serotype. This suggests that serotype I is the epidemic serotype in Japanese aquariums. Since serotypes I and II are evenly distributed in aquaculture farms, the epidemic serotypes in aquariums and farms may be different.

## INTRODUCTION

Scuticociliatosis is an important parasitic disease in global aquaculture industries, and scuticociliates have been isolated and/or detected in the diseased fishes. The disease is frequently observed in the Japanese flounder, Paralichthys olivaceus, one of the major fish species cultured in Asian countries such as Japan, Korea, and China. A lethal systemic scuticociliate infection has been reported in aquarium-captive zebra sharks (Stegostoma fasciatum), Port Jackson sharks (Heterodontus portusjacksoni), and Japanese horn sharks (Heterodontus japonicus) (Stidworthy et al. 2014). In other aquariums, the disease was observed in two different kinds of sea dragons (Phycodurus eques and Phyllopteryx taeniolatus) (Rossteuscher et al. 2008). Thus, controlling the disease is important in not only aquaculture farms, but also aquariums.

The causative agent of the disease is *Miamiensis avidus*, and there are at least three serotypes (Serotype I, II, and III) of this ciliate (Song *et al.* 2008) . The three serotypes are distinguishable by the serotype-specific antigenic polypeptides (serotype I, 30 kDa; serotype II, 38 kDa; serotype III, 34 kDa). Because there is no effective chemical treatment method for scuticociliatosis, vaccination may be the key for the prevention of the disease. However, vaccines showed beneficial effects only against infection

with a homologous serotype (Piazzon *et al.* 2008). Therefore, a survey for pandemic serotypes would be important for the development of an effective vaccine.

## MATERIALS AND METHODS

## Scuticociliate

Scuticociliatosis naturally occurred at four geographically separated aquariums in Okinawa, Fukuoka, Chiba, and Tokyo in Japan. At necropsy, multiple macroscopic lesions, which were often hemorrhagic, and ulcerations were observed; these lesions were found to be confined to the skin. Histopathologically, epidermal ulcers were associated with necrosis and inflammation of the underlying dermis and musculature. Numerous ciliates were observed in these lesions. In several fishes, these ciliates had invaded the blood vessels and were detected in the gills and internal organs including the ovaries, epithalamus, and brain. The scales of the affected fishes were used to culture the ciliates in Eagle's minimum essential medium containing penicillin (10 unit /ml) and streptomycin  $(10 \,\mu g/ml)$  at 23°C.

## Detection and serotyping of M. avidus from affected fish by PCR

Genomic DNA was automatically extracted from all 52 isolates by using the mag LEAD 6GC and Mag DEA Dx SV systems (Precision System Science Co., Ltd, Japan). Species-specific PCR was performed by the method described by Tange et al. (2010). The primer set, Ma-F (5'-GTA ACT GAT CGA ATC TCT TCA C-3') and Ma-R (5'-TTC CCG TTC ACG CAA GCG T-3'), was used, and PCR was performed using 1 cycle at 95°C for 5min, 40 cycles at 95°C for 1 min, 58°C for 1 min, and 72°C for 1.5 min, followed by 72°C for 5 min. Serotype-specific PCR was performed as reported previously (Motokawa et al., 2018). Three primer sets were used for the amplification: Serotype I-F (5'-CAGCAGCTACTGTTGCTAATCC-3') and Serotype I-R (5'-AGCAGTACATGCGGTAGCA C-3') for serotype I, Serotype II-F (5'-AAATGC CCTGGTACTGAAGC-3') and Serotype II-R (5'-CTGCAGCAGCTAAAGCTACAC-3') for serotype II, and Serotype III-F (5'-CGCCTTAT

TAGCTCTCTTCTTAGC-3') and Serotype III-R (5'-AGCAGTACAAGCATCGGAAG-3') for serotype III. PCR was performed for 30 cycles, with each cycle consisting of treatments at 94°C for 30 sec, 65°C for 30 sec, and 72°C for 1 min. The PCR products were analyzed by 2% agarose gel electrophoresis.

#### RESULTS

Using species-specific PCR, M. avidus was detected from the 52 diseased fishes (26 kinds) collected from different Japanese aquariums. The results of serotyping obtained after performing serotype-specific PCR are shown in Table 1. Among the 52 isolates, 47 isolates belonged to serotype I. Serotype II was not detected in any of the fishes, whereas serotype III was detected in 2 fishes. One Chromis viridis sample showed coinfection with serotypes I and III. Five isolates from four Chromis notatus samples and one Ostorhinchus holotaenia sample were not classified into any serotypes.

#### DISCUSSION

This is the first trial involving a massive surveillance of fish parasitic diseases in Japanese aquariums. We isolated scuticociliates from many different fish species at geographically separated aquariums in Japan. All isolates were identified as *M. avidus* by species-specific PCR and the analysis of the SSU rRNA sequences. The disease is recorded even in the Okinawa Churaumi Aquarium, which is located at a subtropical area where the seawater temperature is around  $20-30^{\circ}$ C. Because the ciliate could not grow at temperatures greater

than  $27^{\circ}$ C (unpublished data), the disease may hardly occur in such regions. However, an outbreak of the disease in the aquarium was observed among deep-sea fishes reared at low water temperatures of around 16–20°C. From the results, we revealed that scuticociliatosis caused by *M. avidus* is an important infectious disease among Japanese aquariums.

Most of the *M. avidus* isolates from aquariums were identified as serotype I based on the results of serotype-specific PCR. On the contrary, the serotype II was not detected in any of the fishes. Interestingly, one C. viridis sample was found to be co-infected with serotypes I and III. This is the first report of co-infection with different serotypes of *M*. avidus in a single fish; however, different serotypes were detected from Japanese flounders in the same region (Motokawa et al., 2018). Since serotypes I and II are evenly distributed in aquaculture farms (Motokawa et al., 2018), the epidemic serotype in aquariums and farms may be different. Additionally, in the present study, five isolates were not classified into any serotypes. The reason for this is unclear; however, these may represent new serotype(s) because 5 genotypes of *M. avidus* were reported from the results

of mitochondrial cytochrome c oxidase subunit 1 gene analysis (Jung et al., 2010). Further investigation regarding the appearance of new serotypes of the ciliate is needed.

One method of controlling scuticociliatosis would be cleaning the aquariums, because M. avidus is a free-living ciliate. However, cleaning is difficult due to the structure and pipes of the tanks (ex. pressure tank) used to house deep-sea fishes. Therefore, vaccination is an alternative for aquarium cleaning for the prevention of the disease. The serotype-specific vaccine against M. avidus was reportedly effective in the cultured fishes, i.e. Japanese flounders and Spanish turbots (Scophthalmus maximus) (Piazzon et al. 2008, publication number in international patent: WO2010044451A1). Because M. avidus is easily cultured in fish cell lines (Narasaki et al., 2018), it may be possible to manufacture formalin-killed vaccines in aquariums. In this study, we found that the epidemic serotype of *M. avidus* in Japanese aquariums is serotype I. Therefore, a single vaccine against serotype I is enough for prevention of the disease. In the near future, we will evaluate the effect of the vaccine for the prevention of *M. avidus* infections in aquariums.

Hostfish	Fish captive location	Year isolated	Serotype PCR
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2016	I
Japanese snapper ( Paracaesio caerulea )	Okinawa	2016	I
Longfinned bullseye( Cookeolus japonicus)	Okinawa	2016	I
Blue green damselfish ( Chromis viridis)	Okinawa	2016	ND*
- (Odontanthias katayamai)	Okinawa	2017	I
Blue green damselfish ( Chromis viridis )	Okinawa	2017	ND*
- (Trichiurus sp.)	Okinawa	2017	I
- (Trichiurus sp.)	Okinawa	2017	I
Blue green damselfish (Chromis viridis)	Okinawa	2017	ND*
- (Trichiurus sp.)	Okinawa	2017	I
Goldflag jobfish (Pristingmoides guricilla)	Okinawa	2017	ī
(Liopropoma aragai)	Okinawa	2017	I
(Plactronthias Equi)	Okinawa	2017	ī
- (Theoreministration)	Ohimawa	2017	ND*
(Triabiuman)	Okinawa	2017	T
- (Incharasp.)	Okinawa	2017	
(Tichierus Virganis)	Otimawa	2017	I
- (Irichurus sp.)	Okinawa	2017	1 T
- (Irichurus sp.)	Okinawa	2017	1
- (Irichiurus sp.)	Okinawa	2017	1
Lavender joblish (Pristipomoides sieboldii)	Okinawa	2017	Į,
- (Trichiurus sp.)	Okinawa	2017	1
Lavender jobfish (Pristipomoides sieboldii)	Okinawa	2017	I
Japanese chromis (Chromis mirationis)	Okinawa	2017	I
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2017	I
Fryingpan snapper (Argyrops bleekeri)	Okinawa	2017	I
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2017	I
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2017	I
Omate jobfish (Pristipomoides argyrogrammicus)	Okinawa	2017	I
Japanese snapper ( Paracaesio caerulea )	Okinawa	2017	I
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2017	I
Japanese snapper ( Paracaesio caerulea )	Okinawa	2017	I
Omate jobfish (Pristipomoides argyrogrammicus)	Okinawa	2017	I
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2017	Ι
Omate jobfish (Pristipomoides argyrogrammicus)	Okinawa	2017	I
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2017	I
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2017	T
Rosy dwarf monocle bream (Parascolopsis eriomma)	Okinawa	2017	Ĩ
Threetooth puffer (Triodon macropterus)	Okinawa	2017	Ť
Yellowtail blue snapper (Paracaesio xanthura)	Okinawa	2017	T
Bight redfish (Centrobervx druzhinini)	Okinawa	2017	T
Blue green damselfish (Chromis viridis)	Fukuoka	2017	т. Ш
Green eel goby (Odontamblyopus lacepedii)	Fukuoka	2017	I
Redlin mullet (Chelon haematocheilus)	Fukuoka	2017	ī
Pockensker (Charicochiennus denter)	Talara	2017	Ť
Consecution A condition 16-1. ( Octobeling has be let control	Totyo	2018	1
Bigger antenner (Bergering suthus associated)	Tokyo	2018	ND.
Fignity sweeper (Farapriacanimis ransonneil)	Tokyo	2018	1
Diagramese butterniynsh ( Chaelodon mppon)	Tokyo	2018	1
Blue green damselfish ( <i>Chromis viridis</i> )	Chiba	2018	1
Blue green damselfish ( Chromis viridis)	Chiba	2018	I&III
Blue green damselfish ( Chromis viridis)	Chiba	2018	I
Japanese whiptail (Pentapodus nagasakiensis)	Okinawa	2018	I
Japanese snapper ( Paracaesio caerulea )	Okinawa	2018	I
Checked swallowtail (Odontanthias borbonius)	Okinawa	2018	Ι
Japanese chromis (Chromis mirationis)	Okinawa	2018	Ι
Okinawa chromis (Chromis okamurai)	Okinawa	2018	I
Black scraper (Thamnaconus modestus)	Tokyo	2018	Ι
Omate jobfish ( Pristipomoides argyrogrammicus )	Okinawa	2018	I
0 4 1 1 5 1 7 D 7 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Oliviaarua	2019	20

## Table 1. Serotypes of Miamiensis avidus isolates used in this study

\*Not determined

#### REFERENCES

- JUNG, S. J., IM, E.,Y., STRUDER-KYPKE, M. C., KITAMURA, S., WOO, P. T. 2011, Small subunit ribosomal RNA and mitochondrial cytochrome c oxidase subunit 1 gene sequences of 21 strains of the parasitic scuticociliate Miamiensis avidus (Ciliophora, Scuticociliatia). Parasitology Research, 108: 1153-61
- MOTOKAWA, S., NARASAKIA, Y., SONG, J., YOKOYAMA, Y., HIROSE, E., SHOKO MURAKAMI, S., JUNG, S. J., Oh, M. J., NAKAYAMA, K., KITAMURA, S. 2018, Analysis of genes encoding high-antigenicity polypeptides in three serotypes of Miamiensis avidus. *Parasitology International*, **67**: 196-202
- NARASAKI, Y., Obayashi, Y., Ito, S., Murakami, S., Song, J. Y., Nakayama, K., Kitamura, S. 2018, Extracellular Proteinases of *Miamiensis* avidus Causing Scuticociliatosis are Potential Virulence Factors. *The Japanese Society of Fish Pathology*, **53**(1): 1-9
- PIAZZON, C., LAMAS, R., CASTRO, R., BUDINO, B., CABALEIRO, S., SANMARTIN, M., LEIRO, J. 2008, Antigenic and cross-protection studies on two turbot scuticociliate isolates. *Fish and Shellfish Immunology*, 25: 417-424

- ROSSTEUSCHER, S., WENKER, C., JERMANN, WAHLI, Т., OLDENBERG, Т., E., SCHMIDT-POSTHAUS, H. 2008, Severe Scuticociliate (Philasterides dicentrarchi) Infection in a Population of Sea Dragons (Phycodurus eques and Phyllopteryx taeniolatus). Veterinary Patholigy, 45: 546-550
- SONG, J. Y., SASAKI, K., OKADA, Т.. SAKASHITA, M. KAWAKAMI, H. MATSUOKA, S., KANG, H. S., NAKAYAMA, K., JUNG, S. J., OH, M. J., KITAMURA, S. Antigenic differences of the scuticociliate Miamiensis avidus from Japan. Journal of Fish Diseases, 32: 1027-1034
- STIDWORTHY, M. F., GARNER, M. M., BRADWAY, D. S., WESTFALL, B. D., JOSEPH, B., REPETTO, S., GUGLIELMI, E., SCHMIDT-POSTHAUS, H., THORNTON, S. M. 2014, Systemic Scuticociliatosis (*Philasterides dicentrarchi*) in Sharks. Veterinary Patholigy, **51**(3): 628-632
- TANGE, N., SONG, J. Y., KITAMURA S. 2010, Detection and Identification of Miamiensis Causing Scuticociliatosis by PCR. *The Japanese Society of Fish Pathology*, **45**(3): 130-132

#### An Evaluation and Design of a Digital Fish Book Based on the Visitor Research

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**ABSTRACT:** The Large Coral Reef Tank in the Tropical Marine Life section of the Port of Nagoya Public Aquarium displays about 3,000 fish from 150 species. Fish nameplates are located inside the aquarium, but the large variety of different kinds of fish complicate identifying each one of them. Therefore, we propose a mobile digital fish book display that simplifies intuitive searches for fish, even for beginners. This study was conducted using three different types of surveys of aquarium visitors: visitor behavior, fish identifiability, and fish features. The results of the first survey indicate that about 60% of the visitors actively searched for fish while they were standing in front of the tank, signifying a relatively high degree of interest in such searches. The results of the second survey identified which types of fish were most often searched for to determine the priority of search rankings. The third survey determined what part of the fish visitors looked at and what features the visitors identified using these elements. I conducted a demonstration experiment and achieved a high search rate and received favorable feedback. In the future, we will improve this search function both in relation to the fish in the Large Coral Reef Tank section and marine life outside the aquarium.

#### INTRODUCTION

One of Japan's largest aquariums is the Port of Nagoya Public Aquarium, which provides knowledge about the ecology and evolution of various ocean creatures. This aquarium is divided into north and south buildings. In the north building, such cetaceans as killer whales, belugas, and dolphins are featured around the theme of the evolution of life. The south building houses various sea creatures from a variety of countries from Japan to Antarctica. In this research, we focused on the Tropical Marine Life section in the south building. The Large Coral Reef Tank in the Tropical Marine Life section, which was remodeled in 2015, currently displays about 3,000 fish from 150 species (Fig. 1).



Fig. 1. Large Coral Reef Tank.

The aquarium's fish tank contains many species, such as huge fish (Humphead wrasse (*Cheilinus undulatus*) etc.), colorful fish (Yellowbrown wrasse (*Thalassoma lutescens*) etc.),

uniquely patterned fish (Blackbelly triggerfish (*Rhinecanthus verrucosus*) etc.), and distinctively shaped fish (Bluespine unicornfish (*Naso unicornis*) etc.). Since many visitors want to know the names of such fish, three types of fish boards have been installed around the fish tanks: both analog and digital fish name boards as well as a digital fish database. However, few visitors search for fish with them because such navigation is difficult for beginners. Therefore, a tool is needed that simplifies the investigation of fish names.

In this research, we developed a Digital Fish Book with which fish beginners can easily and intuitively search and propose a system design based on visitor surveys.

#### PURPOSE OF RESEARCH

The biggest feature of our proposed Digital Fish Book is that it simplifies searching for visitors who are unfamiliar with fish. Below we list the disadvantages of a fish database or a fish name board in aquariums (Fig. 2).



Fig. 2. Analog fish board and digital fish database.

(1) Common fish databases often search by name, classification (e.g., perciformes: pomacentridae), and group (e.g., butterflyfish species). Beginners often have difficulty utilizing such databases.

- (2) Many fish have similar colors and shapes. However, because only a limited number of fish name boards can be introduced, one kind of fish cannot be found while comparing similar fish.
- (3) Because the fish name board cannot be moved, it is impossible to compare the fish in the fish name board with those the visitors are looking at.

Therefore, in this research, we developed a Digital Fish Book that has the following characteristics:

- (1) It can search based on fish features (size, color, shape) that even beginners can understand, avoiding keyword searches based on such scientific jargon as name, classification, and group.
- (2) When searching for fish, a list of similar fish is displayed with the search results to facilitate searches for similar fish while comparing.
- (3) Since it allows fish searches in front of the tank, the application's (iOS, Android) format is used by mobile phones.

In this research, based on these features, we investigated which fish features the visitors were interested in and those that can be distinguished and based our design on the result.

## SURVEYS OF AQUARIUM VISITORS

We conducted four types of visitor surveys (Fig. 3).

#### 1) Visitor behavior survey

(1) Purpose:

We clarified the target of this system and the characteristics of the visitor behaviors.

(2) Survey date:

May 20, 27, July 15, 2016

(3) Survey method:

We video-taped the tank with a fixed-point camera for three days. We analyzed such visitor behaviors as staying time, pointing at fish, and taking pictures (fish, tank, visitors themselves) and scored the behaviors (survey: 1,796 groups).

(4) Result:

60% of the visitors are interested in fish. They talked about the fish, pointed at them, and watched them swimming in the tank. The

remaining 40% include those with more interest or more knowledge in fish as well as those who are not interested in fish.



Fig. 3. Visitor surveys.

## 2) Visitor attention survey about various kinds of fish

(1) Purpose:

We investigated the different kinds of fish that visitors are interested in to clarify why they focus on particular fish.

- (2) Survey date: March 28, 2016
- (3) Survey method:

In front of the tank, we recorded the characteristics of the fish in which the visitors expressed interest and analyzed the reasons (survey: six visitors, about 3 to 5 hours per person.)

(4) Result:

Such characteristics as color and pattern received much attention. Furthermore, we clarified that people who are unfamiliar with fish have difficulty describing three or more features of fish.

#### 3) Survey of visitor interest in fish

(1) Purpose:

We concretely identified what fish the visitors are interested in.

(2) Survey date:

May 20, 2016

(3) Survey method:

We asked 60 visitors about fish characteristics based on such items on check sheets as color, shape, pattern, size, movement, etc.

(4) Result:

Many visitors were interested in such big fish as Bowmouth guitarfish (*Rhina ancylostoma*) or such fish that resemble movie characters as Palette surgeonfish (*Paracanthurus hepatus*). Overall, the range of fish in which visitors show interest is limited.

## 4) Survey of language used by visitors to describe fish

- (1) Purpose:We clarified the language used by visitors to describe the characteristics of fish.
- (2) Survey date:
- May 27, 2016
- (3) Survey method:

We showed visitors photographs of 16 kinds of fish, such as Blackbelly triggerfish (*Rhinecanthus verrucosus*), and clarified how they expressed their characteristics (Fig. 4) (survey: 27 visitors).

(4) Result:

Visitors expressed four types of fish characteristics: size, color, pattern, and tail shape.



Fig. 4. Photographs.

## SYSTEM DESIGN BASED ON VISITOR SURVEYS

Based on the results of our visitor surveys, we designed the following Digital Fish Book, which is a system for mobile phone applications for Android and iOS.

- (1) The visitors searched for fish by actively observing them and selecting search items.
- (2) We designed three search items. (Actually, since three search results cannot accurately extract, we set it to four items.)
- (3) We placed a popular fish in the corner of the top page. Visitors can see it immediately without searching.
- (4) We set up four search items: size, color, pattern, and tail fin shape.

The Digital Fish Book design is shown in Fig. 5.



Fig. 5. Digital Fish Book.

The following are the fish search words.

Table 1	Search	words.
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Item	Search words
Size	oversized, large, medium, small,
	unknown
Color	16 colors, including white, red,
	and blue
Pattern	none, horizontal, horizontal
	stripes, vertical, vertical stripes,
	diagonal, diagonal stripe, mesh,
	spotted, other
Tail fin shape	line, fanned, bifurcated, up and
	down, single, unknown

Search words were given one by one by looking at pictures of fish. 195 kinds were registered in the Digital Fish Book database.

## **EVALUATION AND IMPROVEMENT**

Three system developers tried to search for ten distinctive fish such as Blotched foxface (*Siganus unimaculatus*) at the front of the tank. The search rate was 43%.

Possible causes for the low search rate include

failing to correctly set the color keywords and incorrectly observing the tail fin shape.

Search words were initially given using fish pictures taken by the Port of Nagoya Public Aquarium. However, when comparing such

photographs to the actual fish in the tank, the colors may appear different due to the influence of light or changes to the tail fin shape during swimming. Therefore, keywording based not only on scientific correctness but also appearance was done for each fish in the tank (Table 2).

Table 2. Search words of fish.

	Blue ring angelfish (Pomacanthus annularis)	Blotched foxface (Siganus unimaculatus)
		12º
Size	large, medium	large, medium
Color	yellow, purple, blue, brown, white	yellow, black, white
Pattern	diagonal stripes, other	horizontal, horizontal stripes, diagonal stripe, spotted
Tail fin shape	lined, fanned	lined, bifurcated, fanned

By correcting the keywords for ten fish, we improved the correct answer rate to 100%. When the search was conducted for fish other than these ten species, the correct answer rate was 88%.

## **EVALUATION BY VISITORS**

We used our Digital Fish Book for visitor evaluations. Its details are as follows.

(1) Purpose:

We examined the search rate by visitors and evaluated the usability of our Digital Fish Book.

- (2) Survey date: July 10, 13, 17, 21, 2018
- (3) Survey method:

Visitors searched for two fish using the Digital Fish Book and then responded to questionnaires about its usability or enjoyment (survey: 106 people).

(4) Result:

The following are the ages of the visitors who completed surveys: 21% in their 10s, 42% in their 20s, 18% in their 30s, 6% in their 40s, 8% in their 50s, and 5% in their 60s. The search rate of the visitors was 73.3%. The survey results did not differ by age.

Table 3 shows the Digital Fish Book evaluation, which is indicated by the averages of the five stages.

Table 3. Evaluation of Digital Fish Book
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Question	Average
Was the Digital Fish Book easy to use?	4.1
Was it easy to see? (usability)	4.2
Did you enjoy using it?	4.2
Did it increase your interest in fish?	4.2

We obtained the following comments from users:

- Since the buttons and icons are small, selecting them was difficult.
- •Since the font size was too small, it was hard to read.
- •The design of the fish size button was especially difficult to understand.
- •The re-search navigation was too complicated.

Future work will implement a more user-friendly design.

#### CONCLUSION

We developed and evaluated a Digital Fish Book based on visitor surveys that focused on the parts of interest in fish features and what features can distinguish them. Then we designed an original search method and a keywording system. We will continue to evaluate our Digital Fish Book by improving its correct answer rate and increasing its usability.

#### ACKNOWLEDGEMENT

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## Putting People and Research Together. Seven Aquarium Symposia Spanning Twelve Years at Atmosphere and Ocean Research Institute, the University of Tokyo. What We did, What We Learned, and What More to Do.

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**ABSTRACT:** Every other year since 2005, the authors have put together symposium on aquariums. The aims and goals of these symposia are to enhance and strengthen the research and educational capability of aquariums by providing an open, multi-disciplinary platform, where researchers from various fields can get together, present their research and exchange opinion related to aquatic organisms and aquariums. Each symposium was given a different theme and presenters were selected accordingly, from aquariums, universities, museums and related research institutions. Invited renowned specialists of each respective field gave a keynote presentation at each symposium. Each symposium attracted more than 100 participants from all over Japan, even from Taiwan, making it one of the largest symposia held at AORI. Two books, "Work at Aquariums. An in-depth, behind the scene tour of aquarium exhibits.", and "Research Activities at Aquariums. An intelligent world not limited to aquarium exhibits." were published based on these symposia. This series of symposia has met its goal as an adhesive to bond people of different discipline and affiliation together.

## INTRODUCTION

Currently, 60 Japanese aquariums are member of JAZA (Japanese Association of Zoos and Aquariums). There are many more non-member aquariums. Over 33 million people visit aquariums in Japan annually. This means one in every four Japanese visit aquariums each year. A quantitative indicator of how popular aquariums are as a recreational and educational institution specializing in aquatic organisms. A total of 15,800 species, from invertebrates to mammals are displayed in Japanese aquariums and many species have reproduced under captive conditions. This shows that Japanese aquariums have accumulated extremely high level of knowledge and techniques over the years as an aquatic organism research institution. Many Japanese aquarium curators possess doctorate degrees. Aquariums are a treasure trove of research material and opportunities. More and more research collaboration of aquariums with universities, museums and related institutions should be carried out.

#### PURPOSE OF SYMPOSIA

These symposia were convened with one

simple purpose. To provide a stage for researchers, educators, administrative staff of aquariums, universities, museums, education and research institutions to present their research, exchange ideas and opinions and form a human network. There are no restrictions for participation, thus anybody can attend and listen to the presentations.

#### PREPARATION

All symposia were conducted as part of a Joint Usage/Research Center Program at either Ocean Research Institute (ORI) or at Atmosphere and Ocean Research Institute (AORI), the University of Tokyo, Japan. Venue of symposia were, ORI in 2005, 2007 and 2009, and AORI in 2011, 2013, 2015 and 2017 (Table 1).

Application to Joint Usage/Research Center Program were made during the previous year of the planned symposium. This was convenient for the conveners because one year, we applied for the symposium and the next year, we held the symposium. This cycle was not over-burdening and easy to keep our pace because each year we had a different task to carry out. Selection of invited key note presentations and oral presentations were made at the initial planning stage of each symposium.

After receiving official notification that proposed symposium was adopted, announcements were made for poster presentations which are open and not by invitation.

#### THE SEVEN SYMPOSIA

The past seven symposia, each and every one was a success. Titles, dates, number of presentations and participants are presented in Table 2.

Each symposium had different themes. 2005: Research at aquariums. 2007: Environmental issues and aquariums. 2009: Research and education related to aquatic organisms at aquariums. 2011: Biodiversity and aquariums. 2013: Collaborated research with aquariums. 2015: Animal ethology and aquariums. 2017: Exhibit and research at aquariums.

2005 and 2007 symposia were convened by Genjirou Nishi (GT) and Toshiro Saruwatari (TS). Kazuhiro Sakamoto joined the two as convener of symposium held in 2007. Ikuo Ueda (IU) and Hiroshi Sakurai cooperated as members of the program committee. From 2009, TS, GN and IU functioned as conveners of the symposia.

33 aquariums from all over Japan made presentation at the symposia. See Table 2 for list of those aquariums and the number of presentations made. Locations of each aquarium are shown in Fig.1.

19 universities, 8 research institutions, 4

museums and 4 schools and others, total of 35, participated in the symposia. Table 3 provides a list of these institutions and Fig. 2 shows their locations.

As can be seen from Figs. 1 and 2, this series of symposia has succeeded in bringing aquarium curators and researchers of related fields of research together from all over Japan. We had one presentation

from Taiwan in 2005 making it an international symposium.

The number of participants of every symposium exceeded 150, with maximum participation of 222 in 2005. Participants were not limited to curators and researchers, but the ordinary mass and students with interest in aquatic biology and aquariums.

We are already seeing results of these symposia taking shape in the form of new multidisciplinary research projects. Through its twelve year history, aquarium symposium held at AORI, the University of Tokyo has established its presence among aquariums and researchers alike, as a place to meet and exchange ideas and interests. The purpose and the goal of symposia to bond researchers are met. And the symposium shall continue as a unique undertaking bonding people of various disciplines and walks of life together with aquariums. A one of a kind symposium in the world. Next symposium is planned in December 2019. We look forward to your participation and presentation.

			No. of	Present	ations	No. of	
	Dates	Symposium Title	Invited	Oral	Poster	Participants	Venue
1	December 5,6 2005	Aquariums as research institution of aquatic organisms. Potential of mutual use of their research resources.	0	24	4	222	ORI
			One prese	ntation f	rom Taiwa	n.	
2	December 6,7 2007	Environmental issues and aquariums. Current status, tasks and future perspectives.	0	20	0	186	ORI
3	December 7,8 2009	Research and education on aquatic organisms at aquariums. Current status and prospects.	3	18	0	185	ORI
4	December 12,13 2011	Biodiversity and aquariums. Research, exhibit and education	2	19	7	193	AORI
5	December 3,4 2013	Collaborated research with aquariums. Current status and hopes for future developments.	2	16	0	153	AORI
6	December 11,12 2015	Aquarium and animal ethology. Research, exhibit, education.	2	13	12	179	AORI
7	December 11,12 2017	Exhibits and research at aquariums. Exploring their interaction.	1	15	24	220	AORI

Table 1. A list of aquarium symposia held at ORI and AROI.

ORI: Ocean Research Institute, the University of tokyo. AORI: Atmosphere and Ocean Research Institute, the University of tokyo.

Table 2. A list of aquarity which made presentations at the symposit
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	Year and Number of presentations						
Aquarium	2005	2007	2009	2011	2013	2015	2017
1 Aqua World Ibaraki Prefectural Ooarai Aquarium	1	-	-	2	-	1	1
2 Chitose Aquarium, the Birthplace of Salmon	-	1	-	-	-	-	-
3 Enoshima Aquarium	1	1	1	1	2	2	1
4 Fukushima Ocean Science Museum, Aquamarine Fukushima	2	1	1	1	1	2	1
5 Gobius Shinjiko Nature Museum	-	-	1	-	-	-	-
6 Hekina Seaside Aquarium	1	-	1	-	-	-	-
7 Himeji City Aquarium	1	-	-	-	-	-	-
8 Inokashira Park Zoo	1	-	1	-	-	-	-
9 Joetsu Aquarium, Umigatari	-	1	-	-	-	-	-
10 Kagoshima City Aquarium	1	-	-	-	-	-	1
11 Kamogawa Sea World	1	-	-	-	-	-	-
12 Kujukushima Aquarium	-	-	-	1	-	-	-
13 Kushimoto Marine Park	1	-	-	-	-	-	-
14 Lake Biwa Museum	1	1	-	1	-	-	1
15 Marine World Uminonakamichi	1	1	1	-	-	-	-
16 Nagasaki Penguin Aquarium	-	-	1	1	-	-	-
17 NIFREL	-	-	-	-	-	1	1
18 Niigata City Aquarium, Marinepia Nihonkai	-	-	-	1	1	-	1
19 Niji no Mori Koen, Osakanakan	-	-	-	1	-	-	-
20 Notojima Aquarium	-	-	-	1	-	-	-
21 Okinawa Churaumi Aquarium	1	1	2	-	1	1	1
22 Ooita Marine Palace Aquarium, Umitamago	-	1	-	1	-	-	-
23 Osaka Aquarium Kaiyukan	1	1	-	1	1	-	1
24 Port of Nagoya Public Aquarium	1	1	1	1	1	1	1
25 Shimane Aquas Aquarium	-	-	-	1	-	-	-
26 Shimonoseki Kaikyokan	-	1	-	1	-	-	-
27 Suma Aqualife Park	-	-	1	2	-	2	1
28 Toba Aquarium	1	1	-	1	-	-	-
29 Tokai University Marine Science Museum	1	2	1	2	1	1	1
30 Tokyo Sea Life Park	-	-	2	1	2	2	1
31 Tsuruoka City Kamo Aquarium	1	-	-	-	-	-	1
32 Uozu Aquarium	-	-	1	-	1	-	1
33 World Freshwater Aquarium, Aquatotto Gifu	-	-	-	1	-	1	1

Table 3. A list of related institutions which made presentations at the aquarium symposium.

	Year and Number of presentations						
Institution	2005	2007	2009	2011	2013	2015	2017
1 Sagamihara Aquarium, Sagamihara Hureai Kagakukan	-	-	-	1	-	-	-
2 Biodiversity Research Center, Academia Sinica, Taiwan	1	-	-	-	-	-	-
3 Japan Sea National Fisheries Research Institute	-	-	-	-	-	-	1
4 National Institute for Educational Policy Research	-	-	1	-	-	-	-
5 National Research Institute of Far Seas Fisheries	-	1	-	-	-	-	-
6 National Research Institute of Fisheries and Environment of Inland Sea	-	-	-	1	-	-	-
7 Okinawa Churashima Foundation Research Center	-	-	-	1	-	-	-
8 Tokyo Zoological Park Society	-	1	-	-	-	-	-
9 Coastal Branch of Natural History Museum and Institute, Chiba	-	-	-	-	-	1	-
10 Iwate Prefectural Museum	-	-	-	-	1	-	-
11 Kanagawa Prefectural Museum of Natural History	-	-	1	-	-	-	-
12 Wakayama Prefectural Museum of Natural History	1	1	-	-	1	-	-
13 Group for Making a School of Fujisawa Rice Fish.	-	-	1	-	-	-	-
14 Bunrin Junior High School, Bunkyo Ward	-	-	-	-	-	-	1
15 Iwate Prefectural Miyako Fisherise High school	1	-	-	-	-	-	-
16 Atmosphere and Ocean Research Institute, the Univerisity of Tokyo	-	-	-	2	1	3	2
17 Chukyo University	-	-	-	-	-	1	-
18 Department of Marine Bio-Science, Fukuyama University	-	-	-	-	-	-	1
19 Faculty of Fisheries Scineces, Hokkaido University	1	-	1	-	-	-	-
20 Faculty of Regional Studies, Gifu University	-	-	-	-	1	-	-
21 Faculty of Science, Yamagata Univerity	-	-	-	-	1	-	-
22 Gene Research Center University of Tsukuba	-	-	-	-	1	-	-
23 Kitasato University School of Marine Biosciences	-	-	-	-	-	1	-
24 Nippon Veterinary and Life Science University	-	-	-	-	-	1	-
25 Ocean Research Institute, the University of Tokyo	1	2	-	-	-	-	-
26 Ochanomizu University	-	-	-	-	-	-	1
27 Sado Marine Biological Station, Niigata University	-	-	-	-	-	1	-
28 School of Marine Science and Technology, Tokai University	-	-	-	-	-	-	2
29 Seto Marine Biological Laboratory, Kyoto University		-	-	-	1	-	-
30 Tateyama Station, Tokyo Univeersity of Marine Science		-	-	-	-	5	7
31 Teikyo University of Science	-	-	1	-	-	-	-
32 Tokai University	-	-	1	-	1	-	-
33 Tokyo University of Marine Science	-	-	1	-	-	-	9
34 Wako University	-	-	1	-	-	-	-
35 Zakotou	-	1	-	-	-	-	-



Fig. 1. Locations of aquariums which made presentations at the aquarium symposia. Numbers in each symbol correspond to numbers in Table 2 indicating each aquarium

## OUTPUT

Two books were published based on presentations made at the symposia.

NISHI, G., SARUWATARI, T. 2007, Work at Aquariums. An in-depth, behind the scene tour of aquarium exhibits. Vi+243 pp. Tokai University Press. (in Japanese).

This book is widely accepted as a book describing in detail the actual work of aquarium curators. It is currently in its third print.

SARUWATARI, T., NISHI, G. 2009, Research Activities at Aquariums. An intelligent world not limited to aquarium exhibits. Vi+238 pp. Tokai University Press. (in Japanese). This book is comprised of chapters describing examples of research conducted at aquariums. It is regarded as an introductory book on marine biology because many pages were spent describing the underlying knowledge and science of each research activity.

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Fig. 2. Locations of institutions which made presentations at the aquarium symposia. Numbers in each symbol correspond to numbers in Table3 indicating each institution.

publishing the two symposium volumes.

We must not forget to thank the staff and curators of Aquamarine Fukushima and Tokyo Sea Life Park, especially, Yoshitaka Abe, Masamitsu Iwata and Akiko Tsuchihashi for putting together the 10<sup>th</sup> International Aquarium Congress which provided the stage for this presentation and manuscript.

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## Chytrid Fungus and Ranavirus in Chinese Giant Salamanders: Routine Checks, Symptoms and Treatments

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**ABSTRACT:** This paper outlines our experience in cases of chytrid fungus and ranavirus infection in the largest of amphibian species, the Chinese giant salamander (*Andrias davidianus*) (IUCN Red List Critically Endangered; CITES I). A fatal case of Chytridiomycosis occurred in 2014 with the chytrid fungus was discovered during necropsy of a salamander. The affected salamander was found dead and with thick viscous mucous on its skin. Chytrid fungus test results were positive. Sally, who had shared the same tank and life support system with the infected one, looked normal until she was tested positive for chytrid fungus. Treatment (Sporanox (itraconazole) bath) was provided and the test result afterward was negative. In 2017, we encountered a case of ranavirus infection in 10 confiscated salamanders that were temporarily held in our quarantined facility. At that time, initial symptoms included swelling, redness and lesions. All the salamanders were confirmed with ranavirus infection and then euthanized to avoid any possibility of disease transmission. After this case, we have enhanced our preventive measures. Besides routine screening tests for chytrid fungus, we have also included screening for Ranavirus in before they can be transferred to our facilities.

## INTRODUCTION

In recent years, Chytrid fungus and Ranavirus have been health concerns to our Chinese giant salamander (*Andrias davidianus*) (IUCN Red List Critically Endangered; CITES I) collection in Ocean Park, Hong Kong. The amphibian collection in Ocean Park had been free from Chytrid fungus and Ranavirus until 2014 and 2017 respectively. This paper aims to share our experience in the handling of both pathogens in our Chinese giant salamander collection.

In 2014, a skin swab sample from the necropsy of a Chinese giant salamander had tested positive for *Batrachochytrium dendrobatidis* (Bd) using PCR screening. Subsequent screening of the rest of the collection had detected another two carriers, including Sally, an adult sized giant salamander, even though they were clinically healthy. Treatment with Itraconazole bath was used to clear the fungal infection from our collection.

In 2017, a batch of 10 juvenile Chinese giant salamanders was found infected with Ranavirus during the quarantine period. They were confiscated from the pet trade by the local authority, the Conservation Agriculture, Fisheries and Department (AFCD) and were held at the Ocean Park quarantine facility. Shortly after arrival, the salamanders started developing progressive necrotizing lesions on their limbs and oral cavity resulting in high mortality in the group. Necropsy findings confirmed that they had Ranavirus infection. Given concerns on the transmission of the virus, the remaining animals from the batch were humanely euthanized.

## CHINESE GIANT SALAMANDERS AT OCEAN PARK HONG KONG

Four Chinese giant salamanders, including an adult female named Sally, are currently held in Ocean Park. These salamanders are either on exhibit at the Giant Panda Adventure facility or being held in a back of house facility. Ocean Park occasionally receives requests from AFCD to provide temporary holding for Chinese giant salamanders that are confiscated from either the local pet trade or illegally imported as food. Quarantine procedures for any collected animals are carried out in an isolated facility to prevent disease transmission.

#### **CHYTRID FUNGUS**

An annual veterinary check on our Chinese giant salamanders includes screening for Chytrid fungus (both *Batrachochytrium dendrobatids* and *B. salamandrivorans*) to monitor and prevent any transmission of the disease to other animals in the collection. The samples are tested by our Environmental Lab using PCR assay. If the results are positive, the animal would be treated by our veterinarian using the itraconazole bath protocol outlined as below.

In 2014, a salamander was found dead with thick mucous on its skin and Chytrid fungus was first detected in our collection from skin swab test during the necropsy. Interestingly the histopathology report had found no evidence of Chytrid fungus lesions and the cause of death was attributed to unrelated disorder. Sally, an adult female (~1.4m and ~30kg) that had been in our collection since 2003, and another young salamander had shared the same tank and life support system with the infected individual. Though both salamanders were without symptoms, their skin swabs had tested positive for Chytrid fungus (Bd).

Treatment using itraconazole bath (Une et al. 2012) was provided to both of them and it was found to be effective. Chytrid fungus was detected from Day 1 to 5. From day 6 to 10, the test results were negative. In this case, a single course of treatment over ten-consecutive-days was effective to clear the Chytrid fungus infection.

## **Operational procedures for treating Chytrid** fungus-infected salamanders

- 1. Preparation of medication:
- 1.1. Distilled water was chilled with freeze packs to 18-20 °C, which was similar to the temperature in the animal holding tanks.
- 1.2. Amphibian ringer powder was prescribed and added to the chilled water.
- 1.3. The solution was then poured into a pre-marked bucket for 15 liters. To prepare the medicated solution, Sporanox (Itraconazole, 10mg/ml) was used. 150ml of Sporanox was added to 15 liters of water, which is 0.01% concentration, to make up the treatment bath solution.



Fig. 1. Sporanox (Itraconazole, 10mg/ml)



Fig. 2. 3M Quick Swab

#### 2. Treatment procedures:

2.1. Two oversized black plastic bags (prepared one inside another) were prepared for treating Sally due to her long body length. It is recommended that the most suitable treatment container be chosen based on animal size (e.g. length). For instance, a circular tarpaulin would be prepared for smaller salamanders.

- 2.2. Swab samples were taken prior to treatment so as to screen for Bd. Each swab is rolled 5 times on each lateral body fold, plantar aspect of each hind foot, and on the ventral body (cranial to cloaca).
- 2.3. The medicated bath solution was added to the bag and the salamander remained in the bath for 10 minutes. In order to avoid having to prepare a large volume of the medicated bath and also for practical reasons, the animal was not totally submerged. Small buckets or cups were used to pour the medicated solution over the exposed areas. The duration of soaking in the medicated bath was extended from the recommended 5 minutes in the literature to 10 minutes and with no side effects noted. This was done to maximize the therapeutic effect of this medication with the smaller volume of medication used.
- 2.4. Should the same container be used for treating another animal in a similar manner, the container should first be rinsed with distilled water and also disinfected after each bath session.



Fig. 3. Skin swab sampling for Sally

3. Disinfection of salamander holding tanks 3.1. Disinfection was done when animals were moved out from their holding tank for treatment, on the 3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> day since treatment had begun. 1% sodium hypochlorite was applied to the tank system for at least 5 minutes. The level of the disinfectant solution was kept at only 1/3 the depth of the tank to maintain safety while using the bleach. The walls of tank were thoroughly with brushes that

were immersed in the disinfectant. The filter was then backwashed and rinsed. Once disinfected, the tank was emptied and filled with clean water. Subsequent test results for residual chlorine had to be negative before animals could be returned to the tank.

3.2. All reusable items were disinfected by soaking in 1:1000 Virkon solutions for at least 5 minutes. The surrounding area and floor was disinfected as well.



Fig. 4. Black plastic bag used for treatment and using a small bucket for pouring medicated solution over exposed areas



Fig. 5. Circular tarpaulin for treatment

## RANAVIRUS

A confiscated batch of 9 juvenile Chinese giant salamanders was presented to Ocean Park. These salamanders weighed about 14 g and were about 17 cm in length, with one young individual at 28cm long. There were wounds found on the limbs of some of the juveniles and some had minor reddish swollen regions on sides of their lateral bodies. For the larger specimen, it had also found to have a serious injury on the lower jaw measuring about 2cm by 2cm, and the wound appearing with yellowish rotting epidermal tissues. Before they were brought into our quarantine facility the skin swab samples of these salamanders tested negative for Chytrid fungus on 8<sup>th</sup> March 2017. After about 10 days in our quarantine facility, more individuals started developing necrotizing wounds on their limbs and lower jaws. Important events of this incident are shown in the following table.

This was our first encounter with Ranavirus infection. Despite the initial presentation of lesions; which were initially suspected to be wounds, this viral infection was not immediately diagnosed.

## QUARANTINE

The quarantine procedure is the most important first-line of protection against the above pathogens. Keen observation on epidermal, appetite and behavioral changes are critical for early detection of the disease and in instituting of treatment.

Based on experience outlined above, apart from screening for Chytrid fungus before animals can be transferred to our facilities, we have since also required the screening for Ranavirus on all amphibians so as to enhance preventive measures.



Fig. 6,7. Picture showing necrotizing lesion on the oral cavity and limbs



Fig. 8. Picture showing necrotizing lesion on a hind limb

Table 1. Chronology of the disease outbreak

happe	ned in 2017.
Date	Event remark
11 <sup>th</sup>	The animals were brought to an isolated
Mar	facility in Ocean Park as per quarantine
	procedures.
$14^{\text{th}}$	The larger specimen in the group was found
Mar	dead. Tentative diagnosis was hepatitis with
	suspected infection.
21 <sup>st</sup>	1 <sup>st</sup> juvenile died of trauma with degloving
Mar	injuries on its limb.
22 <sup>nd</sup>	
Mar	2 <sup>nd</sup> juvenile died of trauma again.
25 <sup>th</sup>	3 <sup>rd</sup> juvenile died and a Ranavirus infection
Mar	was suspected.
27 <sup>th</sup>	4 <sup>th</sup> death; suspected a Ranavirus infection;
Mar	ruled out other bacterial/viral/parasitic skin
	disease possibilities.
29 <sup>th</sup>	Severe necrosis of limbs. Prognosis poor.
Mar	Humanely euthanized. Suspected a
	Ranavirus infection.
31 <sup>st</sup>	Found dead with wounds, stomatitis and
Mar	glositis. Suspected a Ranavirus infection.
$1^{st}$	Rest of the salamanders (3 individuals) had
Apr	swollen limbs and all were in a weak
_	condition. Humanely euthanized.
	Suspected a Ranavirus infection.

## REFERENCE FOR THE CHYTRID FUNGUS TREATMENT

UNE Y, MATSUI K, TAMUKAI K, GOKA K. 2012. Eradication of the Chytrid fungus *Batrachochytrium dendrobatidis* in the Japanese giant salamander *Andrias japonicas*. *Disease of Aquatic Organisms*. **98**:243-247

## Development of an Evaluation Method for Local Rating of Sustainable Seafood

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**ABSTRACT:** This research helps local consumers choose seafood caught in a way that supports healthy ocean and human health for the present and future generations. So this research develops an evaluation items in three ways: Sustainable fisheries, safety and regional sustainability. Furthermore, the rating system has three levels. We consider the local rating system that recommends local production and consumption of seafood for sustainable fisheries as a model case. There are 19 species that could be evaluated this time, but we will improve

so that we can evaluate more species in the future.

#### INTRODUCTION

In recent years, certification and rating evaluation of seafood for sustainability, such as Marine Stewardship Council (MSC) certification and Seafood Watch rating system by Monterey Bay Aquarium, are widely used in the world for supply increase and resource reduction (FAO, 2018).

In Japan, Marine Eco-Label Japan Council (MEL) issued in August 2012 and WWF Japan's Sushi Guide are exists as a certification system and rating system, respectively. But there are criticisms about them. The objectivity, transparency and update frequency of these evaluation systems are not satisfactory compared to the recommended list in Europe and the United States. In addition, the Ocean Health Index (OHI) advocated by Conservation International pointed out that consideration of radioactive substances after Fukushima Nuclear Power Plant accident was insufficient.

Aquamarine Fukushima is developing a unique rating system called Happy Oceans. The restaurant uses its evaluation for several aquariums. The educational effect can be expected by providing aquarium centered on topping on sushi rice with stable resource amount.

In the case of Fukushima, what kind of seafood is recommended for sustainable fisheries and consumer sustainability? In this research, we propose a new rating system to improve Happy Oceans by adding scientific basis and considering regional revitalization.

#### **OBJECTIVE**

This research develops an evaluation items in three ways: Sustainable fisheries, safety and regional sustainability. Furthermore, the rating system has three levels (Table1).

We consider the local rating system that recommends local production and consumption of seafood for sustainable fisheries as a model case.

Table 1. Three levels of the evaluation system.

Level	Mean
Green	Best Choice
Yellow	Good Alternative
Red	Avoid

#### **METHODS**

The rating list should be updated once a year. Therefore, we developed methods for everyone as simple to understand and easy to obtain data for evaluation.

The selection of the evaluated fish species has been done by regional sustainability results.

#### **1. Sustainable fisheries**

From International Union for Conservation of Nature and Natural Resources (IUCN) Red List, threatened species is evaluated the population reduction rate:

Critically Endangered (CR): An observed, estimated, inferred or suspected population size reduction of  $\geq$ 80% over the last 10 years or three generations, whichever is the longer, where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible, based on (and specifying) any of (a) to (e) under A1.

Endangered (EN): An observed, estimated, inferred or suspected population size reduction of  $\geq$ 50% over the last 10 years or three generations, whichever is the longer, where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible, based on (and specifying) any of (a) to (e) under A1.

Vulnerable (VU): An observed, estimated, inferred or suspected population size reduction of  $\geq$ 30% over the last 10 years or three generations, whichever is the longer, where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible, based on (and specifying) any of (a) to (e) under A1.

(IUCN, 2012)

Table 2. Evaluation method of the sustainable fisheries.

maneries.	
Level	Evaluation method
Green	An observed, estimated, inferred or suspected population size reduction of <30% the last 10 years
Yellow	An observed, estimated, inferred or suspected population size reduction between 30% and 50% over the last 10
Red	An observed, estimated, inferred or suspected population size reduction of ≥50% over the last 10 years

According to the above criteria based on the population reduction rate, we developed the evaluation method (Table2). From Fisheries Agency's resource assessment, we confirmed the reduction rate of resource from fiscal year 2005 to 2015.

## 2. Safety

Since 2012, a radioactive cesium of the maximum limits for seafood is 100 Bq/kg.

The limits for general foods were established after considering the differences in the amount of food consumed according to sex and age groups and the impact of radioactive materials on health. The calculation is based on the annual additional radiation dose that would not exceed approximately 0.9 mSv even if 50% of the foods contained a certain level of radioactive materials and if such foods are ingested continuously (limits for radioactive materials in food). The value of "100 Bq/kg" was consequently determined based on the most conservative limit (meaning lower limits) established for males aged 13-18. Accordingly, this is considered the safe limit for people of both sexes and all age groups (Consumer Affairs Agency, 2018).

In order to investigate the contamination situation of seafood due to emission of radioactive materials from Tokyo Electric Power Company (TEPCO)'s Fukushima Daiichi Nuclear Power Plant, the Fisheries Agency investigates sampled fisheries once a week at major ports in Fukushima Prefecture and neighboring prefecture. Since April 2015, no marine fish species exceed the limit of 100 Bq/kg.

According to the above scientific knowledge of health risks, we developed the evaluation method (Table3). 500 Bq/kg is the previous limit. From March 2011 until May 2014, some marine fish species exceeded the previous limit. But now no contaminated fish has been sampled, therefore we think that health risk is low.

Table 3.	Evaluation	n method o	of the	safety
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Level	Evaluation method
Green	<100 Bq/kg
Yellow	100 - 500 Bq/kg
Red	>500 Bq/kg

#### 3. Regional sustainability

We calculated the self-sufficiency rate using wholesale market annual report of Iwaki City in Fukushima Prefecture. Definition of selfsufficiency rate is below. According to the continuity of resultant value, we developed the evaluation method (Table4).

Table 4. Evaluation method of the regional sustainability.

Level	Self-sufficiency rate
Green	≥10%
Yellow	Positive but $< 10\%$
Red	0%

Self-sufficiency rate = X/(X+Z),

X: the amount of seafood caught in Fukushima prefecture and also distributed in Fukushima prefecture.

Z: the amount of seafood caught outside Fukushima prefecture and distributed in Fukushima prefecture.

#### RESULTS

#### 1. Sustainable fisheries

Table 5. Result of the sustainable fisheries.

Fish spacios	resource
r isii species	reduction rate*
Paralichthys olivaceus	-412.9
Tanakius kitaharae	-54.9
Pagrus major	5.3
Gadus chalcogrammus	20.8
Gadus macrocephalus	-174.1
Todarodes pacificus	24.7
Scomber japonicus	-166.6
Sebastolobus macrochir	-1.2
Sardinops melanostictus	-1158.7
Etrumeus teres	-141.1
Makaira nigricans	-5.4
Eopsetta grigorjewi	46.2
Cololabis saira	44.2
Chionoecetes opilio	43.4
Tetrapturus audax	31.3
Thunnus orientalis	52.8
Scomber australasicus	53.4
Trachurus japonicus	58.1
Engraulis japonicus	80.4
Octopoda Leach	NA
Chelidonichthys spinosus	NA
Salangichthys microdon	NA
Argyrosomus argentatus	NA
Lophius litulon	NA
Thunnus albacares	NA
Katsuwonus pelamis	NA
Thunnus obesus	NA
Konosirus punctatus	NA
Arctoscopus japonicus	NA
Oncorhynchus keta	NA
Seriola quinqueradiata	NA
Odontobutis obscura	NA
Lateolabrax japonicus	NA
Anguilla japonica	NA
Pandalus eous	NA
Oncorhynchus gorbuscha	NA
Clupea pallasii	NA
Sebastes baramenuke	NA

\* Negative numbers mean resource increase. NA means there is no data available.

## 2. Safety

The contamination levels are shown in Table 6. We assumed "NA" is safe (Green) because these fish are probably caught in far offshore, but we will check other data source in the future.

Table 6.	Result of	the rad	ioactive	contamina	ition
level.					

	Maximum		
Fish species	Contamination		
	level of		
- tot of total	Cs134+Cs137		
	during April 2017		
Danali obstana oliva o ava	- March 2016		
Paralichinys ouvaceus	15		
Tanakius kitaharae	8.2		
Pagrus major	6.1		
Gadus chalcogrammus	undetectable		
Gadus macrocephalus	0.76		
Todarodes pacificus	8.1		
Scomber japonicus	0.4		
Sebastolobus macrochir	undetectable		
Sardinops melanostictus	undetectable		
Etrumeus teres	undetectable		
Makaira nigricans	NA		
Eopsetta grigorjewi	0.39		
Cololabis saira	undetectable		
Chionoecetes opilio	undetectable		
Tetrapturus audax	NA		
Thunnus orientalis	undetectable		
Scomber australasicus	undetectable		
Trachurus japonicus	0.58		
Engraulis japonicus	undetectable		
Octopoda Leach	13		
Chelidonichthys spinosus	NA		
Salangichthys microdon	NA		
Argyrosomus argentatus	NA		
Lophius litulon	NA		
Thunnus albacares	NA		
Katsuwonus pelamis	NA		
Thunnus obesus	NA		
Konosirus punctatus	NA		
Arctoscopus japonicus	NA		
Oncorhynchus keta	NA		
Seriola quinqueradiata	NA		
Odontobutis obscura	NA		
Lateolabrax japonicus	66		
Anguilla japonica	32		
Pandalus eous	NA		
Oncorhynchus gorbuscha	NA		
Clupea pallasii	NA		
Sebastes baramenuke	NA		

## 3. Regional sustainability

The result of Sustainable fisheries is as follows (Table 7).

Table 7	Rate of the	self_sufficiency	$(\mathbf{X}/(\mathbf{X} \perp \mathbf{Z}))$
	Kate of the	sen-sumerency	$(\Lambda/(\Lambda \pm L)).$

	Rate of the
Fish species	self-sufficiency
Tish species	(X/(X+Z))
Paralichthys olivaceus	34.9
Tanakius kitaharae	33.6
Pagrus major	13.2
Gadus chalcogrammus	6.7
Gadus macrocephalus	6.7
Todarodes pacificus	6.0
Scomber japonicus	5.9
Sebastolobus macrochir	2.7
Sardinops melanostictus	2.6
Etrumeus teres	2.6
Makaira nigricans	0
Eopsetta grigorjewi	33.6
Cololabis saira	32.6
Chionoecetes opilio	22.7
Tetrapturus audax	0
Thunnus orientalis	16.8
Scomber australasicus	5.9
Trachurus japonicus	4.4
Engraulis japonicus	2.6
Octopoda Leach	72.7
Chelidonichthys spinosus	31.1
Salangichthys microdon	22.6
Argyrosomus argentatus	21.2
Lophius litulon	19.7
Thunnus albacares	17.2
Katsuwonus pelamis	15.1
Thunnus obesus	5.8
Konosirus punctatus	4.9
Arctoscopus japonicus	2.5
Oncorhynchus keta	2.3
Seriola quinqueradiata	1.9
Odontobutis obscura	1.3
Lateolabrax japonicus	1.2
Anguilla japonica	0.7
Pandalus eous	0.6
Oncorhynchus gorbuscha	0
Clupea pallasii	0
Sebastes baramenuke	0

## DISCUSSION

## **1. Sustainable fisheries**

There are few species that can be evaluated for international species evaluated by Japanese Fisheries Research and Education Agency. We could add species whose resource assessments are not shown in Fisheries Agency.

## 2. Safety

We will continue to check the Fisheries Agency's investigate results on radioactive contamination of seafood.

Table 8.	. Result	of the	local	rating.
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Fish species	1. SF	2. S	3. RS
Paralichthys olivaceus	G😂	G😂	G😂
Tanakius kitaharae	G😁	G😁	G😂
Pagrus major	G😂	G😂	G😂
Gadus chalcogrammus	G😂	G😂	Y🙂
Gadus macrocephalus	G😁	G😁	Y🙂
Todarodes pacificus	G😂	G😁	Y🙂
Scomber japonicus	G😂	G😁	Y🙂
Sebastolobus macrochir	G😂	G😁	Y🙂
Sardinops melanostictus	G 🏵	G😁	Y🙂
Etrumeus teres	G 🏵	G😁	Y🙂
Makaira nigricans	G😁	G😂	R 🕄
Eopsetta grigorjewi	YÜ	G😂	G 🏵
Cololabis saira	YÜ	G😂	G 🏵
Chionoecetes opilio	YÜ	G😂	G😂
Tetrapturus audax	YÜ	G😂	R 🔅
Thunnus orientalis	R 🕄	G😂	G😂
Scomber australasicus	R 🕑	G😁	Y🙂
Trachurus japonicus	R 🕑	G😁	Y🙂
Engraulis japonicus	R 🕑	G😁	Y🙂
Octopoda Leach	NA	G😁	G
Chelidonichthys spinosus	NA	G😁	G
Salangichthys microdon	NA	G 😂	G😁
Argyrosomus argentatus	NA	G☺	G⊜
Lophius litulon	NA	G☺	G⊜
Thunnus albacares	NA	G☺	G☺
Katsuwonus pelamis	NA	G 🏵	G😂
Thunnus obesus	NA	G😁	Y <sup>(1)</sup>
Konosirus punctatus	NA	G😂	Y🙂
Arctoscopus japonicus	NA	G😁	Y🙂
Oncorhynchus keta	NA	G😁	Y😐
Seriola quinqueradiata	NA	G😁	Y🙂
Odontobutis obscura	NA	G😁	Y🙂
Lateolabrax japonicus	NA	G😂	Yœ
Anguilla japonica	NA	G	Yœ
Pandalus eous	NA	G	Yœ
Oncorhynchus gorbuscha	NA	G	R
Clupea pallasii	NA	G	R
Sebastes baramenuke	NA	G	R③

#### 3. Regional sustainability

We will obtain data directly from the wholesale company so increase the number of evaluation species. Also, we will consider food mileage as an evaluation method.

## CONCLUSION

This local rating helps local consumers choose seafood that's fished in ways that support a healthy ocean and your health, now and for future generations. There are 19 species that could be evaluated this time, but we will improve so that we can evaluate more species in the future.

The result of sustainable fisheries, safety and regional sustainability are shown in Table 8. NA means there is no data available.

#### ACKNOWLEDGEMENT

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#### REFERENCES

Consumer Affairs Agency, 2018, Food and Radiation Q&A Mini (Fourth Edition), 9

Food and Agriculture Organization of the United Nations (FAO), 2018, The State of World Fisheries and Aquaculture (SOFIA) 2018, 2-83

International Union for Conservation of Nature and Natural Resources (IUCN) Species Survival Commission, 2012, IUCN RED LIST

CATEGORIES AND CRITERIA (Version 3.1, Second edition), 16-21

# New Exhibitions of Beautiful Southeast Asian Jellyfish at Aquaria: Collection and Life Cycle

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**ABSTRACT:** Scyphozoans are very attractive as ornamental animals at public aquaria. Almost all European and American species are exhibited at world aquaria. However, few Southeast Asian scyphozoans are exhibited, although some of them are very beautiful species and deserve exhibition. We have been studying edible jellyfish biology in Southeast Asian countries. In the process of the sampling survey, we found some ornamental scyphozoans that are suitable for exhibition at public aquaria. Mature jellyfishes were collected from the fields to obtain the planulae. Collected planulae developed to polyps in the laboratory. Ephyrae liberated from strobilae developed into medusae. Medusae raised from polyps in the laboratory or collected in the field and imported into Japan were exhibited at Enoshima Aquarium and Kamo Aquarium as Japan's and the world's first exhibition. Our on-site husbandry and exportation methods are a breakthrough for jellyfish exhibitions in public aquaria and jellyfish sciences globally.

## INTRODUCTION

Jellyfish exhibitions are very popular worldwide. Husbandry and display methods for jellyfishes have been progressing, and the number of species of exhibited jellyfish has also increased (Adachi, 2016; Knowles, 2016; Lange et al, 2016: Murakami, 2016). Scyphozoans, especially, are very attractive as ornamental animals at public aquaria. Almost all European and American species are exhibited at aquaria worldwide. However, few Southeast Asian scyphozoans are exhibited, although some of them are very beautiful species and deserve exhibitions. The biggest reasons for this are that no aquariums have full-scale jellyfish exhibitions in Southeast Asia, and Southeast Asia is too far from Europe and the United States to export exotic jellyfish in good condition, and that there are no professional jellyfish aquarists or biological scientists.

We have been studying edible jellyfish biology for sustainable jellyfish fisheries in Southeast Asian countries (Malaysia, Republic of the Philippines, and Thailand) for a decade under the Japan Society for the Promotion of Science (JSPS) cooperative research programs between those countries and Japan. A public aquarium, Bangsaen Aquarium, is attached to the Institute of Marine Science, Burapha University, Thailand. In the process of the JSPS cooperative research, the Institute of Marine Science, Burapha University concluded academic exchange contracts with Enoshima Aquarium and School of Marine Biosciences, Kitasato University, Japan. Bangsaen aquarium built a full-scale jellyfish display space and a jellyfish research laboratory for the first time in Southeast Asia under the academic exchange contracts (Fig.1). These efforts have made great progress in Southeast Asian jellyfish study and exhibition.



Fig. 1. Full-scale jellyfish exhibition in Bangsaen Aquarium, Burapha University, Thailand

In the process of a sampling survey of Southeast Asian countries, we found some ornamental scyphozoans which are well suited for aquarium exhibition. Japanese aquarists and scientists needed to export these jellyfish to Japan to study the lifecycle and potential for aquarium
exhibition.

This paper shows that our trials to export live ornamental jellyfishes to Japan and to obtain scyphopolyps for life cycle study and regular exhibition.

## MATERIALS AND METHIODS

# Sampling

Sampling surveys were conducted at Bagan Datoh, Perak, Malaysia for *Acromitus hardenbergi*, Palawan Island, the Philippines for *Acromitus maculosus*, and Gulf of Thailand for *Acromitus flagellatus*, *Catostylus townsendi*, *Lobonemoides robustus*, and *Rhopilema hispidum* (Fig 2). Sampling was conducted with permission from the Palawan Council for Sustainable Development (PCSD), Republic of the Philippines; Department of Marine and Coastal Resources (DMCR), Thailand; and under JSPS cooperative projects and academic exchange contracts.



Fig. 2. Scyphozoan species in Southeast Asia. A: Acromitus hardenbergi, B: Acromitus flagellatus, C: Acromitus maculosus, D: Catostylus townsendi, E: Lobonemoides robustus

Jellyfishes were collected using a ladle or finemeshed dip net from a boat or off the beach, and using light-traps at fishing ports or mangrove swamps. After sampling, jellyfishes were packed into plastic bags without air when our hotel or university was near the sampling point, and with oxygen tablets or oxygen gas (oxygen can or oxygen tank) when our hotel or university was far from sampling point (Fig. 3).



Fig. 3. Oxygen sources for packing jellyfish. Oxygen tablet (left), Oxygen can (center), packing jellyfishes using oxygen tank.

# Keeping and collecting fertilized eggs on site.

Jellyfishes transported from the sampling points were released into tanks (baby pools, containers, or FRP tanks) with simple filters (sponge filter or under-gravel filter), which was set up in the hotel room or garden and facility of the university as soon as possible. Transporting time was 10 min to 6 h. Each jellyfish species was kept in a same pool or tank to encourage the fertilization of eggs. Next morning, the rearing water was filtered using a plankton net (50  $\mu$ m mesh) to collect fertilized eggs or planulae (Fig. 4).

## **Artificial fertilization**

Large jellyfishes, such as *R. hispidum*, whose umbrella size was over 50cm, were difficult to bring back to the hotel or university site. Moreover, they are difficult to export to Japan. To export those large jellyfish to Japan, the easiest way is to bring planulae back to Japan. We tried artificial fertilization to obtain the planulae, according to the method of Ohtsu et al. (2007).



Fig. 4. Keeping jellies and collecting eggs or planulae. A, B: Keeping jellyfish on-site. C: Collecting fertilized eggs or planulae. D: Collected planulae.

## Exportation

Before exporting the jellyfishes, we received government permission to export jellyfish for scientific purposes and obtained an export invoice from the cargo company. An official document to export jellyfish under the JSPS cooperative research and a memorandum of understanding (MOU) on academic exchange contracts were prepared.

Jellyfishes kept in a tank were packed in plastic bags with oxygen tablets without air or with oxygen gas on the last day of the sampling trip. Planulae were packed in plastic bottles (500 or 1000 mL) or 50 mL plastic tubes. Packed animals were placed in foam cooler boxes. Then, the jellyfishes were exported as baggage or unaccompanied goods by air cargo.

# Maintaining polyps and making ephyrae

Imported planulae were transferred into plastic vessels (58 mm in diameter, 34.6 mm high) and leave to stand them at 28–30 °C for a week. Planulae settled on the bottom of the plastic vessels and developed into polyps. Polyps were kept at 28–30 °C and 15–30 ppt salinity and fed *Artemia* sp. nauplii every 2 days. The rearing temperature was increased up to 30–32 °C to make polyps develop into strobila. 5-Methoxy-2-methylindole (10  $\mu$ M) or indomethacin (10  $\mu$ M) was used to induce strobilation (Kuniyoshi et al., 2012; Yamamori et al., 2017).

# **RESULTS AND DISCUSSION**

#### **Transport methods**

Four types of packing for jellyfish transport methods were tested (packed without air, with oxygen tablet, with oxygen gas using oxygen can, and with oxygen gas using oxygen tank). Small jellyfishes < 10 cm diameter arrived alive at Japan in all packing methods. However, some of larger sized jellyfishes died in the plastic bags without air and with oxygen tablets. Jellyfishes packed with oxygen were alive and well. To prevent air bubbles entering under the umbrella or in the body of jellyfish, we used to pack jellyfish in a plastic bag without air in domestic transport. Almost all domestic transportation is successful. In our jellyfish transport trials from Southeast Asia to Japan, packing without air was not as successful; these tropical species need high temperatures to survive, which decreases the dissolved oxygen level. Moreover, large tropical species are more active and demanded more oxygen than warm- and cold-water species. The artificial addition of oxygen gas was highly successful for transporting jellyfishes in good condition to Japan.

# Polyps, ephyrae, and medusae

The planulae of all target species (A. hardenbergi, A. maculosus, A. flagellatus, C. townsendi, L. robustus, and R. hispidum) attached to the bottom of plastic vessels and metamorphosed to polyps in the laboratory (Fig. 5). Polyps were in good condition in low salinity (around 20 ppt) and at 28–30 °C. Asexual reproduction was budding, except for that of R. hispidum, whose asexual method of reproduction was podocysts. Strobilation occurred when the temperature increased with food limitation. However, the key stimulus for strobilation was not detected yet. One month later, ephyrae had developed through metephyrae into medusae (Fig. 5).

A. maculosus A. hardenbergi L. robustus



Fig. 5 Polyp and medusa reared in laboratory

#### Exhibition

Medusae imported into Japan were exhibited at Enoshima Aquarium and Kamo Aquarium as Japan's and the world's first exhibition (Fig. 6). Especially we succeeded in the import of largesized *L. robustus*. At the beginning of our project, we thought that exporting large jellyfish (about 30 cm diameter) was impossible. However, a decade of cooperative efforts with local scientists and staff has progressed knowledge of how to collect and keep jellyfish on-site and export them step by step. Our jellyfish husbandry and exportation methods are a breakthrough for jellyfish exhibitions in public aquaria and jellyfish sciences worldwide.



Fig.6. Exhibited jellyfish in public aquarium A: Lobonemoides robustus in Kamo Aquarium B: Acromitus maculosus in Enoshima Aquarium

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# REFERENCES

- ADACHI, A. 2016, Exhibition and Breeding of Jellyfish in Enoshima Aquarium, *Der Zoologische Garten* **85**: 8-13.
- KNOWLES, T. 2016, The History of Jelly Husbandry at the Monterey Bay Aquarium, *Der Zoologische Garten* **85**: 42-51.
- KUNIYOSHI, H., OKUMURA, I., KURODA, R., TSUJITA, N., ARAKAWA, K., SHOJI, J., SAITO, T., OSADA, H. 2012, Indomethacin Induction of Metamorphosis from the Asexual Stage to Sexual Stage in the Moon Jellyfish, Aurelia aurita, Bioscience Biotechnology and Biochemistry 76: 1397-1400.

LANGE, J., TAI, M., KAISER, R. 2016, Husbandry

of jellyfish, from the beginning until today, *Der Zoologische Garten* **85**: 52-63.

- MURAKAMI, T. 2016, History and Introduction of the Kamo Aquarium, *Der Zoologische Garten* **85**: 64-73.
- OHTSU, K., KAWAHARA, M., IKEDA, H. UYE, S. 2007, Experimental induction of gonadal maturation and spawning in the giant jellyfish *Nemopilema nomurai* (Scyphozoa: Rhizostomeae), *Marine Biology* 152: 667-676.
- YAMAMORI, L., OKUIZUMI, K., SATO, C, IKEDA, S., TOYOHARA, H. 2017, Comparison of the Inducing Effect of Indole Compounds on Medusa Formation in Different Classes of Medusozoa. *Zoological Science* 34: 173-178.

# What the Coelacanth Claim? A Significance of In-Situ Conservation

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**ABSTRACT:** There are two species of coelacanth in the world. African coelacanth, *Latimeria chalumnae*, distributed in the huge area of the east coast of the African continent and islands in front of the continent in the Indian Ocean. On the other hand, Indonesian coelacanth, *L. menadoensis*, has been found in few regions in Indonesian waters.

Aquamarine Fukushima, Marine Science Museum has conducted field surveys in Indonesia since 2005 and found a new habitat in Sulawesi Island and a small island located in the north-west of the New Guinea Island. These activities are collaborated with the Indonesian Institute of Sciences as a national scientific academy and the Sam Ratulangi University as a local academy.

Just eight specimens of the Indonesian coelacanth were caught while more than 300 African coelacanth specimens exist. The ecology of the Indonesian coelacanth is still unknown. The local government and economic markets utilize the Indonesian coelacanth as an icon of conservation or sightseeing industry, but it has not succeeded yet because it is difficult to find them which inhabit deep water of more than 150m. Therefore, their conservation is not active yet.

Indonesian Institute of Sciences plans to construct a conservation center for the Indonesian coelacanth, and the Aquamarine Fukushima involves in this project by constructing a small supporting facility. Understanding their life history and distribution is one of the key information to protect the coelacanth.

# INTRODUCTION

The first coelacanth. Latimeria chalumnae. was discovered in South Africa in 1938, and then the second one was captured in Comoros in 1952 (Smith, 1939, 1953). Indonesian coelacanth, L. menadoensis, was discovered in Manado, where it was located in the north of Sulawesi Island, Indonesia in 1997. Then, the first specimen was captured in 1998 (Erdmann et al., 1998). According to the genetic study, the specimen differed from those of African species L. chalumnae (Pouyaud et al., 1999). Two individuals were observed by submersible in a location, 360 km away from Manado in 1999 (Fricke et al. 2000). Aquamarine Fukushima, Indonesian Institute of Sciences, and Sam Ratulangi University have conducted coelacanth research surveys in Indonesia (Iwata et al. 2019). Off the northern coasts of Sulawesi Island and Biak Island which is located in the north west of the New Guinea Island, it was confirmed that L. menadoensis inhabited by the Remotely Operated Vehicle (ROV) observations. In this paper the summaries of the surveys and their results are described.

# **COELACANTH SURVEYS**

#### **ROV** surveys

The surveys were conducted in the Sulawesi Island and the Biak Island (Fig. 1). They started from 2005. Table 1 shows survey periods and areas from 2005 to 2015. Total of 14 surveys were conducted.



Fig. 1. Localities of the Indonesian coelacanth survey using ROV from 2005 to 2015.

ROVs which were able to dive to the depth of 300 m were used for the surveys. The ROV has two vertical, two horizontal and two right-left propellers, and it was controlled from a boat on surface through a 400 m long tether cable. During the field surveys by ROV recording for Indonesian coelacanths, *Latimeria menadoensis*, from 2005 to 2015, 33 individuals were recorded. Out of them, 30 different individuals were identified and three others were unidentified. Among those, six individuals were observed more than twice. All individuals observed more than twice were encountered in the same area, at locations close from each other within hundreds of meters.

When individuals of *L. menadoensis* were observed, some of them stayed stationary at the same place, but some individuals swam away. It seemed that they tried to avoid and escape from the brightness of the light or the sounds of the propellers of the ROV.

Table 1. Periods and areas of the Latimeriamenadoensis survey using ROV						
No.	Date	Survey Area				
1	17/04/2005 - 30/04/2005	Manado-tua Isl. and other islands				
2	06/05/2006 - 19/05/2006	Northern coast of Sulawesi				
3	27/05/2006 - 05/06/2006	Buol				
4	11/12/2006 - 20/12/2006	Buol				
5	27/06/2007 - 05/07/2007	Manado				
6	09/07/2007 - 12/07/2007	Buol				
7	02/12/2008 - 08/12/2008	Talise and Bangka Isl.				
8	12/09/2009 - 09/10/2009	Talise and Bangka Isl. Manado				
9	06/11/2010 - 16/11/2010	Biak Island				
10	05/12/2010 - 18/12/2010	Manado				
11	02/05/2012 - 13/05/2012	Manado				
12	03/06/2013 - 10/06/2013	Manado				
13	19/05/2015 - 30/05/2015	Bitung, Lolak				
14	30/10/2015 - 16/11/2015	Bitung, Lolak				

#### Ingestion of plastic material by coelacanth

Eight specimens of *L. menadoensis* are recorded in recent time in Indonesia. All individuals except the last specimen were caught within Sulawesi Island. The first one was just photographed, and the second specimen was preserved as the type specimen.

All of captured coelacanths were reported to the Coelacanth Conservation Committee. Each specimen has an own CCC number. The sixth Indonesian specimen (Fig. 2A), CCC 287, was caught by line fishing in July 22, 2011 off Tatapaan Island located in Amurang Bay of Sulawesi Island, Indonesia. When it was caught, the fisherman brought it to the local fishery office in Amurang, and it was stored in a freezer for 10 months. It was dissected on May 22 in 2012. Total length of the specimen CCC 287 was 112 cm. It was thin, and the body weight was only 13.1 kg. The eyes were subsided, and we found two pieces of plastic in the stomach. One was a snack bag of potato chips which was 25 cm x 19.5 cm, and another one was a piece of a white plastic bag which was 20 cm x 11 cm (Fig. 2B). There was nothing except them in the stomach.



Fig. 2. Individual (A) and ingested plastic litters (B).

# Conservation of the Indonesian coelacanth

According to interviews to fishermen who have caught Latimeria menadoensis by line fishing in Indonesia, they were still alive at moments just caught. The water temperature of the surface is usually more than 30 °C which is drastically higher than that of the coelacanth habitat. It causes more serious damage to the fish than the change of pressure because the swim bladder of the coelacanth is fat-filled (Powel et al. 2002). Therefore, we are considering to build rescue and study facilities for coelacanth and deep sea fish. In these facilities aquarium tanks which provide cold water environment will be set, and if coelacanths or other deep sea fish are caught, they will be stored in and rehabilitated. Breeding data during rescue is not only expected to bring new knowledges about their life history, but it could also be one of the key information to protect the coelacanth.

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# REFERENCES

- IWATA, M., YABUMOTO, Y., SARUWATARI, T., YAMAUCHI, S., FUJII, K., ISHII, R., MORI, T., HUKOM, F. D., DIRHAMSYAH, PERISTIWADY, F., SYAHAILATUA, A., MASENGI, K. W. A., MANDAGI, I. F., PANGALILA, F., ABE, Y. 2019, Field surveys on the Indonesian coelacanth, Latimeria menadoensis using remotely operated vehicles from 2005 to 2015. Bull. Kitakyushu Mus. Nat. Hist. Hum. Hist. Ser. A: 17: Now printed.
- POWEL, D.C., FARWELL, C., YOUNG, F.A. 2002, Some thoughts of possible methods for the capture and transport of a living coelacanth. *Proceedings of Aquamarine Symposium, The coelacanth, fathom the mystery*, 30-31.
- SMITH, J.L.B., 1939, A living fish of Mesozoic type. *Nature*, 143: 455-456.



Fig. 3. A conservation plan is collaborated between Aquamarine Fukushima, Indonesian Institute of Sciences, and Sam Ratulangi University. Our focus is to construct facilities to rescue and study coelacanths in-situ. A: Ongoing research facility in Lolak. B: Coleacanth Conservation Center in Bitung.

# Possibility of 20 cm Cube Aquarium

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**ABSTRACT:** In Aquamarine Inawashiro totally 50 to 100 species (fluctuating depending on the seasons) of aquatic insects, amphibians, and other freshwater creatures have been exhibited one by one in 20 cm cube aquariums which can be exchanged. Each aquarium is fitted freely on rales which are set out a wall. 51 species in two families of diving beetles are confirmed in Fukushima Prefecture. About 40 species of them and 18 species of 19 species in six families of amphibians like frog and salamanders are exhibited. Since each aquarium temperature is not controlled, it rises up to near 30 °C in summer and fall down to near 0 °C. Therefore, visitors can observe hibernating frogs during wintertime.

# INTRODUCTION

We wondered whether we could install a permanent exhibition on insects, the most diverse species on the earth, and more specifically put a spotlight onto aquatic insects. There are many small species of aquatic insects that range from several millimeters to several centimeters in size and could not be exhibited in a large water tank. There also were no permanent exhibitions like the one that we focused on here up until now. So, we decided to exhibit each species using 20cm x 20cm x 20cm compact cubed water tanks. With removable water tank stands, the water tanks can be installed randomly. The water depth of the aqua terrarium water tanks ranges between 5-15cm (water amount of 2-6l,) and the 70-120 species of insects, including amphibians (fluctuates depending on the season,) are bred in still water at normal temperature without using water supply and drainage or temperature controllers. There are no heating and cooling installation inside the venue to provide an environment as close to nature, with a maximum water temperature of 24.7 °C in the summer and a minimum water temperature of 0.5 °C during the winter to enable visitors to view the living creatures in a state of hibernation.

## Dytiscoidea of Fukushima Pref. Japan

There are approximately 146 species of the Noteridae and Dytiscidae family that inhabit Japan. 51 species (Fig.1) have been confirmed in Fukushima Prefecture, and approximately 40 species among them are exhibited year-round.

Fukushima Prefecture faces the Pacific Ocean to the east and is divided into 3 regions (Fig.2, Hama-dori area, naka-dori area, Aizu area) in the Abukuma Highlands and the Ou Mountain Ranges





Fig. 1. 51 species of the Noteridae and Dytiscidae family confirmed in Fukushima prefecture.

- A(a, Noterus japonicas; b, Noterus angustulus;
  c, Canthydrus politus; d, Allopachria flavomaculata;
  e, Hyphydrus laeviventris laeviventris; f, Hyphydrus japonicus ); B(a, Hydrovatus subtillis; b, Hydrovatus acuminatus; c, Allodessus megacephalus;
- d, Hydroglyphus japonicas; e, Leiodytes frontalis;
- f, Hygrotus chinensis ); C(a, Hydroporus tokui;
- b, Hydroporus uenoi; c, Nebrioporus ancho ralis;
- d, Nebrioporus nipponicus; e, Oreodytes kanoi;
- f, Oreodytes sanmarkii ); D(a, Oreodytes natrix;
- b, Laccophilius difficilic; c, Laccophilius kobensis;

d, Laccophilius lewisius; e, Laccophilius lewisioides; f, Japanolaccophilus niponensis ); E(a, Copelatus weymarini; b, Copelatus teranishii; c, Copelatus japonicas; d, Platambus pictipennis; e, Platambus convexus; f, Platambus fimbriatus ); F(a, Platambus sawadai, b, Platambus stygius; c, Platambus optatus; d, Platambus insolitus; e, Platambus ikedai;

f, Agabus japonicas ); G(a, Agabus conspicuous; b, Ilybius apicalis; c, Rhantus suturalis; d, Rhantus erraticus; e, Eretes griseus; f, Hydaticus bowringii ); H(a, Hydaticus conspersus conspersus; b, Hydaticus grammicus; c, Graphoderus adamsii; d, Acilius japonicas; e, Cybister brevis; f, Cybister lewisianus ); I(a, Cybister chinensis; b, Cybister tripunctatus lateralis; c, Dytiscus marginalis czerskii )



Fig.2. 3Areas in Fukushima Prefecture

that traverse north to south. Hama-dori area where it is mild and has little snowfall. Aizu area where it is much snowfall. Naka-dori areas surrounded by Abukuma Highlands and Ou Mountain Range are intermediate climates of the other two regions.

#### Natural breeding inside the water tanks

The Dytiscidae live on the land during the pupal stage, so it was made possible for some of the small breeds to breed inside the water tanks at the aqua terrarium exhi- bition. The larvae feed on the Dahniidae or Asellus hilgendorfi that have grown inside the water tanks. 15 species (Fig.3.) breed in aquarium over the 4 years. The Aizu area, which is surrounded by the Iide mountain range and the

Echigo mountain range on the west side, is one of Japan's heaviest snowfall areas. Because of these characteristic climate, Fukushima Prefecture is the area where the southern limit of northern species (*Hydroporus tokui*) and the northern limit of southern species (*Canthydrus politus*) live, and the number of types is also the fifth largest area by prefecture.



Fig.3. Adults and larvae of 15 species of breeding Dytiscidae family.—A (a, *Allodessus megacephalus*; b, *Hydroglyphus japonicas*; c, *Leiodytes frontalis*; d, *Hygrotus chinensis*); B(a, *Hydroporus tokui*; b, *Hydroporus uenoi*; c, *Nebrioporus ancho ralis*; d, *Oreodytes kanoi*); C(a, *Oreodytes sanmarkii*; b, *Oreodytes natrix*; c, *Laccophilus kobensis*; d, *Copelatus teranishii*); D(a, *Platambus convexus*;

b, Platambus fimbriatus; c, Agabus japonicas)

#### Amphibians of Fukushima Prefecture in Japan

There are 80 species of the Caudata and Anura family that inhabit Japan, and 19(Fig.4.) of them have been confirmed in Fukushima Prefecture.

#### Frog hibernation exhibition

From late October, when the tempe- rature drops below 10 °C, frogs begin to go underground. From later November, the temperature drops below 5 °C, and they stop moving inside the water tanks. Frogs dislike bright areas during hibernation, so an openable cover (Fig.4. Fig.5.) is attached to the exhibition surface, and by transferring the hibernating frogs to the exhibition surface side, visitors can view them in their hibernation state (Fig.6.) during the winter.

# Fluctuation of annual water temperature/ room temperature/ Outside temperature

Annual temperature in Inawashiro and correlation was found in the variation of room temperature and water temperature of the 20 cm cube aquariums



Fig. 4. 19 species of the Caudata and Anura family family confirmed in Fukushima prefecture.

A(a, Hynobius nigrescens; b, Hynobius lichenatus;
c, Hynobius tokyoensis; d, Onychodactylus fuscus );
B(a, Onychodactylus japonicas; b, Onychodactylus intermedius; c, Cynops pyrrhogaster; d, Bufo japonicus formosus ); C(a, Hyla japonica; b, Rana tagoi tagoi; c, Rana japonica; d, Rana ornativentris );
D(a, Lithobates catesbeianus; b, Glandirana rugose;
c, Pelophylax porosus porosus; d, Pelophylax nigromaculatus ); E(a, Rhacophorus schlegelii;
b, Rhacophorus arboreus; c, Buergeria buergeri )



Fig. 5-6. Its rearing compact cubed water tanks(5&
6) and The hibernating Pelophylax nigromaculatus.
-5, Frog hibernation exhibition; 6, The hibernating

frogs can be viewed when the cover is opened. 7, The hibernating *Pelophylax nigromaculatus*.

corner. The winter season temperature is slightly higher than the outside, and the summer season temperature tends to be somewhat lower than the outside. Although it can say that the exhibition environment is close to the living temperature of these temperature-changing organisms in the natural world and the seasonal change can be felt, the exhibition organism thinks that it is possible to spend the life close to the original environment.



Fig. 8. Water temperature fluctuation in the 20cm cubed water tanks from April 1, 2017 to March 31, 2018.

# CHALLENGES

- The aquatic insects that come on shore to pass the winter take up a small area of the land and are affected by the changes of the temperature, and depending on the species some end up dying while underground.
- The year-round breeding of the Hynobius, a genus of the salamander (four breeds,) was possible, but in regard to the Onychodactylus (three breeds,) the high summer temperatures make it difficult for year-round breeding.
- The majority of aquatic insects range between 1mm-5mm, so there are many species that cannot see the diving organisms when moss is laid out on the emer gent plants and at the water's edge.

# REFERENCES

MATSUI, M., & N. MAEDA, 2018.

- ENCYCLOPAEDIA OF JAPANESE FROGS. 8-271 pp. Bun-ichi Sogo Shuppan, Tokyo. (In Japanese, with English book title.)
- MITAMURA, T., K.HIRASAWA & S. YOSHII, 2017. THE Handbook of Japanese Aquatic Insect, 1: Coleoptera. 8-124pp. Bun-ichi Sogo Shuppan,

Tokyo. (In Japanese, with English book title.)

- MORI, M., & A.KITAYAMA, 2002. Dytiscoidea of Japan (2nd ed.). 9-177 pp. Bun-ichi Sogo Shuppan, Tokyo. (In Japanese, with English book title.)
- YOSHIKAWA, N. 2015. For *Onychodactylus fuscus* that live in Tadami. Tadami-machi Buna

Center Kiyou, (4):2-6. (In Japanese, with English title.)

YOSHIKAWA, N., & M. MATSUI., 2014. Two new Salamanders of the genus *Onychodactylus* from Eastern Honshu, Japan (Amphibia, Caudata, Hynobiidae) *Zootaxa* 3866(1):053-078

# Happy Oceans Campaign, "Preserving and Eating Oceans"

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**ABSTRACT:** We agree on the motion of the Happy Oceans, "Preserving and Eating Oceans", and making a skipjack tuna migrating tropical seas around the world as a symbol of the stable resources. We turn on a traffic light of red, blue, yellow reflecting natural resources of the fish, and declare herewith to contribute sustaining Japanese food culture together with customers. In reference to the information of the Food and Agriculture Organization of the United Nations, FAO, we turn on a green light of approximately 20% resources of moderate use, and turn on yellow light for approximately 50% of resources if they do the same use because it is in a condition of the surplus use to cause the drying up of resources, furthermore, we turn on a red light in 30% of excessive fishery or of drying up states. We will reflect the information such as the fisheries research institutes or the local fisheries experimental stations, for the purpose to reflect more accurate information to turn on a traffic light.

# INTRODUCTION

At Aquamarine Fukushima, we conduct environmental exhibitions based on the concept of "The Oceans and What They Say about Our Future on Earth" through exhibitions of various aquatic organisms. In addition, we also use these aquatic organisms as food resources. Natural resources can be largely classified as finite coal resources and marine resources that can be reproduced. By implementing appropriate management of marine resources, they can be used continuously. The increase of the world's population in recent years, has also led to an increase of the use of marine resources. However, if their unregulated use continues, there is always the possibility that it may lead to the depletion of resources.

## **ABOUT HAPPY OCEANS**

At Aquamarine Fukushima, we are engaged in the Happy Oceans activities in order to convey the current situation of marine resources to visitors based on the slogan, "Happy Relation with Fish and Human." The skipjack tuna is used as the logo symbol for the Happy Oceans initiative (Fig.1). Skipjack tuna are a migratory fish that travel across wide ranges in groups, and Onahama Port, where the aquarium is located, is a popular landing harbor for skipjack tuna.

## HAPPY OCEANS LEAFLET

We have created leaflets regarding the current situation of the volume of resources of 60 different species of fish and shellfish categorized into red, yellow and green color symbols, and transmit this information to our visitors (Fig.2,3). The color symbol standards are evaluated based on the combination of the resource evaluation criteria published by the Fisheries Agency (high/middle/low) and their rate of change in volume (increase/level/decrease.) In addition, we are also considering a new evaluation standard method through a joint collaboration with Yokohama National University.



Fig.1. Happy Oceans logo.



Fig.2. Happy Oceans Leaflet

# **ACTIVITIES OF HAPPY OSEANS**

At Aquamarine Fukushima, we are engaged efforts such regular exhibitions and educating visitors at the on-site shops and sushi restaurant through the Happy Oceans leaflets, offering educational activities, holding lectures for our visitors (once a year) and holding meetings about the fish from the Jomon period to come together and think about the use of new marine resources (once a month,) and the details of these activities are as follows.

## **RESTAURANT: OISHII AQUARIUM**

The fish belonging to the red and yellow categories on the leaflet are offered at this restaurant (Fig.4). Dai Yamamoto, the head chef, agreed with our philosophy and shut down his own restaurant in Tokyo to come work at this restaurant.

# SUSHI SHOP "HAPPY OCEANS"

Sushi is a cuisine that originated during the Edo period, and is prepared by placing a sushi topping (seafood) on top of vinegared rice. Currently, sushi cultures are being developed in various parts of Japan that are unique to that land. "Shiome Sushi" was opened in front of the current rip tanks that is linked to the region (Fig.5). The restaurant is positioned as an important educational area where visitors can experience and gain a deeper understanding of the various fish swimming inside the aquarium using all five senses (Fig.6).

## **EDUCATIONAL ACTIVITIES**

Japan is an island nation that is surrounded by the sea. Fishing activities have been conducted here using rivers and oceans from ancient times. Here, the "Aquamarine Uonozoki children's fishing museum," where visitors can learn about the history of the fishermen and the fishing industry at the "Aquamarine Egg" (Fig7, 8). We are also spreading the purpose of the Happy Oceans initiatives to our visitors here.

# **LECTURES**

Through various themes relating to the ocean, rivers and mountains, we implement lectures that connect researchers with our visitors. For researchers, these lectures are opportunities for them to freely convey their research to the general public, and serve an important role as a place where the general public can learn about the latest research information. Visitors here can learn about previous themes, gain deeper understanding of the mehikari (*Chlorophthalmus borealis*,) learn about radiation and think about the use of marine resources.







Fig.5. Sushi shop in front of the current rip tanks.



Fig.6.Enjoy sushi while watching aquarium



Fig.7. Maiwai (Fisherman's Festive Clothes)



Fig.8. Experience of bonito cutting

# ZAKO ASSOCIATION

The mehikari (*Chlorophthalmus borealis*,) which originally had a low utility value, serves as the official fish of Iwaki(Fig.9). Fish with low

utility value are referred to as zako (small fish.) Ever since the *Chlorophthalmus borealis* was selected as the official fish of the city, its market value has increased. Once a month, a meeting of the Zako Association is held at the aquarium in order to find the next mehikari(Fig.10). By collaborating with the region, we are continuously considering further unused resources and the branding of zako fish.



Fig.9. Mehikari - Bigeyed greeneye (*Chlorophthalmus borealis*)



Fig.10. Donko - Japanese codling (Physiculus maximowiczi)

# CONCLUSION

Aquamarine Fukushima is located between a fishing port and an industrial port, and was constructed as a waterfront redevelopment project. The aquarium itself is a popular tourist spot, but we will continue to transmit our environmental messages from Onohama Port under the label of an "environmental aquarium" while collaborating with the local region to provide lifelong studies and next-generation educational activities.

# "Education of Life" in Aquariums: The Case of Aquamarine Fukushima

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**ABSTRACT:** The Aquamarine Fukushima's philosophy is "The Oceans and What They Say about Our Future on Earth". While the basic policy of our educational activities is to promote "Favorable relationship between people and the ocean", we emphasize environmental education as an important concept. Therefore, we have developed the "JANOME Beach" which is the world's largest touch pool, a biotope called "BIOBIO Kappa Village" which reproduced the environment of the waterside, and a child experiencing hall called "Aquamarine Egg". Nature-based experience opportunities for visitors have been increased with these facilities, and we have been focusing more on to the "Education of Life" since then. In the present day, many of the common nature-based experiences are no longer available by various restrictions and constraints. In the Aquamarine Fukushima, kids can enter barefoot into the water, chase prawns and Japanese killifish, and touch starfish and sea urchins. They can also eat the fish they caught by themselves. It is also possible to observe the birth of living creatures, how dead organisms are foraged by scavengers, and how animals decay and return into the soil. For children who will be responsible for the next generation, we believe that these rich nature-based experiences play an important role in deepening their understanding of harmonious relationship with nature.

# INTRODUCTION

The aquarium is facility where educational activities are conducted to promote environmental conservation, serving as a place to think about the relationships between nature and humans. Through trial and error, each institution conducts a wide range of tasks to determine what and how to provide entertainment to the visitors who are expecting fun and comfort. Here at Aquamarine Fukushima, we have continued to strengthen functions to experience nature to develop the next environmentally friendly generation using "sustainability" and "education of life" as the pillars of our educational activities based on the principle of "The Oceans and What They Say about Our Future on Earth."

# TOUCH AND EXPERIENCE

One of the reinforced functions as part of our nature experience is our world's largest outdoor hands-on facility. First, we constructed the "BIOBIO Kappa Village," a biotope that reproduces the environment of the waterside of the village including streams and swamps, in the area adjacent to the building. Visitors can enter the streams and follow and interact with freshwater organisms such as Japanese rice fish and frogs. The facility is outdoors, so it is also possible to experience the four seasons of the year through the visible organisms such as dragonflies and families of Indian spotbilled ducks. Next, we built an extension of the "JANOME Beach," a touch pool located outside of the biotope that reproduces the seaside nature of the shore, tideland, and beach. Visitors can get barefoot and touch seaside creatures such as starfish and sea

cucumber. You'll also see children chasing after or running away from the large striped mullet that approach them in the water.



Fig. 1. JANOME Beach



Fig. 2. BIOBIO Kappa Village

These facilities are provided not only for the visitors, but also as a place to implement educational programs for school groups. The space can be used to host hands-on training as an "entrance to nature" for students who can learn how to catch organisms, study their natural habitats and measures suitable for each of the habitats.

## VIEW AND EXPERIENCE

Here at Aquamarine Fukushima, we celebrated our 10th anniversary by constructing the "Aquamarine Egg" children's experience hall in 2010. The facility features a wide range of exhibitions to explain and display the various means that the organisms have learned to survive, and introduces various organisms of the world, ranging from amphibians that have evolved from fish, to mammals. At the exhibitions you can see a wide range of natural events, including sharks and squids developing inside their eggs, the miracle of the birth of organisms (Fig.4), frog corpses returning back into the ground (Fig.5), *Nassarius livescens* feeding on dead fish and what happens when organisms die.



Fig. 3. The "Touch creatures of the sea" educational program conducted at JANOME Beach



Fig. 4. Exhibition of Chiloscyllium punctatum eggs



Fig. 5. Exhibition of *Rana catesbeiana* corpses These two exhibitions introduce the natural

phenomena of life based on the theme of "birth and death."

# EAT AND EXPERIENCE

A fishing exhibit was constructed at "Aquamarine Egg," where visitors can experience fishing for horse mackerel and silver salmon. Unlike other leisure facilities, the fishing exhibit at Aquamarine Fukushima has several rules. The first rule is that you must eat whatever fish you catch. You are also forbidden from releasing any fish that you catch. In other words, it is not simply an experience to enjoy fishing as you can only catch as many fish as you can eat. Visitors can then experience preparing the fish that they caught, as children can experience using a knife for the first time. We often hear people say that they feel sorry for the fish when removing the head, but this serves an opportunity to think about the meals that you eat everyday. In Japan, there is a custom to say "itadakimasu" (an expression of gratitude) before each meal, and this is meant to educate people about the importance of receiving the life of other organisms and to not leave any food on your plate as a sign of respect. Experience the way of living in nature by preparing and eating the food that you catch.



Fig. 6. Cooking experience

# CONCLUSION

Up until recently, these types of experiences with nature were commonplace on an everyday basis. However, due to various limitations and restrictions in our modern age such as environmental problems and social trends, these experiences have gradually disappeared. That is why we believe it is our responsibility to play an important role in teaching the next generation of children about the education of life and deepen their understanding of symbiosis with nature and the use of sustainable resources through various experiences at the aquarium.

## History, Conservation and Research Program of Indonesian Coelacanth

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**ABSTRACT:** Coelacanth or living fossil was firstly discovered in Indonesia seas in 1997, however its holotype specimen (Indonesian Coelacanth, *Latimeria menadoensis*) was found in 1998. Till nowadays, 8 specimen of coelacanth from Indonesia were listed with the CCC no 174, 175, 215, 225, 254, 287, 299 and 305. Observation on this fish has been conducted since 2005, and its results show its distribution and habitat in Indonesian waters. Several detailed information on specimen collections are described, and some remarkable findings during the last 21 years field observations are also reported. Research collaboration program for Indonesian Coelacanth for the period 2017-2019 includes several topics such as Meristic and morphology study of Indonesian Coelacanth; Evolutional analysis of the lateral line scale and associated neuromast in Osteoichytes; Quantitative study of antropogenic chemicals accumulated in Coelacanth; Coelacanth Indonesia distribution survey uses eDNA technique.

Keywords: History, Conservation, Research collaboration, Indonesian Coelacanth.

## BACKGROUND

This species of fish is known as the "living fossil fish" or "sea king fish", because it is estimated that all groups of fish from the order Coelacanthiformes have been extinct for 70 million years ago. However, this conjecture vanished, after the fish was found on the coast off South Africa in 1938. This specimen was identified and given the scientific name *Latimeria chalumnae* (Smith, 1939). Then, for 60 years, the east coast of Africa became the world's only concern for research of sea king fish, and until now it has been known that this type of fish is spread on countries such as Kenya, Tanzania, Comoros, Mozambique, Madagascar and South Africa.

Since 1998, the phenomenon of the east coast of Africa as the only distribution area for 'Raja Laut' fish has changed, because it was found in the waters of Manado Tua (North Sulawesi) by Mr. Lameh Sonathan (ERDMANN et al. 1999). The captured specimen were later made as the holotype of coelacanth from Indonesia, with the scientific name *Latimeria menadoensis* (POUYAUD et al., 1999). Since then, the coelacanth research has begun in Indonesia, especially in the waters of north Sulawesi. This paper informs the progress that has been achieved from observing the coelacanth (*L. menadoensis*) in the period 1998-2018, and planning for developing the future research on the biology and ecology of the coelacanth.



Fig 1. Distribution of sea king fish in the world and the first year found (Source; IWATA et al., 2009).

#### UNEXPECTED DISCOVERY

The first report on the existence of sea king fish in Indonesia in 1997 was taken by Dr. Mark V Erdmann (University of California at Berkeley, USA) and his wife, Arnaz Mehta. This finding was basically unexpected and happened by accident, when they saw a fish with a unique shape was on a cart in the Manado Bersehati market. At that time, Mark and his wife only had a chance to take a photograph of the fish. However, Mark had suspected that this unique fish was a "coelacanth". To confirm their finding, the photos of the fish were later shown to several ichthyologists. Then, it confirmed that it is king sea fish or known as living fossil fish (ERDMANN, 1999). Based on the results of the photograph, the Coelacanth Conservation Council (CCC) provided a sequence number for these specimens with CCC no. 174. Other additional information from this species, such as the total length was approximately 130 cm and the total weight was 30 kg (ERDMANN, 1999). A year later, on July 30, 1998, Lameh Sonatham, a fisherman from Manado Tua Island accidentally caught a sea king fish, and then this specimen was used as the holotype of Latimeria menadoensis (POUYAUD et al., 1999). The scientific name for this fish was proved by Comptes Rendus de L'Academie des Sciences in March 1999. This second specimen was numbered CCC 175, with a total length of 124 cm, weighs 29.2 kg and is female with 3 eggs that have developed in the abdomen (ERDMANN et al., 1999). The specimen was then transferred to Bogor and then preserved and stored in the Bogor Zoological Museum located in Cibinong Science Center - LIPI (Holotype MZB 10003)



Fig 2. Raja Laut fish collection at Bogor Zoological Museum as holotype (CCC no. 175) (Doc : Zoological Museum of Bogor).

The Sulawesi coelacanth was firstly published in Nature on September 24, 1998, as a cover story. At the same year, in Manado, Indonesian also involved in commemoration of the International Year of the Ocean 1998 (IYO 98) launched by the UN / IOC-UNESCO. The activity was inaugurated by President Habibie, where he signed the charter "The Ocean Charter" and "Bunaken Declaration" stated the importance of the development of the archipelago's marine future. News, posters and information about the findings of the big sea king fish were immediately exhibited and soon became the world's big news which was covered extensively in various media including CNN, ABC News, National Geographic. Discover magazine even placed this finding as the top science stories of 1998.

The specimens of the three sea king fish from Indonesia were only discovered 10 years later. On Saturday May 19, 2007, a king sea fish was caught by a fishing rod from local fisherman Justinus Lahama and his son, Delvi Lahama, on the Malalayang beach (Manado city). The total length of this fish is 129 cm and weighs 51 kg, while the sex is female. In the stomach of this fish is found 25 eggs. This specimen is given CCC registration number 215 (Figure 3). It means that in the last 10 years there have been 40 sea king fish caught throughout the world. The third specimen was exposed at Aquamarine Fukushima, Japan (November 2007 - March 2008), and around 50,000 people were visited while the fish had been in Japan. In May 2009, this specimen became a mascot in the World Ocean Conference and Coral Triangle Initiative (CTI) Summit in Manado.



Fig 3. Head part of king sea fish (CCC 215) caught May 19, 2007 on Malalayang beach, Manado. A hook still remains in its mouth (Doc: Augy Syahailatua).

On November 22, 2008, a report from the Talise area (55 nautical miles from Manado) that a sea king fish measuring 110 cm in length and weighing 20 kg, was caught. This specimen becomes the 4th and registered with the code CCC no. 225 (Figure 4). Currently this specimen is collected by Sea World in the Taman Impian Jaya Furthermore, on Ancol Complex (Jakarta). September 16, 2009, at this location one king sea fish specimen was caught, with a total length of 116 cm and weighing 27 kg. The registration number for this specimen is CCC no. 254 and is the 5th specimen from Indonesia (Figure 5). Currently these specimens are stored in the Faculty of Fisheries and Marine Sciences - Sam Ratulangi University, Manado.



Fig 4. Specimen of sea king fish CCC No. 225 caught on November 22, 2008 in the waters of northern Talise Island (Doc: Augy Syahailatua).



Fig 5. Sea King Fish CCC 254 caught on September 16, 2009 in the northern waters of Talise P. (Doc: M. Iwata).

On July 20, 2011 at around 05.00 a coelacanth was caught again by a fisherman named Rafles Tampi in the Popontolen waters. The caught fish measures 112 cm and weighs 13.1 kg. This specimen is given CCC registration number 287. Then the seventh fish was caught on November 5, 2014 around P. Gangga, North Sulawesi with a body length of about 130 cm and weighing around 37.8 kg. The fish is registered coded CCC 299. On July

1, 2018 again an angler named Dave Lenny Santoso managed to catch a coelacanth using a fishing rod at 8:30 a.m. Because they did not know this fish is coelacanth, the angler immediately cut the fish, so that the fish had not yet been measured and weighed. However, body parts are still available for DNA analysis. The results of DNA analysis confirmed that the fish is a coelacanth. The registration number for this specimen is CCC no. 305.

Table 1. Coelacatiun fish caught in indonesian waters since 1997-2018									
No.	CCC	Date	Location	Sex	Total Length	Body Weight			
1.	174	Sep 18, 1997	Manado	Unknown	130 *	30 *			
2.	175	Jul 30, 1988	Manado	F	124 cm	29,2 kg			
3	215	May 19,2007	Manado	F	129 cm	51kg			
4	225	Nov 25, 2008	Talise	Μ	110 cm	20 kg			
5	254	Sep 16, 2009	Talise	М	116 cm	27 kg			
6	287	Jul 21, 2011	Amurang	М	105 cm	13,1 kg			
7.	299	Nov 5, 2014	Gangga Is.	F	130 cm	22 kg			
8.	305	July 1, 2018	Raja Ampat	Unknown	Unknown	Unknown			

Table 1. Coelacanth fish caught in Indonesian waters since 1997-2018

\* Approximate value from Erdmann (1999)

# RESEARCH OF INDONESIAN COELACANTH

The discovery of the sea king fish in the waters of North Sulawesi attracted the attention of national and international scientists. So, in 1999, the Max Planc Institute (Germany) and LIPI conducted a situ-observation using the research vessel Baruna Jaya VIII and Submersible "Jago". Firstly, they observed in the waters around Manado Tua Island. where the first two specimens of king sea fish found. But they could not find any coelacanths in their habitat. Then, they moved to the coastal area of Buol near Toli-Toli town, and finally they recorded the presence of two sea king fishes at 145 meters depth around Buol waters (border between Provinces of North Sulawesi and Central Sulawesi). Furthermore, biological and ecological survey of sea king fish a marine king fish biology was designed by researchers from LIPI Centre for Oceanography, Aquamarine Fukushima (Japan), and University of Sam Ratulangi to understand the distribution of this fish in Indonesia waters, particularly in Sulawesi waters. This survey began in 2006, and by using ROV (Remotely Operated Vehicle), we succeeded recording the presence of several sea king fishes lived at depths of 150-200 m in the Sulawesi Sea.

Recordings of sea king fish in the waters of Manado Bay on June 27, 2007 were considered impressively, because these findings could be attributed to the capture of CCC no. 215 in a relatively similar location. Thus veryfied that Manado Bay is one of the coelacanth habitat. Then, in November 2009, the coelacanth research team also succeeded in recording the presence of marine fish measuring 31 cm in total length, which is thought to be the sea king fish at the juvenile level. This finding also adds to the information that the possibility of spawning location of sea king fish is also around the Manado Bay.

Coelacanth is not only found in the waters of north Sulawesi. In November 2010, the team from Fukushima Aquamarine (lead by Dr. Masamitsu Iwata) in collaboration with Center for Oceanography, Indonesian Institute of Science (lead by Dr. Kasim Moosa) discovered these ancient fish in the waters southeast of Biak Island (Province of Papua).

#### **CONSERVATION STRATEGY**

According to all information obtained from coelacanth study, it could be seen that the sea king fish are not only caught with hand-lining, but also

with trawls and gill nets (BURTON et al., 1992; HISSMANN et al., 1998; ERDMANN et al., 1998). Besides that in some remote locations, there are still possibilities, fishermen can catch fish with using explosives and chemicals technique. All of these fishing method could be danger to the life of the sea king fish. For this reason, research on the sea king fish must be carried out continuously in order to disclose the distribution and population conditions of this fish. These information are very necessary to implement for protecting the coelacanth at its habitat. Based on several discoveries of king sea fish and the increasing recognition of these fish as one of the ancient fish that live in Indonesian waters, protected strategy for this fish are needed. Legally, this fish has been protected through the Minister of Forestry Regulation No. 7/1999. So that efforts to catch the sea king fish for any reasons are not allowed. Furthermore, sea king fish is also protected by CITES (Convention on International Species of Wild Fauna and Flora). Coelacanth, Latimeria chalumnae has been registered on CITES Appendix II since July 1<sup>st</sup>, 1995.

# FUTURE COLLABORATIVE WORKS

Collaborative works between Indonesian and Japanese researchers has been continued after workshop on coelacanth survey held in Jakarta on November 24, 2015. One agreement from this workshop that coelacanth specimen with registration number CCC 295 is transported to Aquamarine Fukushima Laboratories for some detailed analyzing. The fish was cargo to Japan in August 2016 and then, mini symposium on coelacanth study was conducted at Aquamarine Fukushima in November 2016 to discuss on observation. After this symposium, the research team form both sides had measuring morphometric and dissecting abdomen of the coelacanth specimen, and also sampling some parts from this specimen. Finally, this specimen had fixed into formaldehyde for 6 months, then it would be preserved into alcohol for display at the Aquamarine and Marine Science Museum Fukushima.

Several research topics related to the coelacanth specimen that would be conducted by scientists from Indonesia and Japan 2017-2019, as follow;

1. Meristic and Morphology Study Indonesian Coelacanth (AORI - University of Tokyo, Aquamarine / Marine Science Museum Fukushima, Indonesian Institute of Sciences, Museum of Natural History and Human History Kitakyushu, Fukuoka)

- 2. Evolutional Analysis of the lateral line scale and associated neuromast in Osteoichytes (Jikei University and Indonesian Institute of Sciences)
- 3. Comprehensive survey of gut microbiota and food preference of coelacanth, based on metagneomics
- 4. Quantitative study of antropogenic chemicals accumulated in Indonesian Coelacanth (Ehime University, Indonesian Institute of Sciences, and Agency For the Assessment and Application of Technology)
- 5. Distribution survey of Indonesian Coelacanth using eDNA (Univ Riukyu, Indonesian Institute of Sciences, and University of Sam Ratulangi, Indonesia.

Based on these research programs, there are several scientific publication would be produced, such as:

- 1. A Prototype a seen from the comparative morphology of lateral line scale and the accompanying neuromast in the body of *Osteichthyes*;
- Field Survey on the Indonesian Coelacanth, *Latimeria menadoensis* using remotely operated vehicles in from 2005 to 2015
- 3. Observation of the first juvenile Indonesian coelacanth, *Latimeria menadoensis* from Indonesian waters with a comparison to fetuses of *L. chalumnae*
- 4. A detailed morphological measurement of the seventh specimen of Indonesian Coelacanth, *Latimeria menadoensis*, with a compilation of current morphological data of the species;
- 5. Osteology of Indonesian coelacanth, *Latimeria menadoensis* with a comment on the origin of extant coelacanths
- 6. The first investigation of Polychlorobiphenyls (PCBs) in Multiple Tissues of Coelacanth from Indonesia;
- 7. First assessment of persistent organic pollution (POPs) in Organ of Coelacanth from Indonesia: Concentration and Patterns of PBDEs;
- 8. Organoclorine contamination concentration in Multiple Tissue of Coelacanth from Indonesia;
- 9. Biodiversity on gut microbiota on Coelacanth fish;

10. Mapping the distribution of Indonesian coelacanth fish using e DNA study

# REFERENCES

- BRUTON, M. N; A.J.P. CABRAL and H. FRICKE 1992. First capture of a coelacanth, *Latimeria chalumnae* (Pisces, Latimeriidae), off Mozambique. *African J. Sci.* 88: 225-227.
- ERDMANN, M; R. CALDWELL and M.K. MOOSA. 1998. Indonesian 'king of the sea' discovered. *Nature* 395: 335.
- ERDMANN, M.V. 1999. An account of the first living coelacanth known to scientists from Indonesian waters. *Environmental Biology of Fishes* **54**: 439–443, 1999.
- ERDMANN, M.V.; R.L. CALDWELL; S.L JEWETT and A. TJAKRAWIDJAJA. 1999. The second recorded living coelacanth from north Sulawesi. *Environmental Biology of Fishes* 54: 445–451.
- HISSMANN, K.; H. FRICKE and J. SCHAUER. 1998. Population monitoring of the Coelacanth (Latimeria chalumnae). Conservation Biology, 12 (4): 759-765.
- FRICKE, H.; HISSMANN, K.; J. SCHAUER.; J. ERDMANN.; M. K. MOOSA and R.PLANTE. 2000. Biogeography of coelacanths. *Nature* 403, 38.
- IWATA, M.; S. YAMAUCHI; K. FUJII; K. YOSHIMURA; A. KOMODA; M. K. MOOSA; A. SYAHAILATUA; D.H. KUNARSO; K. W. A. MASENGI; I.F. MANDAGI; P. A. ANGMALISANG; J. BUDIMAN; F. P. T. PANGALILA and Y. ABE. 2009. Biological Survey on Indonesian Coelacanth, Latimeria menadoensis. Paper presented in the World Ocean Conference, Manado. May 2009 (unpublished paper).
- POUYAUD, L.; S. WIRJOATMODJO; I. RACHMATIKA; A. TJAKRAWIDJAJA; R. HADIATY and W. HADIE. 1999. A new species of coelacanth. C. R. Acad. Sci. III 322, 261–267.

## "World Aquariums #ReadyToChange to #BeatPlasticPollution?"

•Nadia Ounais<sup>1</sup> 1. INSTITUT OCEANOGRAPHIQUE

In 2017, the European Commission, with the support of the Oceanographic Museum of Monaco, the European Union of Aquarium Curators and the World Association of Zoos & Aquariums, built a coalition of 150 aquariums from 38 countries to raise awareness about marine litter and promote the Our Ocean 2017 conference hosted by the European Union (Malta, 5-6 October 2017).

The campaign was officially launched by the EU Commissioner for Environment, Maritime Affairs and Fisheries Karmenu Vella in Monaco on 27 July 2017, in the presence of H.S.H. Prince Albert II, the Head of the United Nations Environment Programme Erik Solheim, and the WAZA CEO Doug Cress.

The "World Aquariums against marine litter" were engaged in various activities: many displayed a tank full of marine litter to shock their visitors, others organized beach cleans, broadcasted movies, created artistic pieces with litter etc.

All aquariums disseminated the main messages on all communication channels, notably on social media. All these activities, in particular the press releases prepared by most aquariums, resulted in hundreds of articles, television and radio programms.

Following this success, the European Commission and UNEP, together with five international partners, will announce a commitment at the Our Ocean 2018 conference, to transform the 2017 campaign into a permanent action with the focus on plastic pollution.

In addition to long lasting communication actions, the coalition will call on all aquariums to change their procurement policies, for example in canteens and shops, to eliminate all single use plastic items. Aquariums will also be encouraged to ally with all potential partners and multipliers to maximize impact by promoting best practices in behavioral change on a local, regional, national and global scale.

Material will be available for all participating aquariums, based among others on the EU campaign "#ReadyToChange" and on the #CleanSeas campaign by UNEP.

# World Oceans Day + Aquariums = Unique Opportunity to Rally the World to Restore Our Ocean

Bill Mott<sup>1,2,3</sup>, Samantha Mackiewicz<sup>2</sup>
1. The Ocean Project
2. World Oceans Day
3. Youth for the Ocean

World Oceans Day (WOD) provides a unique opportunity to convey humanity's dependence on a healthy ocean for our survival and raise the ocean's – and aquariums' – profile among the public. For 16 years, The Ocean Project has been working year-round with its network of 2,000 partner aquariums and other organizations across all sectors to develop WOD as a global rallying point for raising awareness and promoting empowering ways to help with solutions to the many issues, from climate change to plastic pollution.

In 2002, there were a handful of WOD events in a few countries. Hundreds of aquariums and zoos now regularly participate. Following a petition campaign led by The Ocean Project and World Ocean Network, starting in 2009, the United Nations officially recognizes 8 June as WOD. Since 2015, the WOD Youth Advisory Council (YAC) – with 20 young people from 17 diverse countries – has helped grow WOD in innovative ways. We will share results from WOD 2018 – the biggest ever, with over 1,500 events in 125 countries registered on www.WorldOceansDay.org and social media reach of over 4 billion impressions – and discuss plans for 2019 and beyond will be discussed.

Specifically, The Ocean Project will be proposing a new collaboration with the world's leading aquariums to leverage WOD as a platform to unite and synergize the aquarium community, and work closely with the YAC and other leading youth to protect and restore our ocean. By doing so – and based on our research and results from several conservation campaigns with aquariums and zoos that we will share – we believe aquariums will be better recognized as trusted community and national leaders for aquatic and ocean conservation, increase their relevancy among youth, and increase their number of visitors, both onsite and online.

# Effects of Radiocesium Released from the Fukushima Dai-ichi Nuclear Power Plant Accident on Aquatic Environment Around Onahama, Japan

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Aquamarine Fukushima

A large quantity of radioactive materials was released into atmosphere by Fukushima Dai-ichi Nuclear Power Plant accident caused by the East Japan Great Earthquake disaster on 11 March 2011. Some parts of radiocesium have been deposited on the land area of Fukushima Prefecture, Japan, and have been transported to the ocean through river systems. Therefore, it is important to study a long-term impact of radiocesium on river basin and coast marine environments. This study reports the monitoring results of radiocesium activity in river waters from the two river systems (Natsui and Same Rivers) and in seawater at Onahama Bay from July 2011 to October 2016. Dissolved Cs in the river waters was collected with ammonium molybdophosphate (AMP)/Cs compound. The radioactivity was measured for the dried AMP and suspended solid samples by using gamma-ray spectrometry with low background Ge detectors equipped with a multichannel analyzer during 1 to 3 days. Decay correction of radioactivity for <sup>134</sup>Cs and <sup>137</sup>Cs was carried out at each sampling date.

Total (dissolved + particulate phases) radiocesium activity has been decreased with increasing time after the accident, though the higher radioactivity was observed after the rainy events. The radioactivity of <sup>134</sup>Cs and <sup>137</sup>Cs was 0.37 to 284 mBq/kg and 1.5 to 894 mBq/kg, respectively. The percentage of particulate <sup>137</sup>Cs to total ranged from 11 % to 98 %. There is a good positive correlation between particulate of <sup>137</sup>Cs and turbidity. These results suggest that a particulate phase of <sup>137</sup>Cs is major existent forms and is related to the variation of total radioactivity fluctuation in the Natsui River. The Same River shows almost similar temporal variation. The coastal seawaters also exhibited a decrease of <sup>137</sup>Cs radioactivity with time after the accident from 192 to 6.5 mBq/kg.

# Current Status and Strategic Research Plan of Marine Plastics Debris in Indonesia

Zainal Arifin<sup>1</sup>, Dede Falahudin<sup>1</sup>, Reza Cordova<sup>1</sup>, Corry Manulang<sup>2</sup>, Dwi Hindarti<sup>1</sup>
 1. Research Centre for Oceanography – LIPI, Jakarta, Indonesia
 2. Centre for Deep Sea Research – LIPI, Ambon, Indonesia

Marine plastics and other man-made marine debris have become re-emerging pollution issue after more than 30 year dormant. In Indonesia, the first study on marine plastics and its associated debris was done in May 1985. The research has started again in 2017 after the issue of micro-plastics debris became one of the ocean problems that must be eliminated after the UN Ocean Conference in New York in 2017. Although Indonesia is ranked to be the second nation to produce marine debris, researches in Indonesia have been very scarce and scattered. Our present paper is to attempt to assimilate information related to marine pollution, especially marine micro-plastics and to summarize a strategic plan set by the government of Indonesia. The government of Indonesia issued several decrees on waste managements and national action plan (NAP) controlling marine plastic debris as well as public awareness. On science and innovation fields, research on distribution, source and its effects of marine debris on marine biota has been carried out. The innovation technology is also explored such as, a use and reuse of marine plastic debris and alternative technology.

# ARCTIC 2100

•Sylvain Ghiron<sup>1</sup> 1. OCEANOPOLIS - BREST

"Arctic 2100" is an original educational animation aimed at raising visitors'awareness on issues related to the effects of climate change on marine ecosystems. This new technology using virtual reality headsets is attractive, dynamic and spectacular. It makes it possible to deliver more easily a complex scientific message. This foresight on climate change in 2100 is carried out in close collaboration with the Institute for Technological Research b <> com, Green Hill Studio, Océanopolis and scientists whose skills are recognized worldwide. It offers an animation presenting as accurately as possible the state of the Arctic marine environment today, but also how it may be in 2100. By groups of four, equipped with virtual reality and audio headsets, visitors can discover the Arctic seabed during approximately ten minutes. 20 meters below the surface, divers travel in a hyper-realistic synthetic marine environment and interact freely with each other but also with the fauna and flora. The development of this application took almost two years. It was tested in Océanopolis, presented at the EUAC meeting in October 2017. We were also invited by the Ministry of Ecology to present the application at the French pavilion during the COP23 in Bonn, Germany. It was awarded in April 2018 at the biggest show of French Virtual Reality (Laval Virtual) among the eight most innovative animations.

The final version was operated at Océanopolis this summer 2018. About 4000 visitors tested and enjoyed the application.

# International Sawfish Day - Connecting Public Aquaria, Research and Field Conservation to Secure a Future for Sawfish.

OPaula Branshaw Carlson<sup>1</sup>, Katy Duke<sup>2</sup>, Alan Henningsen<sup>3</sup>, Stacia White<sup>4</sup>
1. The Dallas World Aquarium
2. The Deep
3. National Aquarium Baltimore
4. Ripleys Aquarium Myrtle Beach

The first International Sawfish Day, dedicated to increasing awareness of sawfish, was held on October 17, 2017. This annual event was established through collaboration of the Sawfish Studbook and Species Survival Plans of the European and American Associations of Zoos and Aquariums, and through partnership with the Sawfish Conservation Society (SCS). More than 60 participating organizations from around the world including public aquaria, conservation agencies and research facilities held celebrations and events, shared messages and images on social media, and otherwise spread the word about these amazing animals. Social media posts and interactive events at public aquaria and zoos reached hundreds of thousands of people in the days leading up to, including, and following International Sawfish Day. Fundraising efforts by The Deep, SCS and others contributed to sawfish field programs in the Sudan. In addition to the awareness generated for sawfish this program helped to strengthen the collaborative relationships between the public aquarium community and research and field conservation organizations. Plans are in place for the second annual event to be held on October 17, 2018, and the momentum from the first event will be carried forth in future years solidifying efforts to strengthen both in situ and ex situ conservation and research efforts, with the intent that along with increased awareness about sawfish, these actions will generate additional funding in the future for important sawfish conservation and research projects. The role that public aquaria play in sawfish conservation is critical. We serve as conduits for information exchange, and potential sources for funding, but more importantly we have the unique ability to connect people with living sawfish which can make a lasting impression and ultimately help secure a future for sawfish on this water planet.

# Conservation Activities of Endanger Freshwater Fishes Cooperated by Museums, Regions and Companies

•Shigefumi Kanao<sup>1</sup> 1.Lake Biwa Museum

Lake Biwa Museum carries out activities to preserve and conserve the endanger freshwater fishes in Japan, together with the regions and companies. In this presentation, I will introduce the conservation activities of endanger freshwater fish "Hariyo (Three spined stickleback)" *Gasterosteus aculeatus* subsp.2 inhabiting Shiga prefecture, Japan.

Hariyo has decreased in recent years due to deterioration of habitat environment and depletion of spring water. This subspecies is positioned as Critically Endangered (CR) in the Ministry of the Environment Japan Red List 2017. On the other hand, according to research in recent years, it was known that Hariyo inhabiting Shiga Prefecture has different genes for each river system. Therefore, Lake Biwa Museum began activities to conserve each regional population based on genetic consideration. First, we held a study meeting about Hariyo for local residents in areas where Hariyo inhabiting. Also, depending on the region, field studies were conducted in rivers where Hariyo live, and people in the community saw the real fish. Among them, the local residents reaffirmed the value of Hariyo.

In addition, the museum led the ex-sites conservation in cooperation with the region and companies. In this effort, regions, companies and experts have established close relations with each other for proper conservation, and we created a conservation pond within the premises of the area and company. Currently, in these conservation ponds, more than 300 individuals of Hariyo are inhabited, and populations are properly maintained.

For the conservation of endanger freshwater fishes, the museum plays a core role in research and preservation, and by linking with local communities and enterprises, the activities will be further enhanced.

# A Model for Accredited Zoos and Aquariums to Partner with IUCN on Red List Assessments

Alistair Dove<sup>1</sup>, Rob Bullock<sup>2</sup>, Kira Mileham<sup>3</sup>
Georgia Aquarium
The Deep Aquarium
UCN

The IUCN Red List is the international gold standard for assessing conservation status of animals and plants and aims to provide extinction threat assessments for 160,000 species by 2020. However, much of the work behind Red List assessments is conducted by specialist groups, consisting of volunteer taxonomic experts in academia and the NGO sector. Historically, zoos and aquariums have not played a significant role in the Red List process, and this is a missed opportunity for four reasons: first, a significant amount of taxonomic expertise resides among zoo and aquarium staff globally; second, zoos and aquariums typically have an explicit conservation goal, especially among non-profit facilities; third, participating in Red List assessments has the potential to dramatically increase the scientific profile of the zoos/aquariums involved; fourth, these facilities have enormous potential to educate the public about red listed species; they come with "built-in" extension capability. We present a model that would distribute a portion of the red listing workload (and associated costs) among zoos and aquariums by contributing a portion of staff effort at a zoo or aquarium to IUCN red listing activities. We have trialed this model at The Deep aquarium in the UK and Georgia Aquarium in the US and it is proving both effective and scalable. This model can greatly increase capacity to conduct Red List assessments and thereby understand the extinction threats faced by the species we care for. Furthermore, it better integrates zoos and aquariums into one of the key conservation tools in the modern arsenal, and provides a workflow to share the results of that work directly with the public through zoo and aquarium programs and public relations. We encourage zoo and aquarium executives to consider contributing staff expertise to the IUCN Red List process for the better conservation of wildlife globally.

## Aquariums and the Quadruple Bottom Line

•Cynthia L. Vernon<sup>1</sup> 1. Monterey Bay Aquarium

Each year, the hundreds of aquariums that are members of various associations around the world (e.g., WAZA, AZA, JAZA) welcome tens of millions of visitors. These public touch points afford us powerful opportunities to support our collective goal of ocean conservation: to make a difference for marine species, ecosystems and sustainability. Using Quadruple Bottom Line (QBL) thinking and practices offer new and sometimes challenging perspectives on how we can advance this goal. QBL's four tenets—a commitment to the people we touch, to managing our financial resources effectively, taking responsibility for the wellbeing of our planet, and contributing to continuous innovation in the face of new challenges—can help us address threats to the ocean from human activities the likes of which we have never confronted before. An overview of QBL thinking will be given, illustrated by examples from the Monterey Bay Aquarium.

# Apply to the CLEAR REEF Social Fund for Marine Research and Conservation

•Philippe De Lacaze<sup>1</sup> 1.CLEAR REEF

During IAC Vancouver 2016, CLEAR REEF created a fully philanthropic FUND meant to donate money to promote local initiatives being brought forward by regular citizens (students, researchers, unemployed, retirees, or any person of good will) seeking a financial help to run a project directly or indirectly related to marine research and/or conservation.

It is meant to be at the crossroads of individuals' social/self-development needs, and marine research and conservation issues.

The Fund is exclusively financed by CLEAR REEF's incomes. No any additional contribution is requested from other partners.

Each year: Around 25 projects are fully financed by the Fund all around the world.

Examples of projects financed so far:

An eco-citizen wishing to promote a local conservation action.

A researcher needing to finance his/her project.

A student seeking some help for his/her studies in marine biology.

An unemployed person wishing to bounce back through a specific training linked to an environmental program.

Who can apply? Any individual - physical person - of any country provided that he/she is not acting on behalf of an organization (company, NGO, state organization, university etc.)

This talk will introduce the Social Fund, its achievements and the way laureates are selected.

# The Oceanic Movements of Yoshi, an Adult Female Loggerhead Turtle, Released After 20 Years in Captivity

OMaryke Musson<sup>1</sup> 1. Two Oceans Aquarium

The Two Oceans Aquarium in Cape Town, South Africa, was home to a female loggerhead turtle (*Caretta caretta*) for just over 20 years. The juvenile turtle, named Yoshi, was rescued in 1997 and weighed only 2 kilograms on arrival. By 2009 the Two Oceans Aquarium increased its turtle rehabilitation efforts significantly and over the following 8 years rescued, rehabilitated and released more than 450 sea turtles of all 5 species found along the Southern African coastline. In 2015 two adult hawksbill turtles were satellite tagged before release and successfully tracked for 14 months post release. These positive results gave the Two Oceans Aquarium confidence to prepare Yoshi, by then weighing 170 kilograms, for release. Yoshi was tagged with a SPOT-293A satellite transmitter and released off Cape Point (34°31'44.72''S; 18°7'18.24''E) on the 16<sup>th</sup> of December 2017. Since her release Yoshi has moved up the west coast of Africa covering more than 5000 kilometers. She has maintained an average speed of 1.1 km/hr. The media coverage and support the Two Oceans Aquarium received due to the release of this legendary sea turtle has been tremendous and has contributed greatly to increasing marine conservation awareness and the plight of sea turtles worldwide.

# A New Giant Aquarium for a Theme of the 21st Century

•Stephane J. Henard<sup>1</sup>, Philippe Vallette<sup>1</sup> 1. Nausicaa Centre National de la Mer, Boulogne sur Mer, France

In May 2018 the French National Sea Centre did open to the public a new massive expansion. The central piece of this new building is a 10 Million litres tank which recreates the ecosystem of the high seas.

Such structure is an architectural, aquatic and technical challenge; hammerhead sharks, manta rays and shoals of sardine are displayed together. Several windows including an 18 meters long tunnel and a 20 metres long by 5 metres high flat panel give very different views of the tank.

The theme of this new exhibit is management of the high seas, one of the major issues of the 21<sup>st</sup> century. The tank resembles the emblematic island of Malpelo off the coast of Colombia.

The high sea seems like a vast desert, but is crossed by highly migratory species and inhabited by creatures which travel enormous distances to feed or reproduce. Small fish which provide very large quantities of food for larger marine creatures are clustered at depths of between 200 metres and 1,000 metres. The high sea, which covers more than half of our planet's surface, offers huge scope for exploration and science. It also hugely expands the possibilities for the human race. In this 21<sup>st</sup> century, it is necessary to manage resources in the High Sea, which covers half of the Planet's area. If the resources in the Sea become an alternative to those on the Land, we need to ensure that they are used in a sustainable way while also seeking to eliminate the sources of conflict that this may cause by making it fair. Discussion have just startedat the United Nations, on methods of governance of the high seas.

# Welfare Assessment of Mixed Species Aquariums

°Craig Thorburn<sup>1</sup>, Nicolas de Graaff<sup>2</sup>

1. SEA LIFE Conservation Welfare and Engagement Asia Pacific 2. Zoo and Aquarium Association

Globally Zoo and Aquarium Associations are continually seeking to provide value to their membership and demonstrate to the general public that their members maintain a high standard of animal welfare for all animals in their care.

Aquariums in particular maintain unique and diverse populations of aquatic species often in single exhibit spaces and seek an industry-relevant approach to assessing and managing animal welfare.

The Zoo and Aquarium Association in the Australasia region has developed an Accreditation Program to substantiate its members' animal welfare and supporting practices.

Whereas traditional assessments of animal welfare have relied on avoidance of negative states, the ZAA Accreditation program has employed a body of knowledge called the Five Domains and actively investigates positive experiences for individual animals. The assessment tool has been developed to facilitate assessment of any species in any scenario.

SEA LIFE operates 4 large public aquariums within the ZAA membership and has worked with the ZAA Accreditation team in applying the Five Domains welfare assessment program to assess welfare states of animals in large ocean tank mixed species displays.

This presentation describes the implementation of an assessment tool that has enabled aquarium staff to gain an appreciation of external factors that likely influence and animal's welfare state and provides examples where SEA LIFE staff have gained a better understanding of areas where welfare may have been compromised and or enhanced.

# A New Challenging and Immersive Tank: Night Dive on a Fluorescent Coral Reef!

Olivier Brunel<sup>1</sup> 1. Musee oceanographique de Monaco

The oceanographic Museum of Monaco was one of the first to keep live corals in aquarium. Since 1989 and the founder expedition to the red sea, aiming to collect corals, we grow and breed corals for our exhibition tanks, thanks to our 30 year experience. In March 2017, we opened a new challenging coral tank, presenting fluorescent corals in a dedicated tank and room.

This tank and the exhibition are unique for several reasons:

- It is a  $5m^3$  suspended and open tank, offering a top view on the corals and fish, as well as an underneath view
- It is a unique piece, offered by Nippura, 100 % transparent and made of PMMA with a round artistic shape. This gives the impression of an invisible tank. Children can lay underneath to enjoy and rest.
- Immersive experience of the visitors: the scenography of the entire room recreates a night dive on a fluorescent coral reef. LED light spots are held by fake divers hung on top of the tank.
- Transparency of the tank makes the visitor feel like they are diving around the reef.
- Several screens show "homemade" slow motion coral movies revealing the incredible biology and colors of corals.
- More than 30 fluorescent coral species maintained with blue/white LED spots

One year after opening, the feedback is really positive:

- Corals are nice and grow well in the tank,
- Visitors love the tank, which became one of their preferred,
- The suspended fake divers gave us the opportunity to develop a win-win partnership with our new sponsor.

# **Modern Jomon Zoos and Aquariums**

•Etsuo Narushima<sup>1</sup> 1. Japanese Association of Zoos and Aquariums

The Jomon period is the time in Japanese prehistory, continued for more than 10,000 years from around 13,000 BCE in Japan. The Jomon period named after Jomon pottery. Jomon means "cord marks". The pottery used in this era was called the Jomon pottery. Its surface is decorated with cord-pattern impressions and is generally accepted as the oldest pottery in the world.

The People in the Jomon period did not rely on agriculture and lived mainly by hunting, fishing and gathering nuts and roots which were safe to eat. In the world history, the Jomon period corresponds to the Neolithic Age. People settled by making villages, living a life coexisting with nature. The Jomon people respected nature surrounding them, such as giant trees, giant rocks, mountains and earth and that idea is still living in Shintoism which is an ancient religion of Japan and still practiced today. Modern Japanese people also believe that spiritual powers exist in the natural world like the Jomon people.

Currently, the human environment is falling into a state of crisis due to our human activities, such as climate exchange, destruction of the rainforest, desertification and destruction of ozone layer etc.

In reviewing our lives that require massive energy, I think that it is necessary to reconsider the view of nature of the Jomon people who had been engaged in a sustainable life and to make use of it in the activities of modern zoos and aquariums.

# Working Hand in Hand with Aquariums. Opening up Numerous New Horizons

•Toshiro Saruwatari<sup>1,2</sup>

1. Atmosphere and Ocean Research Institute, the University of Tokyo

2. Seikei Education and Research Center for Sustainable Development

How does an inhabitant of an ivory tower go out and study fish? Wading with a dip net, hauling nets on a commercial bottom trawler, research cruises on board research vessels. All this involves fieldwork and we need all the help we can get, from professionals sharing common interests and skills. During the course of my career, I have had fruitful and stimulating experiences working together with aquarium curators. I would like to share some of those wonderful experiences I have had with the so-called "wet-thumbs".

Since 1984, I have worked on life history of fish in Lake Hinuma, a brackish lake, together with Ibaraki Prefectural Ooarai Aquarium. The high potential and popularity of an aquarium as a local social education institution became more than apparent during a tidal movement survey using homemade GPS buoy. Local fishermen and anglers all cooperated in recovering the buoys, resulting in a 90+% recovery rate. A joint research on deep sea demersal fish, Greeneye, *Chlorophthalmus* spp. together with Aquamarine Fukushima was also very fruitful. Research cruises on RV Tansei maru and Shinsei maru, cumulated into numerous publications, press coverage, and a couple of awards. This project led me in conducting a joint research on extant Coelacanth with Aquamarine Fukushima, an ichthyologist's dream come true. A joint study on Notothenids with Port of Nagoya Public Aquarium took me to the Antarctic Ocean on board RV Hakuhou maru!

Aquariums are not just counterparts of research, but a wonderful stage for outreach activities as well. Symposiums held at aquariums provide a wonderful opportunity to present to the mass, old and young alike, the stimulating and wonderful world of marine biology. I must say, with true appreciation, that I AM indeed blessed and fortunate to have had all these opportunities and interactions with wet thumbs and their aquariums.
## Efforts for Deep-Sea Creature Exhibition and the New Discovery of Their Ecosystem

ORyousuke Komi<sup>1</sup>, Kotaro Tsuchiya<sup>2</sup>,
Ryosuke Mimori<sup>3</sup>, Yumiko Takahama<sup>1</sup>,
Hiroshi Nakamura<sup>1</sup>
1. TOKYO SEA LIFE PARK
2. Tokyo University of Marine Science and Technology
3. Inokashira Park Zoo

The Tokyo Sea Life Park (TSLP) has exhibited deep-sea creatures since 1989, when the TSLP opened. To display these deep-sea creatures, most of them were collected by TSLP staff. We also study the treatment of creatures that suffer from decompression disease due to the sudden change in water pressure by using a pressurized vessel. Any collected creatures that are unable to swim normally due to buoyancy abnormalities, are placed in a pressurized vessel at a collecting site until they normalize. The normalized creatures are transported to TSLP under pressurized conditions and are transferred to a pressurized water tank equipped with filtered circulation equipment, and adapt gradually into normal pressure conditions. This recompression treatment has been successfully used for species such as Red spikefish (*Triacanthodes anomalus*), Japanese perchlet (*Plectranthias azumanus*), and Japanese codling (*Physiculus japonicas*).

The TSLP has been exhibiting the mysterious ecosystem of deep sea creatures, and has recently made a new discovery. In particular, the reproductive biology of very little known deep-sea creatures. At the TSLP, the spawning of Hilgendorf's saucord (*Helicolenus hilgendorfii*) was observed in captivity. Therefore we discovered the oviparity of *Helicolenus hilgendorfii*, which was considered to be ovoviviparous.

We also succeeded in the long-term breeding of Japanese pancake devilfish (*Opisthoteuthis depressa*) by improving breeding conditions. Advance of its breeding knowledge lead us to acquire many findings on the biology of this animal, including its feeding and reproductive behaviors. The spawning was observed in captivity. We were also able to successfully obtain hatchling from an egg, which is new to science. The juvenile was kept for 5 days, and the first feeding of juvenile octopus was observed.

## Creating an Educational Outline, Interpretation and Signage for the Israel Aquarium in Jerusalem

 OShai David Ben Ami<sup>1</sup>, Nicole Wexler<sup>1</sup>, Noam Werner<sup>1</sup>
 1. The Gottesman Family Israel Aquarium in Jerusalem/ The Tisch Family Zoological Gardens in Jerusalem

Israel's first public aquarium was opened to the public in September 2017 after a long process of planning and construction, The Aquarium is a part of the well-established Tisch Family Zoological Gardens in Jerusalem (also known as the Jerusalem Biblical Zoo). In this Presentation, I will give an overview of the planning process that was in the basis of creating and education plan, signage and other educational messages for the new Aquarium. The presentation will deal with three main topics: creating an education plan for a new aquarium, creating meaningful and interesting signage based on the education plan outline and the challenges of creating the same in three languages. I will explore the process itself including working with professional scientific experts, graphic designers and translators in order to obtain the most understandable science-based result; and investigating various interpretation methods.

## Get Out! - Getting Guests Outside and Doing Science

<sup>o</sup>Ed Mastro<sup>1</sup>, Regina Wetzer<sup>2</sup>

1. Cabrillo Marine Aquarium 2. Natural History Museum of Los Angeles County

Public aquaria are popular and highly regarded venues to explore the diversity and natural habitats that most visitors otherwise will never experience first-hand. Aquaria can promote learning about the health of our very special underwater world and as a result guests are inspired to appreciate this very special world.

Getting guests outside our aquaria walls greatly increases our effectiveness and inspires both our future scientists and to take positive conservation actions. The Cabrillo Marine Aquarium (CMA) is located in the Port of Los Angeles, the largest shipping port on the West Coast of North America. At CMA's doorstep are open ocean beach, breakwater with a fishing pier, and a mitigated salt marsh. CMA and its Natural History Museum of Los Angeles County (NHM) partner have a menu of opportunities to engage the community in actual science in these environments.

CMA uses a dedicated exhibit gallery where students conduct scientific projects in full view of guests. This gallery is designed to grow young animals and young scientists. Our joint field surveys with NHM involve guests in sampling our local sandy beaches, mudflats, and tide pools. Community contributed observations are the basis for current and evolving field guides available through iNaturalist [https://www.inaturalist.org/places/pt-fermin]. Guests work alongside staff to measure organismal diversity and abundance. They sample plankton. They participate in sample collection for environmental genomics (eDNA). All these efforts contribute to a better understanding of how populations and environments change, and what we as a community can do to make it a better world for all.

Today's enterprising aquaria are partnering with like-minded institutions to broaden public understanding of ecosystems, communicating science and conservation messages. Together we connect people and nature to our "ocean city" and "urban ocean".

## Mr.Goodfish, New Tools to Increase Sustainable Seafood Consumption

○Florence Huron<sup>1</sup>, Stefano Angelini<sup>2</sup>, Paco

Franco Del Amo<sup>3,4</sup>, Philippe Vallette<sup>1,4</sup>

- 1. Nausicaa, National Sea Center
- 2. Acquario di Genova
- 3. Aquarium Finisterrae
- 4. World Ocean Network

Initiated by the World Ocean Network and launched in 2010 in France, Italy & Spain, the Mr.Goodfish program provides a new approach to the promotion of sustainable seafood consumption, conveying a positive message based on recommendations to "choose the right fish".

Every three months, a committee of experts - scientists, fishermen, fish farmers, retailers, chefs, etc - meet to establish recommendations of wild and aquaculture species through easily understandable criteria for the public. It addresses all chain of custody: fishermen, retailers, chefs, fishmongers or canteens, as possible means to disseminate the message to a larger audience.

Coordinated by Nausicaá, Mr.Goodfish is widely relayed by many partners: Foundation Prince Albert II de Monaco, professional organizations, and consumers associations. These supports add to the program's credibility. The program is also working with others international organizations, through the Global Seafood Ratings Alliance, a coalition of non-governmental organizations (NGOs) each focused on improving the health of the world's oceans and the fisheries they support by implementing programs that rate and promote sustainable seafood products.

Through this communication with national and international institutions, Mr.Goodfish contributes to public debates on environmental issues. The message is addressed to general public within three aquariums: Nausicaá in France, Acquario di Genova in Italy and Aquarium Finisterrae in Spain, through a permanent exhibit.

Mr.Goodfish participated in many events: annual taste week or World Ocean Day, etc. It participated in national and international exhibitions (Expo Milano 2015, Salon de l'Agriculture, etc) and also organized cooking contests in partnership with Chefs' associations.

A website www.mrgoodfish.com and communication tools have been created to develop the program. A dedicated app has been launched this spring 2018. The Mr.Goodfish app makes it easier to get the recommendations for sustainable seafood, get informed about events and locate or share businesses that serve sustainable seafood recommended by Mr.Goodfish.

## **Observation of Breeding Behavior in Captive Emperor Penguin**

○Yosuke Zaitsu<sup>1</sup>, Yuka Ueno<sup>1</sup>, Naeko Kasugai<sup>1</sup>, Nozomi Toyamasaki<sup>1</sup>, Masanori Kurita<sup>1</sup>, Hiroshi Nitto<sup>1</sup> 1.Port of Nagoya Public Aquarium

The Port of Nagoya Public Aquarium started rearing Emperor Penguins (*Aptenodytes forsteri*) in 1998. However, breeding has not been successful. In nature they form huge colonies during the breeding season. They move around the colony freely but do not make nests so it is difficult to observe a particular individual continuously during breeding season. This study aimed to understand the breeding behavior of Emperor Penguins by continuous observation of individuals under captive conditions by video recording.

We observed two pairs of Emperor penguins during the breeding seasons (April to May) from 2012 to 2015. We recorded breeding behaviors in 4 categories: "bowing", "presses female's neck", "lies down" and "mounts".

"Bowing" was observed 95 times during one breeding season. It was seen in April only a few times. In May it was observed more frequently and in the 7-9 days before egg-laying, it was seen as many as 24 times in one day. "Presses female's neck" was counted 98 times during one breeding season. We recorded this behavior a maximum of 11 times in one day, 7-9 days before egg-laying. "Lies down" was observed 14 times during one breeding season. It was observed only during the 6-17 days before egg-laying (maximum 4 times per day). They laid an egg 6-8 days after the last "lies down". They did not lay any eggs in the year when "lies down" was not observed. "Mounts" was observed only 9 times during 4 years of observation. It was observed following the "lies down," however, most attempts failed. The egg laid in 2015 was a fertile egg.

Our data suggests that an optimum period for insemination is the period when "lies down" behavior was observed. Therefore, in future attempts at non-invasive artificial insemination, the optimum timing may be determined by observing "lies down" behavior.

## The Experiences and Learnings of Using Chloroquine to Treat Cryptocaryon Within SEA LIFE

<sup>o</sup>Emily Madge<sup>1</sup>

1. SEA LIFE Marine Programs and Engagement, Merlin Entertainment Group

Cryptocaryon is a commonly known and aggressive pathogen found in public aquaria worldwide that has proven very difficult to manage in mixed species exhibits such as ocean displays.

Traditionally combinations of treatments such as copper, hyposalinity, increasing temperatures and medicated diets are used to 'control' and minimise levels of this disease, but this presents challenges with large, mixed species tanks containing invertebrates and elasmobranchs of which many cease to feed.

In response to recent experiences in dealing with Cryptocaryon in ocean tank displays the need for an effective pharmaceutical that is fast acting, measurable, and has minimal impact on target and non-target species, or effect the clarity of the water is desirable.

SEALIFE investigated and researched the use of Chloroquine to combat the pathogen. After discussion with veterinarians and other SEA LIFE sites and aquarium institutions, it was applied as a bath treatment to the main, mixed species Tropical Marine System in SEA LIFE Chongqing.

This presentation explores the experiences, both positive and negative in using this treatment method; this includes dose rates, operational challenges, accurate testing equipment, impact on fish health and the removal process.

## Approaches to Improve Artificial Breeding of Eudyptes chrysocome chrysocome

OKonomi Ito<sup>1</sup>, Shigeyuki Hayashi<sup>1</sup>, Shoki Murakami<sup>2</sup>, Yoshikazu Kobayashi<sup>1</sup>, Nami Tomisawa<sup>1</sup>, Daiki Nojima<sup>3</sup>, Sotaro Kawakami<sup>3</sup>, Madoka Yoshizawa<sup>3</sup>, Tatsuya Yamamoto<sup>4</sup>, Takaomi Ito<sup>1</sup>
1. Osaka Aquariumu Kaiyukan
2. NIFREL
3. Tokyo Sea Life Park
4. Tama zoological Park

Rockhopper Penguins, *Eudyptes chrysocome chrysocome*, are classified as vulnerable (VU) by the International Union for Conservation of Nature (IUCN) Red List 2017 of Threatened Species. The reproductivity of captive Rockhopper Penguins failed to rise in Japan, resulting in risk of decreasing captive population. Kaiyukan and Tokyo Sea Life Park carried out a study for the technical establishment of artificial captive breeding and the result is reported here.

In this study, fluctuation in calcium, triglyceride and inorganic phosphorus concentrations in the blood was monitored during breeding season from March to May. Mating behaviour was observed from nesting to incubation period through video recording. Using these two methods, the optimal timing for artificial insemination (AI) and day of egg laying were estimated.

AI was conducted using semen from male penguins from Tokyo Sea Life Park. Under physical restraint, samples were collected by exerting digital pressure at the genital papillae. The samples were then diluted with Beltsville (Sexton, 1977) and maintained at 4-10 °C or less for 5-6 hours while being transported to Kaiyukan where semen were injected into female penguins. DNA tests were conducted on blood samples to confirm the identity of the male penguin.

Calcium, triglyceride and inorganic phosphorus concentrations in the blood increased from six weeks before egg laying and peaked at four days prior to egg laying. The day of egg laying could be estimated by monitoring these changes. Based on the observation of mating behavior, the optimal timing for AI was estimated 5-10 days before egg laying. Upon conducting AI 7-10 days prior to egg laying, one chick hatched. AI was confirmed successful for this chick as the parent was the semen donor.

Following this attempt of AI using frozen semen, we will continue further studies with the aim to contribute to the conservation of this species.

# Observations on the Maintenance of Sexually Mature Scalloped Hammerhead Sharks in a Public Aquarium

Helen Ka Wun Chau<sup>1</sup>, Toni Wing Tung Hui<sup>1</sup>, Ashley Sin Tung Kwok<sup>1</sup>, Ken Wai Kin Lee<sup>1</sup>, Lell Man Ho Luk<sup>1</sup>, Walter Yiu Ming Tang<sup>1</sup>, Anthony Kim Ho Chang<sup>1</sup>, Suzanne M. Gendron<sup>1</sup> 1. Aquarium Department, Ocean Park Corporation, Hong Kong China

The existing collection of Scalloped Hammerhead Sharks (*Sphyrna lewini*) in Ocean Park Hong Kong was acquired from Kagoshima, Japan in November 2009. These sharks have become one of the most iconic species on display since the opening of the Grand Aquarium in 2011.

In the past 9 years, marked growth was recorded in this species, which resides in an exhibit environment with over 160 species including elasmobranchs and teleost fishes. The Hammerhead Sharks (five males and three females) were noted to have displayed mating related behaviours as early as 2013 and mating events were observed since 2015.

In this presentation, we describe the three females' measurements, and behaviours observed in individuals and in a group, and include (1) the period when the females were subjected to mating interest (i.e. the timeline involving trailing behaviors, mating-related bite attempts, inappetence, and copulation), (2) mating interest observed in the males (i.e. chasing and other behaviours observed in group, periods of inappetence, and the sequence of females being targeted as the subject of mating interest within the period), (3) observations on the sequence of mating events, from the trailing behaviour presented by males, which occurred at the beginning of events, to the death of the females. The purpose of outlining the facts mentioned is to foster advancement in husbandry and breeding management of this species under human care.

## Study of Reproductive Biology to Create an "Artificial Uterus" to Provide Nutrients for Great White Shark Embryos

Keiichi Sato<sup>1,2</sup>, OTaketeru Tomita<sup>1,2</sup>, Ryo Nozu<sup>1,2</sup>, Masaru Nakamura<sup>2</sup>, Minoru Toda<sup>1</sup> 1. Okinawa Churaumi Aquarium 2. Okinawa Churashima Research Center

Premature deliveries of elasmobranchs sometimes occur in aquariums around the world, but it is difficult to maintain the fetus alive when it is born too small because of the lack of sufficient equipment to recreate the intrauterine environment. To create an artificial uterus to save and accommodate premature fetuses, we studied a variety of uteri from sharks with different modes of reproduction. In general, the gestation of viviparous sharks and the maternal input to intrauterine embryos is complex, especially in lamniform sharks, which includes the great white shark (*Carcharodon carcharias*); oophagy is one of the primary modes of embryonic nutrition. According to our recent studies, the nutrition of embryos for these types of sharks is more complex than previously thought, as embryos likely rely on changing the source of nutrition over the course of their development. We posit three likely nutrition sources: (1) a lipid-rich fluid that is secreted from the uterine epithelium but only during early gestation before the onset of oophagy, (2) embryos use the abundant uterine fluid before using the encased nutrient eggs at the early stage of development, and (3) uterine fluid before the onset of oophagy. The lipid-rich secretion in great white sharks is a unique mode of shark reproduction and resembles that of the trophonemata in pregnant mobulid rays. Currently, we are in the process of developing an artificial uterus to accommodate an embryo after the oophagous phase, and we are analyzing uterine fluid from the salmon shark (Lamna ditropis) to better understand the mechanism in the uterine epithelium that supplies oxygen to the embryos.

## National Aquarium's New Animal Care and Rescue Center: An Upgraded Space for Ethical Animal Care and Behind-the-Scenes Access for Guests

•John Racanelli<sup>1</sup> 1.National Aquarium

Public attitudes have evolved. Today, visitors to public aquariums want more than an experience separated by a pane of glass. National Aquarium guests visit to experience other worlds: vibrant reefs, river gorges, lush rain forests. Until now, few visitors have experienced 'our world' behind the scenes, but that's exactly what they're craving. Visitors want to know what goes into caring for our world's aquatic treasures, learn about the animals in detail, and understand the stories behind our beautiful exhibits.

The National Aquarium recently opened an innovative new Animal Care and Rescue Center, the purpose of which is twofold:

- 1. To provide a new home for the heart of our work—i.e., comprehensive, individualized care for all off-exhibit and rescued animals, as well as saltwater production at 56.78 m<sup>3</sup>/month (15,000 gal/month), and exhibit fabrication. The new Center can accommodate up to 5,000 animals at any given time.
- 2. To allow guests to see the inner workings of our organization. This second function responds to the public's expressed desire to have more access to our work, a desire we greatly value and readily accommodate as it increases empathy for marine animals and supports our mission to inspire conservation.

The new, 5,234 m<sup>2</sup> (56,339 ft<sup>2</sup>), state-of-the-art Animal Care facility was renovated to our exact specifications and built with public tours in mind. A first-of-its-kind in the industry, this new facility has viewing windows lining its primary hallways, which provide transparency and allow a unique opportunity for paying guests and student groups to view animal care work previously unseen by the public. Unprecedented access allows guests to see the Aquarium's commitment to standard-setting animal care firsthand, while also learning how their favorite exhibits are carefully monitored and maintained.

The National Aquarium's New Animal Care and Rescue Center provides guests an opportunity to observe the heart of all we do and, it is our hope, enhance their overall understanding of animal welfare, aquatic science, and ocean conservation.

## National Aquarium Standard Development in China

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There are about 200 public aquariums in China currently. The number of aquariums has grown rapidly from the mid-1990s through now. This rapid growth presents a challenge: balancing the welfare needs of animal collection and husbandry with the demanding requirements of aquarium construction and aquarium management. Two national systems of aquarium standards, focused primarily on marine mammals, have been developing since early 2000. One system, called the "National Vocation Standards for Marine Mammal Trainers", was issued by the Ministry of Labor and Social Security in 2005. The second system focuses on aquarium construction and animal welfare, including minimum spatial requirements for facility, water quality standards, studbook requirements, etc. Parts of this second series standard were issued by the Ministry of Agriculture and Rural Affairs in 2013, while additional standards are under review or development, which will be releasing successively in the future.

### Public Aquariums in Russia. Past, Present, and Future

Liubov Konstanskaya<sup>1</sup>, Andrey Oppolitov<sup>1</sup>
 MCo "Planeta Neptun" St.Petersburg Oceanarium

It is little known that at the close of the 19<sup>th</sup> Century, Russia was one of the leading aquarium countries in the World. Unfortunately, a tragic chain of events during the first half of the 20<sup>th</sup> Century set aquarium science in Russian back, as the rest of the World moved forward. During the era of the Soviet Union, public aquariums were represented by small expositions in zoos. However, at the beginning of the 21<sup>th</sup> Century, the situation started to change: large commercial aquariums were designed, built, and opened for visitors in St. Petersburg and Moscow, and even in far-flung regions of Russia, such Siberian Kogalym and Novosibirsk. In addition, some public aquarium projects were supported by the Federal Government, for example the Primorsky Aquarium in Vladivostok and the aquarium in the Museum of the World Ocean in Kaliningrad. As this trend of growth continues, the public aquarium community in Russia must strive to optimize welfare standards for the animals in their care, and optimize exhibition quality and service for their visitors.

## NIFREL: Making a Living Museum Using Abstract Ecology Technique

OHiroshi Obata<sup>1</sup>, Hiroyuki Doi<sup>1</sup>,
 Hideto Nakagawa<sup>1</sup>, Kiyoko Onda<sup>1</sup>
 NIFREL

Located inside Osaka's Expocity, NIFREL is a unique type of zoological exhibit born of the challenge to design a new and unique way of engaging with nature. Whereas the exhibition at Osaka Aquarium KAIYUKAN provides a dynamic experience using a technique of environmental immersion, NIFREL uses a technique of abstract ecology, providing an experience where visitors are directed to a core, common attribute of exhibit species. The primary concept of NIFREL was to invoke "The Sense of Wonder" (as per Rachel Carson 1965), with seven zones constituted under common themes of animal diversity, color, shape, ability, etc., regardless of natural habitat or climatic origin. By creating a new type of museum, with mixed elements of aquarium, zoo, and art, we hoped visitors who were not interested in conventional aquariums and zoos, would become more aware of, and engaged by, the many surprises and mysteries in nature. The intent of NIFREL was to actively challenge traditional zoological exhibits by taking unprecedented actions—e.g., having artists of multiple genres expressing an exhibit theme during limited live installations. Opening on 19 November 2015, with a total floor space of ~7,200 m<sup>2</sup>, NIFREL received two million visitors during its first year of operation, and an additional 1.2 million visitors the following year. It appears that the original purpose of NIFREL, to reach a new audience, was successful. NIFREL challenges our community through a new expression of biology and by breaking the traditional norms of aquarity. We will continue to challenge these norms in an effort to engage and remind visitors, as much as possible, about the magnificence of living things and nature.

## Catching the Next Wave: Aquarium Business in Evolving Market Conditions

•Elena Kazlas<sup>1</sup> 1.ConsultEcon, Inc.

Aquariums are part of a worldwide industry and are a known attraction type for the general public. The aquarium community continues to evolve, with increasing global awareness of climate change, overfishing, and an urgent need for ocean conservation, as well as an increased development of smaller, forprofit aquariums, with a purely commercial orientation. Aquariums tend to be price and attendance leaders in their respective markets. However, as market conditions change, aquariums, like other attractions, need to remain relevant to a growing, aging demographic, made possible through advances in medical science, as well as engaging to a large, younger population. With the advent and distraction of social media, aquariums play a vital role, not only in ocean conservation and education, but also as place for respite and wonder, away from 'screens'. Aquariums and other visitor attractions are using many techniques to address these market changes: increasing earned revenue potential, attracting new audiences, and sustaining attendance. Based on a survey of industry leaders, and drawing on previous surveys conducted in 2008, 2012, and 2016, this presentation will review: (1) new markets for aquarium developments; (2) models for business and market sustainability in a changing demographic; (3) new opportunities for aquariums to grow both visitor numbers and earned revenue; and (4) the risk of market saturation and the future of the global aquarium industry.

#### PLANKTON 3D: New 3D Technologies for Science Outreach Plankton as You've Never Seen It Before!

OAnne Rognant<sup>1</sup> 1. OCEANOPOLIS

Plankton are invisible to the naked eye, yet they play key roles in aquatic ecosystems and global biogeochemical cycles. They represent 95% of marine biomass and exceptional diversity: viruses, bacteria, microscopic algae, reproductive cells, larval fishes etc. Plankton produce half of the atmosphere's oxygen and therefore contribute to regulate our climate. They form the bottom level of aquatic food chains.

Despite their fundamental role, it can be difficult to present them in aquaria and other centres for science culture or schools, since the capture of this living matter and its manipulation under the microscope poses a number of constraints. Indeed, there are no microscopes able to observe planktonic organisms, from viruses to larvae!

Over thirty-five 3D models of plankton, from viruses to jellies, were created based on microscope imaging and discussions with science educators at Océanopolis and experts at the Roscoff Biological Station in France.

The PLANKTON 3D programme proposes novel plankton exhibits using innovative technologies (RA-RV-ED Kinect) and offers a response to the question: Can new technologies facilitate learning about an object of science, the understanding of a complex message, and outreach in general?

This talk is an opportunity to look at virtual and augmented reality and 3D modelling as promising educational tools that can catch public attention and boost learning. It is also a chance to present innovative, immersive, interactive exhibits designed to engage the public with the fascinating world of plankton.

#### **Global Resources in ZIMS - Support for Species Conservation Programs in Aquariums**

•James Peter Donlon<sup>1</sup> 1.Species360

Medical, physiological and biochemical information regarding a multitude of species is a critical component in the practice of aquatic medicine and in advancing species conservation outcomes. But, unfortunately, such information is not always readily available. Increasing the speed and volume of health care information and experience across the aquatic medicine community is a conservation challenge that needs to be solved. Electronic medical records offer opportunities for both real-time sharing of information between institutions and data mining of existing records to build unique global information resources.

ZIMS is the most widely used records system within the aquarium community. Partnering with the Institute of Museum and Library Sciences, Species360 focused on extracting, summarizing and organizing information contained within millions of medical records to produce resources that could support and improve veterinary care in aquariums and advance conservation initiatives. The project successfully produced 3 completely new medical resources (Anesthesia Summaries, Drug Usage Extracts, Morbidity and Mortality Analysis) and significantly enhanced an existing resource (Expected Test Results). For each of the 4 resources, an algorithm extracts relevant records, discards outliers, calculates values, and assembles the remaining data into a searchable compilation of medical experience that is useful to species conservation efforts.

The global nature and size of the ZIMS database ensures that each resource is the most comprehensive summary available on that subject. Questions about pharmaceutical usage or anesthesia protocols, topics in aquatic medicine that historically relied extensively on personal experience or information shared directly by colleagues, can now be answered in seconds from within ZIMS. As a result, this unique compilation of medical experience and knowledge is critically useful to medical staff and research partners in pursuit of insitu and ex-situ conservation goals.

## Continued Development of Collections of Deepwater Fishes and Invertebrates by a Combination of Submersible and Scuba

Forrest A. Young<sup>1</sup>, Koji Ishigaki<sup>2</sup>,
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Dynasty Marine Associates, Inc.
Blue Corner

Both SCUBA and submersible technologies have been in existence for almost 7 decades. However, recent continued development in techniques have allowed extensive collection of both public display specimens and the description of over 30 new species of fishes and invertebrate across many taxa to depths in excess of 300m. These techniques will be described in detail as will the great reduction in hyperbaric risk profile for the SCUBA diving teams.

## Constant Exhibition of Endangered Freshwater Fishes at Gifu World Freshwater Aquarium

•Hajime Oshima<sup>1</sup> 1.Gifu World Freshwater Aquarium

In 2018, there are 60 public aquariums that are members of the JAZA, more than 36 million people visit a year. Therefore, it is effective to educate the existence of endangered freshwater fishes itself and the circumstance that led to extinction. It is now difficult to see endangered freshwater fishes in the wild, exhibiting the living them at the aquarium is a conservation activities that only the aquarium can do.

However, fishes kept in aquariums have been mostly acquired from wild or purchased from ornamental fish distributors. Endangered freshwater fishes are included in this. However, many of them have a life cycle of 1 to 2 years, which can lead to destruction of natural habitats if you rely on introduction from wild. Therefore, in order to be able to exhibit all the time, it is required to establish reproducible breeding techniques, and it takes much time and labor.

On the other hand, it is necessary to prioritize and efficiently breed with limited costs, because it keeps various organisms besides endangered freshwater fishes.

At Gifu World Freshwater Aquarium, 36 species of endangered freshwater fishes of Japan described in the Red List of the Ministry of the Environment and Gifu prefecture are made permanent exhibition type, and we have kept efforts to secure the captive numbers and maintain the exhibits.

Acheilognathus longipinnis and Pseudorasbora pugnax are positioned as priority species at Gifu World Freshwater Aquarium, captive breeding is carried out as ex-situ conservation activities assuming reintroduction while considering genetic diversity and genetic adaptation to captivity.

As for other species not aiming for wild reversion, we explored more efficient breeding methods for exhibition enlightenment, and as a result, 15 of 36 species (41.6% of total), (11 Cyprinidae, 1 Salmonidae, 1 Gasteroseidae, 2 Gobiidae), have been constantly exhibited by establishing reproducible breeding techniques.

## MARINA Exhibition, or How to Explore the Seven Seas and Make Marine Research and Innovation Relevant to All

Iwona Gin<sup>1</sup>, OFlorence Huron<sup>1</sup>, Helin Haga<sup>2</sup>, Sander Kask<sup>2</sup> 1. NAUSICAA, National Sea Experience Centre 2. AHHAA, Science Centre

Helping citizens of all ages understand the importance of the world's ocean and how marine Responsible Research and Innovation (RRI) can unlock its huge potential is the aim of the MARINA travelling interactive exhibition. It emphasises the role of public engagement, science education, open access, ethics, gender balance and governance in empowering society to meet current challenges and contribute to the well-being of humanity through sustainable use of marine resources.

A special focus is given to marine biotechnologies, deep-sea mining, sea transportation and marine renewable energy while the exhibition showcases how the treasure from the sea benefits our daily life whether we live on the coast or in the hinterland.

Consisting of hands-on displays, the MARINA exhibition encourages active participation of the visitor who has to take decisions and act upon them. In this way they learn to engage in building a sustainable future based on Blue Growth and thus become a marine literate, Blue Society.

The exhibition is developed in the framework of the MARINA project by the science centre AHHAA in Estonia and Nausicaa Sea Experience Centre in France.

## Safe Handling of Stingrays

•Robert Jones<sup>1</sup>, Clem Kouijzer<sup>2</sup> 1. The Aquarium Vet 2. 1-2-1 (Animal Handling) Products Ltd.

One of the greatest Health and Safety issues in public aquariums is the safe handling of stingrays. There have been many serious injuries including one fatality in public aquariums.

The anatomy of the stingray barb and its venom will be presented. We will examine a variety of safe handling techniques and personal protection equipment (PPE) as well a prototype device designed to cover the barb during handling and to protect aquarists.

## Larval Culture Technique of the Japanese Giant Spider Crab Macrocheira kaempferi

•Kazutoshi Okamoto<sup>1</sup> 1. Shizuoka Prefectural Research Institute of Fishery

The Japanese giant spider crab *Macrocheira kaempferi* is the largest crustacean in the world and one of the most important species for exhibition. The development of this crab culture and resource management for sustainable fisheries is our prime focus in ensuring the continuity of this crab availability. In order to produce many juvenile crabs artificially, it is necessary to study the optimum conditions for larval rearing. There are 1 prezoeal stage, 2 zoeal stages and 1 megalopa stage.

The influence of temperature on the survival and growth of larvae of the crab was investigated in the laboratory. *Artemia* nauplii were given as prey. Survival temperature ranges for zoea and megalopa were 12 to 23 °C and 11 to 18 °C, respectively. The duration of each stage increased exponentially at lower temperature. Molting interval was longest for megalopa, followed in order by the 2nd zoea and 1st zoea in that order. The optimum rearing temperature for all the larval stages was 15 to 18 °C. The duration of the planktonic larval stage could range from one to three months.

Larvae of the crab were reared from hatching to the first crab stage in the combination of several conditions, e. g. rearing water, food, bottom substrate, water temperature. The highest survival rate as obtained in a group in which filtered seawater with antibiotics was used, *Artemia* nauplii were given, coral sand was placed in the rearing container after megalopa stage, and water temperature was maintained at 18 °C during the first zoeal stage and at 15 °C after the second zoeal stage. In this group, the best survival to the megalopa and first crab stages were 90 % and 67.5 %, respectively. The number of bacteria should be maintained lower than 10<sup>4</sup> CFU/mL to rear the larvae.

## Natural History Education in the Aquariums

 OHongwei Ding<sup>1</sup>
 BEIJING YIHUA MARINE SCAPE SCIENCE AND TECHOLOGY DEVELOPMENT Co., LTD.

Based on the theory of new museology, the museum has experienced a change of humanism ideology from emphasizing "things" to "people", and museum education has become the primary task of museum functions. This change of the museum education also puts forward new requirements for the transformation of the exhibition system and education mode. The exhibition system of the aquarium needs to be oriented by public natural education requirements to construct and plan a new exhibition science communication system. Based on the needs of nature education, this paper will elaborate the methods of autonomous inquiry learning, natural observation, natural experience and natural exploration etc. and the methods of natural education in the Aquarium.

## **Cost Management in Public Aquariums - Challenges and Opportunities**

•Aleksei Kazakevich<sup>1</sup> 1. Aqua Logo Engineering

Aqua Logo Engineering is an outsource maintenance team for the Moskvarium, Oceania and Aquatica public aquariums in the Russian Federation, and the Ocean Plaza Aquarium, in the Ukraine. Aquarium investors are critically interested in cost efficiency. The primary factors that affect aquarium management costs and therefore management decisions include the following:

1. What determines management costs?

- 1.1. General design decisions building structure, its ergonomics, and engineering.
- 1.2. Conceptual decisions types of exposed inhabitants, collection sizes and diversity, and regulatory requirements.
- 1.3. Technology decisions selected filtration scheme, electricity and water requirement, and number of filtration circuits.
- 1.4. Maintenance methods choice of engineering and animal husbandry teams, and planning and decision-making principles.
- 2. What are the critical components of management costs?
- 2.1. Water and electricity.
- 2.2. Food it is often difficult to forecast animal diet, food quality, and food cost. Feeding costs frequently rise over the time due to biomass growth.
- 2.3. Water quality maintenance consumables (e.g., salt, coal, chemicals, UV-lamps) these costs are easy to predict, but impossible to ignore.
- 2.4. Spare parts and replacement of high-wearing components, as well as unforeseen repairs.
- 2.5. Animal collection replenishment these costs are possible to calculate and are necessary. Staff and/or a management company must be motivated to ensure the longevity of the animal collection.
- 2.6. Staff working with animals and visitors.
- 3. How can one influence the expenses of an already existing exhibition?
- 3.1. Reducing the consumption of seawater.
- 3.2. Optimizing water treatment practices.
- 3.3. Developing staff qualification systems.
- 3.4. Implementing a dispatching system.
- 3.5. Investments in animal welfare.

Conclusions: A significant part of aquarium operation costs can be optimized during aquarium design. However, there is a variety of techniques to further optimize aquarium operations and reduce costs once an aquarium is already operating.

## The Conservation Activities for Amphibians in Tokyo by Ueno Zoological Gardens Inokashira Park Zoo, Tama Zoological Park and Tokyo Sea Life Park

OJunichi Nakazawa<sup>1</sup>, Masaaki Kodama<sup>1</sup>, Hiroshi Nakamura<sup>1</sup>, Momoko Hotta<sup>1</sup>, Ryousuke Komi<sup>1</sup>, Satoru Matsumura<sup>1</sup>, Yasuko Miyazaki<sup>1</sup>, Sachiko Suzuki<sup>1</sup>, Mayuka Ishigami<sup>1</sup>, Kokoro Sato<sup>1</sup> 1. Tokyo Sea Life Park

Recently, amphibians have been decreasing worldwide. In Japan, the decrease of amphibians is especially notable in Tokyo. Tokyo Zoological Park Society takes charge of conservation activities for 11 species of amphibians in Tokyo.

As to ex-situ conservation, the three zoos and the aquarium gain merit by conserving individuals from the same habitat because we can easily exchange breeding individuals to maintain genetic diversity and the population size.

In 2006, Chytridiomycosis, which caused some catastrophic damages in overseas, was confirmed in Japan. Also, Invasion by Rana virus disease was confirmed in 2008.

Chytridiomycosis does not seem to spread among Japanese wild amphibians. However, we aim to establish a system to maintain captive populations as insurance to prepare for invasions by Ranavirus and other infectious diseases.

Therefore, establishing breeding methods is an urgent issue. Currently, 10 amphibian species have bred successfully.

In-situ conservation of Cynops pyrrhogaster was started at approximately 20 km away from the downtown Tokyo in 2002. As the results of breeding condition researches and conditioning the environment, the estimated population has been increasing.

Through the amphibian exhibitions, we try to introduce visitors to the charm of the animals and the current situation they are in. We carry out amphibian themed programs such as the special exhibitions, the field observations and the staff talk shows and field programs to tell the environmental importance of the amphibian habitats.

# Japanese Pygmy Squid *Idiosepius paradoxus*: Its Life History and Potential as a Model for Cephalopod Research, Exhibition, and Education.

•Takashi Kasugai<sup>1</sup> 1.Port of Nagoya Public Aquarium

The pygmy squid, genus *Idiosepius*, is the smallest living cephalopod inhabiting coastal beds of seagrass and algae in the Indo-West Pacific region from South Africa to Japan and southern Australia. *Idiosepius paradoxus*, Japanese pygmy squid, is distributed in the northern areas such as Japan. In the *Zostera* beds of the temperate coast of central Honshu, Japan, *I. paradoxus* have at least two generations within one year, namely, the small-sized summer spawning generation and the large-sized spring spawning generation.

The Japanese pygmy squid is an ideal model not only for cephalopod researches but also for exhibition and education purposes. They are collectable in abundance with a small drag net in the eelgrass beds throughout the year and are tolerant to long transportation. The adults can be maintained in a small tank, which makes various behaviours visible. For example, their unique behaviors such as using ink for predation and external digestion as well as copulation and camouflage are often observable in captivity. They also have a unique habit of attaching the dorsal mantle to the eelgrass by means of an organ secreting an adhesion substrate and laying eggs on the surface of eelgrass or even aquarium walls, from which many fertilized eggs can be constantly collected. A single individual lays up to 2000 eggs over a 70 days period in captivity. The eggs and egg capsules are transparent and can be reared in a shallow glass dish, which enables us to observe the embryonic development under the microscope.

The cephalopods exhibit not only numerous unique behaviors but also a unique body plan, both of which have been intensely investigated by researchers majoring in embryonic and post-embryonic development, neurobiology, physiology, and life history. *I. paradoxus* is an ideal, attractive model species for exhibition and education of such research outcomes in the aquariums.

## Breeding King Crab, Chaceon granulatus, for the Sustainable Deep-sea Animal

Koji Ishigaki<sup>1</sup>, Jumpei Yabuki<sup>1</sup>, Yura Tsubouchi<sup>1</sup>, Taiyo Ishigaki<sup>1</sup>, Hiroshi Miyake<sup>2</sup>, Satomi Komori<sup>2</sup>, Tomoyoshi Sanada<sup>2</sup>, Ayako Oda<sup>2</sup>
1.Blue Corner Inc.
2.Kitasato University

Recently, deep-sea animals for the ornamental purpose in public aquariums have been getting popular. It has been much more concerned about over-fishing by deep-sea trawl fishing and replacement of deep-sea animal exhibit with higher mortality. Therefore we should consider the sustainable deep-sea animal exhibit in public aquariums with conservation of deep-sea ecosystem in the future. We have been trying for breeding deep-sea crustaceans because of not only ornamental market, but also high demand for consumption market.

A deep-sea trawl fisherman offered one Japanese golden crab, *Chaceon granulatus* bearing enough number of fertilized eggs caught by trap fishing at 300 m depth in Suruga Bay. The eggs were cultured at different water temperatures, 9 °C and 12 °C until zoea period between Blue Corner Inc. and Kitasato University. Zoea was hatched in captivity on 47<sup>th</sup> day from the start of the keeping and it took 55 days from zoea to young crab by at least 7 times molting. It had survived in captivity for 22 days.

It has been found through this experiment as follows. The appropriate water temperature is 20-22 °C until the young crab period. It might be better to set water temperature for 18-20 °C after the young crab period. Good food for megalopa-young period is krill, *Euphausia superba*. Need to set up the scaffolding for megalopa to be molt successfully.

## Using High-Throughput Sequencing & Time Series Analysis to Explore the Change of Micro-Ecosystem in Aquariums

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 2. General marine ecological engineering (Beijing) Co., Ltd.
 3. QingDao UnderwaterWorld
 4. Beijing Aquarium

With the progress of management technology on culture environment of aquarium, the correlation between microorganism and culture management has been improved. Researches on micro-ecology formed by various kinds of microorganisms in the environment has been a hot research topic recently. Currently, there are several research problems in the study of microorganism in aquarium, including sampling methods, range of identification, ecology predication.

In this study, detection of aquatic water and the original source water was conducted in two different kinds of aquariums in China, Beijing aquarium (inland areas) and Qingdao aquarium (coastal areas) for a period of 18 months. The aquariums detected were all over 1,000 tons, including freshwater aquaculture ponds and marine aquaculture ponds, inside impounding reservoirs and the sea water outside aquariums. We use the high-throughput sequencing technology to detect and classify all microbial species in the studied water environment to further assess the microbial safety and stability in the current water body. According to the result, a time series model for the aquarium microecology was established.

Our results indicate that, 1) A total of 1840 genus microorganism were identified, including 41 Resident genus and 52 normal Pathogenic bacteria. According to the management records, the abundance of regular detected pathogenic bacteria such as Escherichia coli and *Vibrio* were lower in aquarium. 2) Significant micro-ecology was identified in different circulating water system (seawater versus freshwater) and different part of circulating water system (Ponds/ Cistern/ Source water) based on the high-throughput sequencing results and prediction of PICRUSt ecology model. 3) Based on the model formed by the difference of abundance of microorganism, our data could be used to predict the micro-ecology of circulating water system, laying the foundation for the intervention and guidance of further microorganism adjustment and controlling. Ultimately, a healthy micro-ecological culture environment will be established through rational management.

## Microbial Environmental Control and Countermeasures for Large Aquaculture Water in Aquarium of Inland Areas

•Li Hao<sup>1</sup>

1. General marine ecological engineering (Beijing) Co., Ltd.

The aquarium of inland areas is a relatively closed aquaculture ecosystem. The recycling of organic matter is an important biochemical process. There is a very close relationship between the production, presence and migration of organic matter, the composition of aquatic organisms (microorganisms, plankton, and fish) and life activities. The status and changes of the microbial environment will greatly affect the water quality and fish health. In the water environment with natural conditions, the composition and concentration of bacteria are different due to different sources, locations, forms, and seasons of the water body. Under the interference of breeding behavior, bacteria in the water environment will undergo major changes. This change presents a more complex and complicated relationship.

Based on years of operation of large-scale pools in Beijing Aquarium, this paper combines the regular monitoring and data collection of large-scale pools in recent years, summarizing the management of biological aquaculture in marine aquariums at various levels, including microbiology, breeding individuals, and breeding environments. Existing water environment control technologies have advantages and disadvantages in the scope of application and practical application. The new methods for the treatment of microbial problems in aquatic organisms are summarized, and the deficiencies and solutions of current technologies are analyzed, and the development trend of microbial detection in aquatic environments is also forecasted.

## Cooperative Effort for the In-situ and Ex-situ Conservation of the Common Murre

•Daiki Nojima<sup>1</sup>, Yasunori Takenaka<sup>3</sup>, Mari Iwahara<sup>2</sup> 1. Tokyo Sea Life Park 2. Haboro Ranger office Ministry of the Environment

3. Iriomote Ranger office Ministry of the Environment

The Common Murre is a large Alcidae, collectively nests on subarctic islands of the North Pacific and the North Atlantic Ocean and cliffs of the coast and catches fish by diving. In addition to being listed as a National Endangered Species of Wild Fauna and Flora, it is listed as Endangered Class IA in Red Data Book 2017 of the Ministry of the Environment.

As the Restoration Project of the Common Murre, the Ministry of the Environment has induced murres to breed on Teuri Island, the only remnant breeding colony in Japan, by using the decoys and a vocalization system, and has been monitoring their breeding activities since 2003.

On the other hand, at the Tokyo Sea Life Park, in addition to improving breeding techniques, it became a member of the Restoration Project for the Common Murre since 2017 and provides knowledge obtained from the breeding. In addition, under the auspices of the Japanese Association of Zoos and Aquariums, we purchase and install decoys in the habitat, and we are implementing measures in collaboration with the Ministry of the Environment. In addition to conducting lecture meetings continuously, we actively engage in educational activities such as participating in local events in the town within the habitats.

Based on these, I will introduce the approach aiming at conserving the natural environment surrounding the seabird, to make it possible for the species to stay in a stable state in the natural state by collaborating within the in-situ and ex-situ conservation.

## Proposal for Comfortable Light for Marine Animals and Plants.

Hidetaka Kato<sup>1</sup>, Kazuo Oda<sup>1</sup>, Kiyotaka Yokoi<sup>1</sup>
 Kyocera corporation

We will introduce the results of experiments pursuing high-quality light substituting for mercury lamps and metal halide lamps, which are currently in use in aquariums.

Examples include eelgrasses cultivation experiment, sea lettuces cultivation experiment, corals breeding experiment, on the theme of photosynthesis. At the same time, we will also introduce the results of beautiful color change of corals bred under the high-quality light.

## **High-Quality Lighting for Corals**

Kazuo Oda<sup>1</sup>, Kiyotaka Yokoi<sup>1</sup>
 KYOCERA Corporation

Zooxanthellae are dinoflagellates that live in symbiosis with corals. When photosynthesizing, zooxanthellae provide corals with essential nutrients for coral growth. Normal coral growth depends heavily on the health of their symbiotic zooxanthellae. To test the effect of light on coral growth, zooxanthellae abundance and photosynthetic pigments (chlorophyll a and peridinin) were studied in corals maintained at 24 °C and illuminated with Kyocera's Natural White's LEDs (KNW) and a conventional LED (CLED) at 400 µm/cm<sup>2</sup>/sec. Trials were conducted for two months, for each lighting regime, using two coral species, *Montipora digitata* and *Acropora* spp., obtained from Okinawa. Corals incubated under KNW retained their original color, while corals incubated under CLED became pale over the two-month trial. Zooxanthellae density, chlorophyll a, and peridinin increased by 32 - 47%, 17 - 44 %, and 26 - 125%, respectively, when incubated using KNW, as compared to CLED. Results indicate that the similarity of KNW to natural sunlight provide a highly suitable environment for the long-term growth of corals. It is anticipated that KNW will provide a great tool to study and better understand coral physiological responses, as well as mechanisms of coral bleaching.

# The Exhibition, Research and Education of *Lamellibrachia satsuma* in the Kagoshima City Aquarium

Ayuta Yamaki<sup>1</sup>, Tomoko Yamamoto<sup>2</sup>, Takahiro Furukawa<sup>2</sup>, Hiroshi Miyake<sup>3</sup>, Takehiro Shimura<sup>3</sup>, Toshihiro Maki<sup>4</sup>, Yuka Koyano<sup>5</sup>, Akira Sasaki<sup>1</sup>
1. Kagoshima City Aquarium
2. Kagoshima University
3. Kitasato University
4. Tokyo University
5. Enoshima Aquarium

Vestimentiferan tubeworms (Annelida: Polychaete) inhabit sulfide-rich environments like deep-sea hydrothermal vents or seeps, and harbor sulfur-oxidizing symbiotic bacteria intracellularly. This kind of animal is called chemosynthesis-based animals. The vestimentiferan tubeworm *Lamellibrachia satsuma* has been discovered at depth 82 m in Kagoshima Bay in 1993 as the first species of Vestimentiferan tubeworms in Japan.

Lamellibrachia satsuma has been exhibited for over 20 years since the Kagoshima City Aquarium opened in 1997. L. satsuma has been kept in an aquaria and sodium sulfide solution was supplied for chemosynthesis. This species was found to settle on whale bones that were experimentally deployed beside L. satsuma habitats in Kagoshima Bay in 2010. It is suggested that hydrogen sulfide which is generated by degradation of oil contained in whale bone, attracted larvae of L. satsuma and supported chemosynthesis. Since 2013, we have been trying to rear the L. satsuma collected by this method. This method helps to maintain the original population of L. satsuma in Kagoshima Bay, because of the method collects the new population on the whale bone.

The *L. satsuma* habitat in Kagoshima Bay is the shallowest area where vestimentiferan tubeworms inhabit in the world. For the reason, Kagoshima bay and *L. satsuma* are a good for studies of vestimentiferan tubeworms. We have been revealed population characteristics of *L. satsuma* in Kagoshima Bay. This study showed that the individuals of *L. satsuma* in Kagoshima Bay use various-origin sulfide which is generated from volcanic activity and degradation of sediments by bacteria even in the same colony. Furthermore, we have been shown the relative age composition in and among the colonies of *L. satsuma* by using concentration of lipofuscin which is a kind of protein.

The educational projects and special exhibition of the *L. satsuma* have been also conducted in the Kagoshima City Aquarium.

## Development of a School Travelling Exhibition for Ocean Education

Yumi Watanabe<sup>1</sup>, Kazuyoshi Chiba<sup>1</sup>
 1.Ochanomizu University

The oceans provide us with many benefits such as food, medical products, and oxygen, whereas they have been heavily affected by human activities. To achieve sustainable development of oceans, Japanese government enacted the Basic Act on Ocean Policy (2007) indicating that "enhancement of citizens' understanding of the oceans" and the promotion of "ocean education" in schools. However, education about oceans is not always easy in schools located inland.

To solve these problems, we developed a travelling exhibition in schools entitled "Umi no Megumi wo Itadakimasu". It showed seafoods' stories including biology and food science. Environmental and economic issues are also treated. The exhibition consists of many real objects, graphics, projection-mapping, and interactive exhibition. Most of the exhibition furniture were made of cardboard, enabling it easy to move and set up. This exhibition was completed in November 2017, travelled 5 schools and more. The exhibition has bringing out the distinctive activities from each school, which showed the possibility as a powerful tool for ocean education.

### Introduction of Membrane Filtration to LSS for the Aquarium-Technologies to Ensure Advanced Water Quality and Water Reuse -

OKenichi Futami<sup>1</sup>, Kazuaki Shimamura<sup>1</sup>, Kiwamu Nishimura<sup>1</sup>, Taketoshi Kimura<sup>1</sup>, Sumiyo Sato<sup>1</sup> 1.Swing Corporation

Recently, the typical technical trend for the aquarium includes the adoption of large-size tank for exhibition and closed recirculation system for life support system (LSS), which is expected to be increasing more and more in the future. Together with these developments, it's supposed that the supply with more clear water for large-size exhibition tanks and the recovery and reuse of backwashing wastewater for LSS should be required. Based on these future requirements, the traditional sand filtration for recirculated LSS is not enough, and the introduction of membrane filtration as one of alternative technologies for LSS was discussed in this study.

For the large-size exhibition tank for fish culture with the volume of several thousand m<sup>3</sup>, the visual water turbidity could be observed even with the "extremely good water quality" defined by the common water analysis, which was caused mainly by the micro particles with the diameter less than 1µm according to our findings. Furthermore, the water turbidity was also related to the structure of exhibition tanks and the numerical relationship was also proposed in this study. In order to remove the micro particles resulted to the water turbidity, it was supposed to introduce membrane for the better filtration performance than traditional sand filter. The pilot-scale experiments were carried out and the performance of membrane filtration was confirmed. The introduction of membrane filtration to the reuse of backwashing wastewater has been adopted successfully in many fields, and the same application to the LSS in aquarium was also demonstrated successfully.

In this study, the introduction of membrane filtration to LSS in aquarium was demonstrated to meet the future requirements of both advanced water quality for large-size exhibition tanks and the wastewater recovery and reuse for LSS.

## Husbandry in Primorsky Aquarium: Motion to the Purpose

 OMarat R. Khaidarov<sup>1</sup>
 Primorsky Aquarium, National Scientific Center of Marine Biology, FEB RAS

The Scientific and Educational Complex «Primorsky Aquarium» is a part of the National Scientific Center of Marine Biology, the Far Eastern Branch of the Russian Academy of Sciences. Main building designed in shape of shell has total interior space exceeding 37,000 m<sup>2</sup>. 135 tanks of the Aquarium's nine permanent exhibits hold more than 25,000 m<sup>3</sup> of water. Primorsky aquarium is home for representatives of all oceans and the climatic zones of the Earth. It is about 500 species. They are kept in guarantine in the Scientific-and-Adaptation facility (SAF) tanks before moving into the Aquarium's exhibits. It is supports the work of the Main building across the board providing complex solutions of the problems such: adaptation, quarantine and breeding. It includes all crucial services such as veterinary, ichthyopathology and hydrochemistry labs. Capacity of the SAF allows if it's necessary place the animals and make reconstruction of any exhibitions of the Main building. SAF's structure provide optimal zoning for adaptation and quarantine: warmwater hall (water temperature from 20 to 25°C), coldwater hall (water temperature from 10 to 16°C) and ultracoldwater room (water temperature from 5 to 8°C). Infrastructure of SAF presumes freshwater and marine water in each hall. Besides halls with tanks SAF includes separate ornithology room for penguins(for Humboldt penguins quarantine), greenhouse (plants stock and guarantine for the Tropical Rain Forest Exhibit), algological facility for the kelp forest tank and livefood (artemia, daphnia, mysida, rotifers, microalgae).SAF was built for 3 years earlier than Exhibition building. This made it possible to train qualified aquarium staff and prepare all exhibitions for opening.

## Closure of a 50 Year Old Iconic Aquarium Including the Movement and Transport of Six 200+Kg Grey Nurse Sharks (*Carcharias taurus*) Aged 25 to 40 Years.

•Craig Thorburn<sup>1</sup>, Robert Townsend<sup>2</sup> 1. SEA LIFE Marine Programs and Engagement, Merlin Entertainment Group 2. SEA LIFE Sydney Aquarium

In 2017 Merlin Entertainments, after thoughtful consideration, decided to close the 53 year old Manly SEA LIFE Sanctuary (opened in 1965 as Marineland) in Sydney Australia. Engineering reports came back indicating a cost effective rebuild was not possible due to the building core structure being beyond repair. For this reason the site was closed at the end of January 2018. Home to over 1,000 different fish, invertebrates, reptiles, and birds required careful planning to relocate them to new zoo or aquarium locations. Of these 1,000 different animals, six were 25-40 year old Grey Nurse Shark (GNS, *Carcharias taurus*) resident of the main Oceanarium. These sharks were all in excess of 3.5m and 200+ kg. It is no longer possible to obtain this species from the wild in Australia, nor is it possible to import them, in accordance with the Australian Department of the Environment Recovery Plan for Grey Nurse Shark. The movement of the Grey Nurse Sharks required the development of systems and equipment that would allow the sharks to be submerged and supported by water through all transfers, allowing swimming behaviours during transport and avoid traditional capture stress implications. Through careful planning, a few hiccups, many long days and nights, and 17 interstate transports over 1,200km, Merlin Entertainments closed Manly and relocated over 1,000 animals including the six aging Grey Nurse Sharks with zero transport related mortalities.
## Genetic Analysis and Conservation Activities in Pseudorasbora pumila

Noriko Ishikawa<sup>1</sup>, Mitsuru Sakaizumi<sup>2</sup>
 1.NIIGATA CITY AQUARIUM/MARINEPIA NIHONKAI
 2.Niigata University

Dwarf topmouth minnow *Pseudorasbora pumila* (Shinai-motsugo in Japanese) is a rare freshwater cyprinid fish categorized into "Critically Endangered" in the Red List by the Ministry of Environment, Japan. Japanese Association of Zoos and Aquariums has been working on breeding and preservation of this species since 1995.

In 2012, we conducted molecular phylogenetic analysis of this species, and revealed two genetic groups, the Pacific side and the Japan sea side populations. The Japan sea-side population consisted of five subgroups. Based on the results, wild populations to be preserved in captivity have been reviewed, and *ex-situ* conservation considering genetic diversity is now encouraged. We will discuss several problems that prevent us from achieving the aim. We introduce regional conservation groups aiming *in-situ* conservation of the species, and involvements of zoos and aquariums in *in-situ* conservation and prospects of *ex-situ* conservation by them will be also discussed.

#### 'Where Do My Fish Come From?'

•Rob Hicks<sup>1</sup> 1. Merlin Entertainments SEA LIFE

There is an increasing focus on the source of animals for aquariums across the world, but do we truly understand where and how our animals come to us?

In the past 4 years SEA LIFE centres (52 across the globe with 23 million visitors) have been working on a process that allows us to assess the sources, improve the welfare and look for new opportunities to further develop the ethical sourcing of our animals. A task that we originally believed would be relatively straightforward has had some challenges, but is now gaining traction across the key sources and is creating a significant change in the traceability of our animals. So much so that other organisations have taken elements of our work to help path the way for change themselves.

The strategy includes development and implementation of:

- Approved sources Including assessment and auditing process for external and internal sourcing of animals
- · Positive change programs that create environmental and social advancement in animal supply
- $\cdot$  Rehome working to offer homes for animals that are either non-releasable from rescue programmes or require rehoming from other establishments.

The work continues, the challenges still arise, but the desire and drive to create change and deliver ethical sourcing is never greater.

By sharing our experiences and learnings we would like to create opportunities for the aquarium community to work together to advance the reach and impact of this work.

## Approaches to DNA Research at Kaiyukan

°Takahiro Inoda<sup>1</sup>, Takaomi Ito<sup>1</sup>, Itsuki Kiyatake<sup>1</sup>, Megumi Ishikawa<sup>1</sup>, Shota Matsumura<sup>1</sup> 1. Osaka Aquariumu Kaiyukan Co. Ltd.

In late years, the research using DNA is utilized in many fields and Kaiyukan begun collaborative research from 2016 while getting cooperation from university and research organization.

Based on collaborative research with RIKEN Center for Biosystems Dynamics Research, the whole *Chiloscyllium punctatum* genome sequencing was completed and we found it consists of 4.7 gigabases.

Massively parallel DNA sequencer was used for decoding and we could obtain all genome sequence information of high completeness from relatively small DNA data.

In a precedent research, the detailed monitoring of development stages was carried out and it became clear that 1.5 times earlier than development of *Scyliorhinus torazame's* eggs.

We tried genome decoding with Okinawa Churaumi Aquarium and RIKEN and the whole *Chiloscyllium punctatum* and *Scyliorhinus torazame* genome sequencing was completed. Concerning *Rhincodon typus* genome, it became highly precise information by decoding again genome sequence that has been already available.

We are proceeding with fish disease study using environmental DNA analysis, field research of *Neophocaena phocaenoides* and development of gene screening tool for coexistence microbe of coral. These research will play an important part in various fields, from marine industry to environmental conservation, and the existence value of aquarium as research facility will also increase. We introduce these approaches.

## World Coral Conservatoire

•Nadia Ounais<sup>1</sup>, Didier Zoccola<sup>2</sup> 1.INSTITUT OCEANOGRAPHIQUE 2.CENTRE SCIENTIFIQUE DE MONACO

Coral reefs are in decline due to climate change. Using the network of World Public Aquariums, we propose to build a "Noah's ark" for corals that will serve as a reservoir for conservation purposes, research laboratories, and restoration of coral reefs. The project consists of creating a world reference center for coral that will house, in a network of public and private aquariums, a unique worldwide collection of the majority of species and strains of corals described to date, in the form of living colonies. The World Coral Conservatoire project involved a consortium of scientists, aquariums curators and field.

#### The World Coral Conservatoire

- Will provide scientists with tagged referenced biological material. For each species, a transcriptome will be produced using next generation sequencing techniques.
- Will help to protect the biodiversity of coral reefs using solution-based approaches that combine Science, Conservation and Reef management
- Will increase the ability of reef organisms to tolerate stress and facilitate recovery from disruption using the "assisted evolution approach" to enhance coral reef resilience.
- Will provide comprehensive information on coral and coral reefs for the general public and also stakeholders.

This work will be carried out by all aquarium partners who will join the project and will be coordinated by a steering committee composed of scientists and aquariums curators. The project needs to build on a consortium that will include research institutes that can help sample, cultivate, sequence and analyze data. This consortium will also include NGOs involved in reef restoration (50reefs initiative, Coral Guardian and much more). It's a five-year project starting with the sampling of 200 coral species. The final goal is to reach at least 1000 species In the World Coral Conservatoire.

## The High Seas: Our Future at Stake How to Get Citizens Interested in the High Seas?

OManuel Cira<sup>1</sup>, Francisco Franco Del Amo<sup>4</sup>,
Philippe Vallette<sup>1</sup>, Judy Mann<sup>7</sup>, Ram Boojh<sup>6</sup>,
Alejandra Cornejo<sup>5</sup>, Paul Van den Sande<sup>3</sup>,
Toyo Emoto<sup>8</sup>, Elisa Atger<sup>2</sup>
1.Nausicaa
2.World Ocean Network
3.International Aquarium Network
4.Aquarium Finisterrae
5.CeDePESCA
6.Society for Environment Education & Development
7.SAAMBR
8 FESTIVAL MONDIAL DE L IMAGE SOUS MARINE

The high seas are an ocean of opportunities. They cover two thirds of the ocean and remain unknown to most of the inhabitants of the planet. In 2015, the United Nations decided to start in 2018 negotiations for a legally binding agreement to protect the biodiversity in the areas beyond national jurisdiction (ABNJ), thus launching the largest multilateral negotiation to come.

Nausicaa opened an exhibition in 2018: "Dive into the unknown waters" focusing on the high seas, and organized an international conference to mobilize civil society.

Nausicaá and World Ocean Network will bring public opinion to the ABNJ negotiations. To foster a sustainable equitable and ethical use of the High Seas an ambitious international agreement is needed; to reach such an agreement there is an important need for the involvement of all interested stakeholders and citizens.

To contribute to the negotiations, Nausicaa plans to mobilize a group of celebrity ambassadors, the "Pioneers of the High Seas" as spokespeople for the High Seas. Nausicaá will enrich its exhibition "Dive into the unknown waters", develop educational programs and organize International Stakeholder Conferences. Nausicaa will join WON efforts to promote the Citizen of the Ocean programme worldwide.

Nausicaá and WON started cooperating with Global Ocean Forum, FAO / Common Ocean and IOC/UNESCO. World Ocean Network, will follow the discussions at the UN and share the questions and suggestions of citizens and professionals.

We call all our colleagues from aquariums worldwide to follow this movement, with the support of the diplomatic and scientist communities, and make this topic shared with all our visitors and partners. We are all citizens of the Ocean; the high seas are our future and our responsibility.

## Husbandry and Transportation of the Bowmouth Guitarfish (Rhina ancylostoma)

•Jeffrey de Pauw<sup>1</sup> 1. De Jong Marinelife

The Bowmouth Guitarfish (*Rhina ancylostoma*) is a fascinating animal that pops up in very few public aquaria. This gentle giant easily reaches lengths of 2.5 meters and with its striking morphology, is easily recognized. However transporting and husbandry of the Bowmouth Guitarfish can become a difficult task when it arrives from another institution or from the wild. Wild caught specimens often have helminths and/or protozoan and in some cases bacterial infections present. The husbandry of the Bowmouth Guitarfish will also be discussed including their adaptation to the type of prey they are able to process. This will be supported with a CT-scan performed on a juvenile Bowmouth Guitarfish. This presentation will provide (new) information that we came by and should be shared with others. This to ensure we offer the best to our animals that are kept on display so visitors can learn about the wonders there are swimming in the ocean.

# Recent Advances in the Physiological Ecology of Pacific Bluefin Tuna Derived from Current Tagging Studies in the Coastal Waters of Japan, Western Pacific Ocean and the Sea of Japan

Charles J. Farwell<sup>1</sup>, Ethan Estess<sup>1</sup>, Ko Fujioka<sup>2</sup>
 Monterey Bay Aquarium
 National Research Institute of Far Seas Fisheries

Collaborative research activities in Japan involving Monterey Bay Aquarium and National Research Institute of Far Seas Fisheries during the past five years have focused on Pacific Bluefin Tuna (*Thunnus orientalis*). Migration, diving behavior and associated physiological changes occurring in both the nearshore Pacific Ocean, at Shikoku Island, and in the Sea of Japan, at Sado Island, Niigata Prefecture, Honshu are the focused research areas of interest for this species.

Highlights of research results from both study areas will be presented utilizing both published and inpress data, including physiological changes occurring in developing young of the year Pacific Bluefin Tuna in the near-shore Pacific Ocean, Shikoku Island.

Migration behavior of older, 3-5 year age class, Pacific Bluefin Tuna archivally tagged in the Sea of Japan, show a return to their spawning site area. Aerial and underwater footage plus research data will be shared in this presentation.

# The Oceans off Fukushima: The Underwater Road to the Continental Shelf

OShinya Yamauchi<sup>1</sup>, Takaharu Ito<sup>2</sup> 1. Aquamarine Fukushima 2. Atelier Mov

The Oceans off Fukushima display corner was reopened on April 27, 2018 after undergoing renovations.

For the renovations, we made substantial changes to the shape and layout of the display aquariums, and gave the corner the title The Oceans off Fukushima: The Underwater Road to the Continental Shelf. A continental shelf is a topographical feature of the oceans floor that is so named because it extends from the coast to a water depth of 200 m. The displays in the corner are comprised entirely of images in which visitors can make a variety of discoveries while taking a simulated underwater voyage in a submarine from the continental shelf to the deep sea.

What is more, we have organized the exhibit around the three keywords of: "twilight zone", "deep sea", and "marine snow" in order to further clarify the images.

"Twilight zone" is a world to which only feeble sunlight can penetrate, and which serves as the entryway to the deep sea.

"Deep sea" generally refers to pitch-black waters where sunlight cannot reach that are generally found at water depths of 200 m or greater.

Such areas contain a world of deep sea creatures that are adapted to a harsh environment that features high water pressure, low water temperature, and so forth.

"Marine snow" was named for the fact that organic matter such as the bodies of dead plankton and their excrement constantly drift down to the deep sea in a manner that makes it appear as if snow is falling.

What is more, at the group of aquariums with round windows that have been patterned after those of a submarine, visitors can catch a glimpse of a wide variety of small deep sea creatures.

# Interactive Experiences in Aquarium: A Way to Create Oceans Health Awareness, Combining Fun, Information and Lifetime Experience

Cucio Conti<sup>1</sup>, Tim Zhang<sup>1</sup>,
Francesco Palaferri<sup>1</sup>
Atlantis Sanya

Introducing the recently open Lost Chambers aquarium in Atlantis Sanya, Hainan Island, China which opened to public in March 2018. This brand-new facility has amazing exhibits and offers its guests the unique opportunity to experience interaction with several species of marine life, and this experience is enriched by expert Aquarists that provide a personalized educational program. This presentation will illustrate the different interactions currently offered and discuss the full experience offered to the guests with special attention to the educational component, the feeding experience, and the different interactive opportunities. This presentation will highlight the power of education thru interaction as a key element to create lifetime memo

# Prophylactic Techniques for Mahi Mahi (Coryphaena hippurus) in Aquarium

Francesco Palaferri<sup>1</sup>,
 Lucio Conti<sup>1</sup>, Tim Zhang<sup>1</sup>
 Atlantis Sanya

In July 2018 our recently opened Aquarium of the Lost Chambers located in the resort of Atlantis Sanya, in Hainan Island in China, received a total of 12 Mahi mahi form a local fish supplier. As not many aquariums in the world are hosting this specie we could not find sufficient information to conduct a successful quarantine prophylactic treatment prior the introduction to the main exhibit. For every new fish in Aquarium it is crucial to do a proper quarantine period to limit the introduction of pathogens to the exhibit. We have noticed that all fishes who come from the wild are carrier of several parasites and bacteria. Mahi mahi are susceptible to several different parasites (Palko et al., 1982; William Jr. and Bunkley-William, 2009) and disease (Leamaster and Ostrowsky, 1988). In the Poster we will describe which kind of Prophylactic treatment was performed to our collection of Mahi mahi and which parasites were identified in the skin scrape sample we collected.

# Long-Term Temporal Variation in Lake Inawashiro Water Quality

°Takayuki Sato<sup>1</sup>, Saori Onuma<sup>1</sup>, Kazunori Nakamura<sup>1</sup>

1. Research Department, Fukushima Prefectural Centre for Environmental Creation

In Lake Inawashiro, the largest acidtrophic lake in Japan, deterioration of water quality based on increased chemical oxygen demand (COD) and detection of coliform bacteria, has accompanied the rapid pH neutralization, which started from the mid-1990s. To elucidate the cause of the deterioration, we investigated long-term temporal variation in water quality data collected continuously by Water and Air Environment Division, Social Affairs and Environment Department, Fukushima Prefectural Government. In particular, we focused on parameters that are highly relevant to internal primary production in the surface water layer at the center of the lake. Our results showed that the surface water of Lake Inawashiro showed a dramatic increase in primary production because the cell density of both phytoplankton and zooplankton increased exponentially since the start of neutralization.

## Sand Filters and Beyond: The Future of Life Support Design

•Paul Cooley<sup>1</sup> 1.PCA Global

Sand filters have been the "Go To" mechanical filtration method for aquatic animal life support system designs for the past 40 years. They are simple, cost effective and with the use of filter aids (including using ozone as a micro-flocculent) can produce excellent water clarity. However, with the increased desire to remove or reduce the use of oxidants in the system, a review of alternative treatment methods has been completed with the objective of optimizing the water quality and minimizing the space requirements, water consumption and capital and operational costs. By looking at existing facilities, installing a full-size pilot plants, and providing a mathematical model to extrapolate the data, an optimization of the full-scale systems was developed. This data will be presented.

#### Field Survey of the Wrought Iron Butterflyfish Chaetodon daedalma on the Ogasawara Islands

Satoru Matsumura<sup>1</sup>, Masaaki Kodama<sup>1</sup>,
 Hiroshi Nakamura<sup>1</sup>, Naoaki Kawahara<sup>1</sup>,
 Hiroshi Arai<sup>2</sup>
 Tokyo Sea Life Park
 Tama Zoological Park

The Wrought iron butterflyfish *Chaetodon daedalma* is endemic species to Japan and mainly distributed around Izu and Ogasawara Islands, Tokyo. Although this species is well known to constitute a temporary aggregation which occasionally consists of more than 100 individuals in spring, detailed field study has not been conducted. Furthermore, the Wrought iron butterflyfish is listed on the red list of threatened species of Tokyo as a concern species because of apprehension about overfishing by aquarium fishery. To provide ecological information which leads to its conservation, Tokyo Sea Life Park (TSLP) has started a field survey around the Ogasawara Islands since 2012. As the result of the social grouping survey focusing on the number of individuals swimming together, we found that they usually form pairs. Also the results of tagging study showed that 26 individuals were tagged and released, and 21 individuals were recaptured including 9 pairs. Consequently, It appears that they inhabits the same home-range for 768.5±348.6 days (n=21) on average and the pairs stay together more than one year including nonreproduction season (645.4±351.5, n=9). Those results suggest that the Wrought iron butterflyfish has a monogamous social system. Also, shearing the information on focal species with local community plays an important role for their conservation. TSLP occasionally holds a lecture meeting on the wrought iron butterflyfish and is planning to build a framework for conducting surveys in collaboration with local recreational dive operations.

## Kamo & Paris Aquarium Story

 ○Alexis Ludovic Powilewicz<sup>1</sup>, Kazuya Okuizumi<sup>2</sup>
 1. Aquarium de Paris
 2. Kamo Aquarium

Kamo Aquarium is the world's first Aquarium for techniques and protocols to breed jellyfish. Paris Aquarium, is a 4 milion liters, 70 tanks, fully subterranean Aquarium, rebuilt in 2004-2006.

First we failed: we started with one jellyfish kreisel tank, one species *-Aurelia aurita-* and one aquarist dedicated to jellyfishes. We went nowhere in 2014 and even missed the 1st International Jellyfish Conference, held at Kamo Aquarium in 2015.

Second we asked for help: We started to work with Jacqueline Goy in 2014. She recommended a visit to Kamo. Through Kaiyukan Aquarium, we were introduced to Kamo Aquarium, which offered on the spot to train our staff on site!

Third we learned: Cooperation between Kamo and Paris Aquariums started. Two Paris staff went one week to Kamo mid-June 2015 and returned two weeks in 2016. This made Paris first jellyfish display possible.

Since then Paris team has been trained three more times, and Kamo Aquarium team visited Paris two times. Paris currently keeps around 50 polyps species and displays them in 19 tanks. We strictly stick to Kamo's teaching & protocoles.

A friendship agreement was signed on the 31st of May 2016. Our teams work together on an almost daily basis. Paris Aquarium can be seen as a backup for Kamo's collection of polyps.

Nowadays Paris Aquarium starts to collect new species and share them with Kamo (*Phyllorhiza punctata*, *Stomolophus meleagris*) and we work on new reliable feeding technics for predatory species.

Conclusion: the keys to our successful cooperation were humility and dedication. Humility because Paris staff had to accept to learn everything from scratch, dedication because it was a huge human investment, time consuming and costly. The rewards are already impressive and we can't stop thanking Kamo's team!

# Amalion, a Lighting That Cares for the Plants

OThomas Schwend<sup>1</sup> 1. BLV Licht- und Vakuumtechnik

Plants are an important element of most zoos, parks and aquariums installations. Finding a lighting, which keeps your plants healthy without compromising the atmosphere of your exhibition can be a challenge. Designated plant lightings are usually pink, disturbing to the human eye, and designed to stimulate extensive growth. White light, on the other hand, is pleasing for the visitors, but inefficient when it comes to photosynthesis. Therefore, we developed a new product line `Amalion`, which gives plants all the light they need and the visitor white light with all the colours they wish for. It emits a full spectrum with all colours in the visible range and, at the same time, provides sufficient photosynthetically active photon flux for plants to grow. In this way, your plants will thrive without interfering with the human experience of an indoor installation.

#### The Role of Aquariums for a Development of Marine Science

OJeongrack Koh<sup>1</sup> 1.LOTTEWORLD AUQARIUM

"Aquarium" comes from the Latin words, a combination of aqua (water) and atrium (place). In the past, an aquarium means an institute of displaying and introducing marine creatures. Nowadays, aquariums not only collect data about natural science and social sciences of marine study, but also organize and classify the data. Furthermore, aquariums play a role in introducing these data to ordinary people in various ways while they carry out research and analysis. It means that the aquarium's role has been expanded even to organizations and facilities which trying to develop marine science as well as marine species conservation center.

In the past, people simply looked into the water tank. Over time, the aquarium has had exhibits in various ways and has set a theme. Then, gradually, the division of the exhibition was made including as experience, behavior and ecological exhibition. During this process, the issue of animal welfare was also presented and this is not only the pleasure of viewing the exhibit but It has become a driving force to develop species conservation and research institutes.

Also, although imports of ornamental fish with the development of the aquarium industry have grown, there are still a lot of confusions in naming fishes with country name and scientific name. This can lead to ecosystem disturbance but also crossbreeding. Crossbreed fishes and variable species which have been raised in aquarium with isolating completely from the nature indicated lots of problems in fish's classification system.

In conclusion, for research projects, Lotteworld aquarium could be a test bed to collect gene information and accomplish a key role of the aquatic species conservation. Predictive biology will become possible based on morphological classification according to species diversity and research data through genetic classification. This type of biology can be used as basic data for species conservation by using role and value of marine life as an educational resource.

10th International Aquariunm Congress

Date: November 5-10, 2018

- Venue: Onahama Fish Market, Event Hall Shiome, Aquamarine Park, and Aquamarine Fukushima Iwaki City, Fukushima Pref., JAPAN
- Parcitipants: 500 delegates (30 countries and regions)
- Organizer: 10th IAC Domestic Committee [Aquarmarine Fukushima, Fukushima Prefecture, Iwaki City, Inawashiro Town, Iwaki Chamber of Commerce and Industry, Iwaki Tourism and City Planning Bureau, Onahama City Planning Citizen Conference, Fukushima Prefectural Federation of Fisheries Co-operative Associations (Onahama Federation of Medium Trawlers), Japanese Association of Zoos and Aquariums, Enoshima Aquarium, Tokyo Zoological Park Society, Tokyo Sea Life Park, Niigata City Aquarium]

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