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 jellies 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 which leaves aquarium visitors 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 & Reeve 1974). Nevertheless, maintaining a ctenophore exhibition has always been challenging. Knowledge of how to culture pelagic ctenophores is limited to a few universities and aquariums working with *Mnemiopsis leidyi*, 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. leidyi* 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.

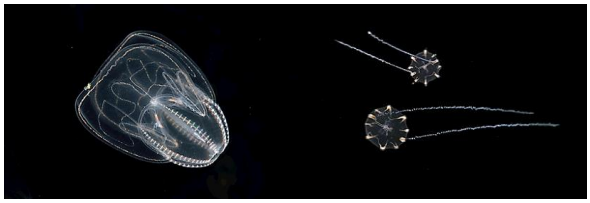

Fig. 1. Adult *Mnemiopsis leidyi* pictured on the left. On the right, the juvenile form of *M. leidyi* referred to as a cydippid.

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. leidyi* 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.

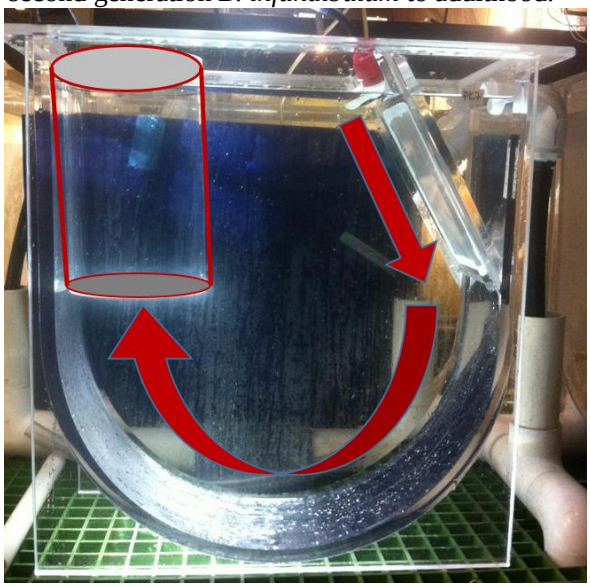

Fig. 2. A cylindrical ctenophore rearing cylinder inside of a pseudokreisel. Seawater flow (red arrows) from a spray bar allows for passive seawater exchange through a 20 micron screen at the bottom of the cylinder.

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 creamy-colored 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.

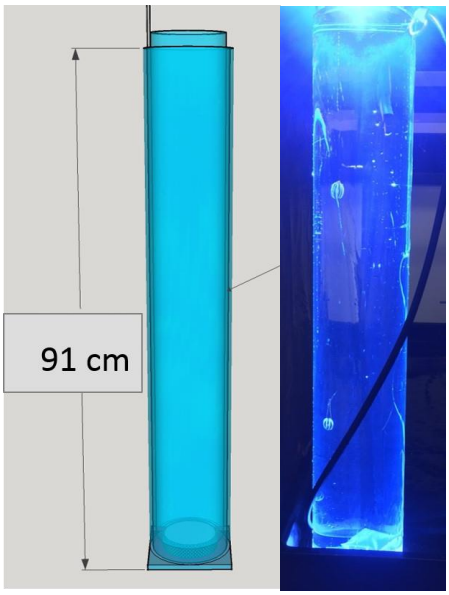

Fig. 3. Diagram of the double cylinder (left), and two adult *Pleurobrachia bachei* spawning inside the double cylinder (right).

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.

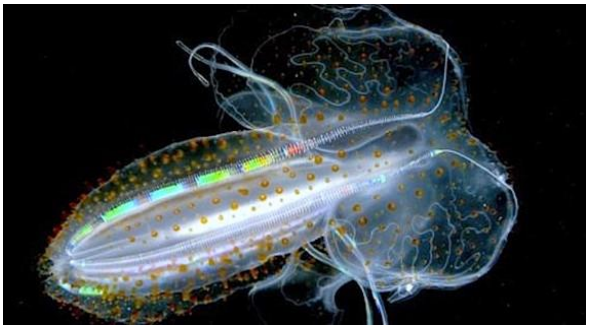

Fig. 4. Adult Spotted comb jelly, *Leucothea pulchra*.

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 leidyi* 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.

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