UNDERWATER WISDOM

A COLLECTION OF ARTICLES AND ESSAYS ON CAREERS THAT EXPLORE AQUATIC WORLDS

by

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Abstract

The articles and essays that make up this thesis feature professionals who seek to understand different aspects of the vastest ecosystem on the planet—the ocean. As scientists and aquarists work to understand the ocean and its inhabitants, they explore how aquatic animals may inform the human condition, uncover unexpected biological mechanisms, and study the elegant and delicate chemistry that allows for underwater existence.

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Introduction

Water is the most powerful element on earth. It shapes the land we inhabit and the lives we live. The strength and beauty that lies within the world's water is what drew me to marine sciences. I believe we have a lot to learn from the ocean and a lot to learn about the ocean. People often underestimate the importance of our world's waters and those who inhabit it, but a select few people understand these implications and dive deeper.

The field of marine science is vast, to match the expanse of the ocean itself. Within this career field are people who are devoted to understanding everything from the chemistry of the water that the animals thrive in, to the biological and genetic makeup of an organism, to the specialization of optimizing an animal's care in captivity.

As I pursued my own education in the marine sciences, my appreciation for the ocean deepened as my world began to revolve around it. Early on I became interested in invertebrates, particularly corals and later cephalopods (squid, octopus, and cuttlefish). I've been afforded a number of diverse opportunities that fueled my passion for both writing and for exploring the ocean environment. In my quest to understand underwater worlds, I found myself learning unexpected things about myself, most notably during my experience as a cephalopod operations intern in Woods Hole, Massachusetts.

There I was afforded opportunities to not only pursue my scientific inquiries, but also to explore my newly identified interest in writing about science. I talked to diverse scientists who studied intricate biological mechanisms of marine organisms with the hopes to be able to inform the human condition. I had conversations with intellectuals who were innovating and diversifying their fields. While I nurtured my own research and raised baby cuttlefish, I also listened to the stories of scientists and learned about their research.

Through this formative experience, I found that my aspirations more strongly aligned with sharing the exciting and unusual stories of these scientists and, more importantly, with communicating the significance of their underwater wisdom to the world.

All of this work was written since 2018 in the Hopkins Master of Arts in Science Writing Program. I would like to say a special thank you to my thesis advisor, Brittany Moya del Pino, for her guidance and invaluable feedback throughout the process and to my faculty advisor, Melissa Hendricks-Joyce, for her assistance along the way. I have an overwhelming amount of gratitude for Taylor Sakmar and Bret Grasse for their guidance during my time as an intern with the Marine Biological Laboratory. I'd like to thank everyone who positively influenced my time there, all whom I interviewed, and all who provided insights along the way. Finally, I'd like to thank my family for always encouraging my education and especially my mom, who knew I was a writer long before I realized it.

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Generations on the *Gemma*

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The September sun glazed over the docks of the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts, causing the ripples on the water to glitter. Across a quiet pond, mallards bobbed through the soft wake, a moored sailboat turning as the direction of the wind changed. The afternoon air wafted a slight smell of seaweed, leaving behind the coarse taste of salt. It was just past 1:30. She was late.

After a while she made her appearance, humming softly, gliding across the water toward the dock. *Gemma* is her name, and she slid into position effortlessly by the command of a seasoned captain. As she pulled alongside the pilings, all hands were on deck grabbing and tying off lines. The captain walked to the back deck and opened a long blue cooler, the tangy smell of marine life rose potently in the air and mingled with the scent of oil fumes from the *Gemma's* overworked engine. Bursts of water and flapping fins hardly fazed the captain as he grasped the squid from the container and transferred them to smaller coolers on the dock. Young researchers and lab assistants in rainboots and windbreakers acted as deckhands, briskly packaging the coolers and wheeling them away.

The *Gemma* is a standard fishing boat with a wide, tall bow and a stern covered in nets, hooks, and lines used for trawling. The hum of the engine quieted as it sputtered to a stop, leaving only the sound of water trickling off the back. The hull is painted forest green, and her name is written in red block letters on a plank of wood, screwed into the side of the boat. Not far from that is a plaque of the MBL emblem, paint faded and peeling away.

The old emblem seemed to be symbolic of the deep history of squidding and fishing in Woods Hole. And so was the captain, an older man who was now emerging from the cabin with his backpack slung over one shoulder and a dog leash in his hand. He clipped it to a black dog with a graying face who was waiting for him on deck, and the two of them began to make their way down the dock, the captain glancing back to give a smile and nod to the remainder of his crew. Woods Hole has its history of squidding, and this is a man who is deeply intertwined with it.

Captain Bill Klimm is exactly the kind of guy you'd think of when you hear the word "fisherman" with his dark blue jeans, a plain grey shirt, and a ball cap shading his slightly sunburnt face. His eyes are a startling shade of blue, so vivid it's like all the time he has spent on the ocean suspended the colors of the water within his eyes. In September, at the age of 82, he was still the captain of the *Gemma*, but would be retiring in less than a month after almost 70 years of working on boats.

It all started with his father, Captain Henry W. Klimm. In 1942 Henry, along with his wife and six-year-old Bill, moved from Hyannis to Woods Hole for a position as a commercial fisherman. As they packed up their bags and made their way west along the Cape, they could never have known the lasting impact they'd have on an entire industry and ecosystem.

Captain Henry quickly became a local legend around Woods Hole, captaining a number of fishing vessels. You could hardly mention his name without someone pointing out his contributions to *Fishes of the Gulf of Maine* (1953) by Henry B. Bigelow and William G. Schroder, which became the main guide for local fishermen and biologists. Captain Henry was taking Bigelow and Schroder out on his vessel at the time, the *Loligo*, to make their observations. He’s also credited with observing a number of species for the first time along the coast of New England, including manta rays, fluke, flounder, and boarfish. Yet one of his most interesting discoveries was the migratory patterns of swordfish.

Bill Mebane, an aquaculture engineer at the MBL, explained that Henry was the person who discovered where the migratory swordfish went in the wintertime. One summer upon their return to the Cape after a long trip out at sea, “he noticed a lot of swordfish had bananas in their bellies,” Mebane recounts excitedly, “big bunches of bananas! Like, a bunch of bananas as big as you are!” Captain Henry thought that the swordfish were grabbing bananas that were washed into the Caribbean or falling off trade ships. Eventually he cruised through the Caribbean one winter and discovered the swordfish and their signature snouts popping up amongst the waves.

Captain Henry is remembered not just for his discoveries, but for his physical presence as well. Dave Remsen, the current manager of Marine Research Services at MBL, has known the Klimm family since he was in high school. He remembers Captain Henry fondly, recalling in amazement how agile he was, bounding up and down the docks and working on boats until his very last days. “When Henry died, he was about to go out lobster fishing with somebody. He’d eaten breakfast, gotten dressed, and was putting his boots on. Then he sat down on his bed and laid back. That’s how they found him.” Captain Henry Klimm is beloved for his contributions to modern knowledge of the Cape’s local marine fisheries, and his son Bill continues to support that legacy.

“By the time I was 12, maybe 13, I had gone to work on [my dad’s] boat in the summertime,” Captain Bill recalled. Or, according to his shipmate and future successor, Bill Mylett, "his mom kicked him out the house, forced him to go work with his dad just so he'd stay out of trouble." Even then, Captain Bill was helping with all the same tasks on the boat that he does now, though without the title of Captain. By the time he graduated high school, he was a natural when it came to boats. "When I got out of school, I was living here in Falmouth, and a construction company had a little towboat. I was going out to work on the towboat and the captain asked, 'Do you wanna drive?'" When the Captain jokingly asked him to maneuver across the sound, Bill was up for the challenge. And that would be the first time Bill Klimm captained a vessel.

He continued to work on commercial fishing boats until his dad called on him for a hand on deck. Captain Henry's shipmates also had children who had been out on the boat, but none of them had a taste for it like Bill did. "My father needed someone to help, that's how I got suckered into here," Captain Bill laughs. Previously a commercial fisherman, Captain Bill's role on the *Gemma* would transition him to obtaining specimens, specifically the longfin inshore squid, for scientific research. This local squid species, *Doryteuthis pealeii,* has been used for decades as a foundation for our basic understanding of how nerves work. These squid are useful for neurobiology courses, anesthetics, work with the eyeball, imaging work with different body parts, and behavior studies. Mylett pointed out, "The squid is prized for its single axon, its main nerve, running down the length of its body. It's one of the largest in the animal world. People doing research on nervous system functioning love them for that type of medical research." The work of these fishermen helps to contribute to a further understanding of this aquatic animal as a human disease model.

So, what exactly is a day out on the *Gemma* like? It starts by catching the opening of the 6:30 a.m. drawbridge from Eel Pond into the Nantucket sound. From there, the crew travels an hour and a half to the western end of the Vineyard sound, right near Martha's Vineyard. Not far from those beaches is where they begin to trawl. Trawling involves casting a long net off the back end, or stern, of the boat. The net drags across the sea floor as the boat moves along, entrapping squid and fish along the way.

Each trawl takes close to an hour. So, depending on the day and how many squid need to be caught, the crew on the *Gemma* does between four and seven trawls. An hour and a half to arrive, an hour per trawl, and another hour and a half to get back. These are long days, often amounting to 10 hours of work. But when asked what a day out on the water was like, Captain Bill answered simply. "Go out, hope the weather's good. It's not much different than going to the beach. You have to work, that's all."

While he may seem like just a simple fisherman providing samples for research, Captain Bill's depth and insights go far beyond his humble surface image. The knowledge and experience that comes with running and captaining a boat in this way is something you can't teach. At this stage, Captain Bill seems reflective of his career, according to Mylett. "You do look at him sometimes and wonder what he's thinking. Sometimes when we're cruising out to go squidding he'll be back on the stern deck just sort of staring out at the scenes going by at the water, the hills, the islands and such. You just wonder what's going through his mind at this stage in his life."

Mylett goes on to say, "He's great, first of all he's just a really nice guy..." Mylett pauses, leans further back in his chair and looks up at the ceiling, blinking a few times. "Sorry, I'm actually choking up a bit. I mean, my goodness. He's 82 years old, and to be doing this kind of work…it's physical work." With his upcoming retirement, Captain Bill has had to reconcile with giving up something he loves doing because of his inability to continue on with the physical rigor of what has been a big part of his identity for most of his life. Mylett recognizes that this is something any older, accomplished person will have to face toward the end of their career.

Mylett hardly has to say more. It's clear that Klimm's colleagues see him as an inspiration. Captain Klimm retired on schedule in October of 2018. Mylett took over for him soon after. Now, after a year and a half of captaining the *Gemma,* Mylett has taken on the additional task of refurbishing the old boat. And as for Klimm? "He still comes around regularly; walks his dog every day, and continues to appear to maintain excellent health," Mylett shared. "Amazingly fit for someone in his 80s."

Seeing Through Synapses

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Glittering blue seas pulling back and forth from the rocky shores of Porto do Son, a coastal city in the Galicia region of Spain, were familiar to local seaman Ramón Sampedro, who was born and raised in the coastal town. These were the same seas he found himself taking in on an August day in 1968 right before he dove off the rocks into the sea below, a plunge that would change everything. Sampedro misjudged the undertow and the area's depth, snapping his neck and sustaining a spinal cord injury that left him quadriplegic.

Sampedro would spend the rest of his life fighting to be euthanized, because for him life as a quadriplegic was not a life worth living. Sampedro's story became well known when 29 years after his accident he filmed his assisted suicide, bringing to light the complicated nature of the right to life and the right to die. Today, spinal cord injury patients have more options than Ramón Sampedro felt he had in the mid-1900s as researchers in the field have worked hard to make advances toward spinal cord injury recovery and treatment methods. Since the time of Sampedro's life changing injury and euthanization, a Marine Biological Laboratory (MBL) based researcher and her team have played a large role in uncovering new methods and potential treatments for spinal cord injury patients through disease modeling in an unusual aquatic organism: the lamprey.

Dr. Jen Morgan sat comfortably at the desk in her spacious office, slices of sun and sea peeking through the half-closed blinds. A personalized ceramic representation of the lamprey, Morgan's study animal, hung near the window. It's a slender and eel-like jawless fish that can grow up to three feet long. Lampreys are considered oceanic parasites as they latch onto prey and suck the host's blood. With horny plated tongues and twelve terrifying rows of teeth that leave cookie-shaped scabs behind on its prey, you'd suspect this creature to have come from a horror movie. Morgan's artistic representation of the lamprey is far more endearing, with its coiled body creating the shape of a heart and the letter J at the same time. Morgan smiled warmly, turning from the artwork toward me. "One of the post docs in my lab designed it and Lori Horb, who works in the Bell Center, made it for me." The gifted piece of personalized art is only the beginning of the testament to Morgan's embodiment of collaboration and service to young scientists.

An associate scientist specialized in neurobiology and the director of the Eugene Bell Center for Regenerative Biology and Tissue Engineering at the MBL, Morgan not only conducts her own research but also supports a research team of between five to 10 people year-round. Her team uses the lamprey as a model organism to study the intricate workings of the nervous system, specifically the processes behind neurodegenerative disorders and recovery from spinal cord injury. The lamprey's large neurons allow for easy access and imaging, making them advantageous for neuroscience researchers. Even better, the molecules, gene structures, and proteins linked to disease processes that can be found in the lamprey are similar to those seen in humans. Morgan finds that using the lamprey as a model generally allows for a level of experimental accessibility that's helpful for looking at the fundamental biology and processes. "[We can] learn something about how the neurons work, how the proteins work, in this idealized system and then you can take the key findings and then see if it holds up in a mammalian nervous system," she said.

Morgan works hard to support and guide the faculty to success. “For me it’s a service role, and it’s for the benefit of the Bell Center and the MBL at large,” she said. Morgan’s desire to give back to the scientific community at MBL stems from her deep-rooted history with the lab. “I had an experience as a young scientist here and I’ve always found the MBL to be a place where I can be very collaborative, very creative.” As she pursued an undergraduate degree in biology at the University of North Carolina at Chapel Hill, Morgan began working in a lab focused on how neurons communicate with each other. Her career as a young intellectual quickly took shape as immersive research opportunities appeared on her horizon. “I got very excited about neuroscience from my very first laboratory experience,” she said. “And part of that early on was an opportunity to come to the MBL as an undergraduate to do my summer research.”

While pursuing her Ph.D. in neurobiology at Duke University, she continued to work with MBL in the summers to conduct her primary research, returning to Duke for the fall to analyze and write up the data. As she progressed along her career path, Morgan’s relationship with MBL deepened, proving to be a lasting thread throughout her academic and scientific career. “Before I had my full-time position here, I already had 15 summers under my belt,” Morgan shared. “So, I’ve been affiliated with the MBL in one way, shape, or form literally through my entire academic career starting when I was an undergraduate. And now this will be my 24th year.”

Morgan’s research at the MBL focuses on neurotransmitter stores maintained at synapses. The key players in this process are first and foremost neurons, the cells in our nervous system that allow for the communication of electrical and chemical signals and by proxy, all essential function of the human body. Then there’s the synapse, the structure that permits the passing of a signal between two neurons. If neurons are cells that can talk to one another, the synapse is host of all their meet-ups. When these interactions occur, neurotransmitter molecules are released. The neurotransmitters carry the actual message that's being sent between neurons. So as one neuron releases these transmitters, they diffuse across a small space and bind to specialized receptors on the other neuron. Signal received. "This talking, back and forth between neurons is in my mind really one of the fundamental processes in how the nervous system works," Morgan said. "The really critical thing here is how do those synapses maintain their stores of neurotransmitters in the vesicles?"

Morgan's investigation into this process added an understanding of some of the molecular mechanisms of a rather simple process of neurotransmitter recycling at synapses, known as endocytosis. Morgan finds the locality of recycling neurotransmitter-filled vesicles at synapses to be a rather amazing process, since most synapses occur at the end of axons, which can be quite long. "You have one neuron in your hindbrain that extends a single axon all the way down to your big toe," Morgan said, excitedly describing the extent of the process. "So you know for your big toe to move and feel, that's like two meters long." Morgan's pointed finger traveled the exaggerated length down her body as she spoke, "If you had to wait for the neurotransmitters to be trafficked all the way down your body to your big toe … that's too slow."

Snapping her fingers in rapid succession, "The nervous system has to be fast," she said. *Snap. Snap. Snap.* "It's working on a millisecond time scale." This process has been proven to be critical, and not just so that we can move our big toe. Vesicle recycling is a highly energetic process that turns out to be an early target in neurodegenerative disease processes. As diseases develop, the recycling process tends to be less precise. Morgan's lab is continuing to explore how vesicle recycling is affected by diseases such as Parkinson's and Alzheimer's.

Recently, Morgan's lab identified a protein linked to familial Parkinson's disease called alpha synuclein as a potential target for spinal cord injury. Alpha synuclein disrupts the process of neurotransmitter recycling at synapses when it accumulates in large quantities. In her research, Morgan found that spinal cord injury induces the accumulation of these proteins, which ultimately leads to neuron death. Since neurons lack the ability to repair themselves, this means permanent damage for the nervous system. Morgan's lab was the first to identify and publish on this. Soon after, another group published supporting evidence after looking at alpha synuclein in the mammalian nervous system.

With the U.S seeing around 17,810 new cases of spinal cord injury a year, this discovery has had large implications for what could happen after someone breaks their neck or damages their back. As it stands, spinal cord injury patients rely on physical therapy for recovery because there are no available FDA-approved drugs that modify neurological repair. Morgan believes that the spinal cord injury field is responsible for building the tool kit to treat patients. "That tool kit must include drugs," Morgan said. After their initial findings surrounding alpha synuclein, Morgan went on to apply that new knowledge. After piloting some test drugs, she's currently going through a patent application process for a small molecule that could be developed for treating spinal cord injury or neurological disease. "The fact that we've identified a target for drug therapy to improve the surviving neurons, improve their growth ability [using the lamprey model] … that's one of the things I'm proudest of," Morgan shared. With several thousand instances of spinal cord injuries each year in the U.S alone, the implications of Morgan's discoveries and research with the lamprey have a wide reach and a range of potential applications.

Morgan's approach is efficient and judicious as she makes advances in her spinal cord research and carves out time to support her research team. Like the ceramic representation of the lamprey, other art hangs in her office. A rectangular painting was mounted on the wall behind her, the background entirely black with pink, purple, and silver strokes crisscrossing their way along the canvas. "That was from a former student of mine. She worked on spinal cord regeneration and this is a representation of one of her images that she painted," Morgan said. With her genuine dedication to the growth of the scientific community, Morgan hopes to continually encourage young minds and make further advances in the field.

Tales of Transport

Picture driving 785 miles, about 18 hours, without stopping to rest. Now imagine doing that with live sharks in the back of the truck. This, among similar situations, is where Travis Snyder often found himself. In 2016, he was in a position that's typically reserved for aquarists and zookeepers: driving a box truck across state lines, from San Francisco to Arizona, for the purpose of delivering aquatic animals. This trip was only one of many. And in addition to having the responsibility of transporting sharks, Snyder had the job of helping to finish building an aquarium from the ground up.

Originally from Wisconsin, Snyder had been working as an assistant aquarist at the Monterey Bay Aquarium (MBA) in California when plans were being drawn up to open OdySea Aquarium. His contract at MBA was about to run out, and the curator at OdySea had recently been in contact with the curators at MBA to discuss exhibit creation. Snyder's colleagues were encouraging him to take the opportunity. They recognized that Snyder's chance to be a part of an aquarium opening was rare, as aquarium openings are infrequent and widely dispersed across the country. "To be a part of designing, building, fabricating, and troubleshooting a brand-new aquarium is unlike anything else," Snyder said. "No one really gets that experience." So, with the support of his coworkers at MBA, Snyder left for Phoenix, where OdySea aquarium was being built.

Snyder was enthralled by the operations of opening the aquarium. He had been hired by OdySea as an animal care specialist, but the work was more than just animal care and maintenance, as he'd been doing at MBA. Many other factors go into opening an aquarium: setting up life-support systems, mixing water, assembling plumbing, building tanks, and designing exhibits. Each aspect then has numerous sub-factors that go into it. "When we do a fish exhibit, we try to mimic the same exact environment that they're normally found in," Snyder said. To recreate these underwater regions, Snyder will first turn to colleagues with scuba diving experience in the area of interest. If none of his colleagues have a connection, then it's time to hit the books. "You need to know what's found in these regions," Snyder said. "The whole point of an aquarium is to show and educate the public on different regions of the world that they may never see."

Then there's the puzzle of finding marine animals in landlocked Arizona. "It's talking to a lot of zoos and aquariums," Snyder shared. When zoos or aquariums have a surplus of animals, they often give them to other institutions to encourage genetic diversity in captive populations and to limit the need to take from wild populations. While the animals themselves tend to be free, transporting them from one institution to the other had to be paid for by the receiving institution. That's where Snyder's skills were pulled in. "He learned very fast, especially with animal transport," said Lyssa Torres, a senior aquarist at OdySea from 2016-2018. "He was just thrown into that."

"Transporting …" Snyder mulled the word over, no doubt flooded with the memories that come along with it. "That's actually a really fun time, in my opinion," he said as he leaned back casually in his chair.

Transporting animals involves a lot of balance, and a good bit of chemistry, he explained. Trips in the box truck are often slow, with the truck barely reaching a max speed of 70 miles per hour on top of frequent stops for bathroom breaks and animal care checks.

Snyder recalled completing a transport to Las Vegas in 100-degree weather with the cab's air conditioning out. "It was brutal," Snyder said. "The only way to cool off was when we stopped to check the water quality, because the animals had their own air conditioning." When transporting, the needs of the animals always come first. "One of the biggest challenges is being in the front driving and just not knowing for sure how the animals are doing in the back," Snyder shared.

From his interest and studies in chemistry, Snyder knew the tricks of the trade. For example, he knew how to calm down elasmobranchs, the subclass identifier for sharks and rays, by increasing the dissolved oxygen (DO) that they respire. "You basically put them in a form of tonic immobility by increasing the DO to more than 150 percent," he said. "It really does calm them down a lot." Increasing DO is done by bubbling the water with pure oxygen, inducing a state similar to hypnosis. The animals stop swimming and slow their respiration. Elasmobranchs are known to enter this state naturally, often when they're mating or trying to evade predators.

Water quality plays a large role, arguably the most important role, in animal safety and comfort. This includes monitoring DO, salinity, temperature, ammonia, and pH just for starters, all of which can be tricky to keep under control during transports. Luckily, Snyder is a bit of an expert when it comes to water quality. He finds himself fascinated by it, the formulas, the mind-blowing mixtures of aluminum and ferric oxides that can treat water and cause no harm to the aquatic life. To him, the chemistry is very simple, and very elegant.

This knowledge of water chemistry made him keen for transports. "One of the worst times, we ran out of sodium bicarbonate," Snyder shared, explaining that sodium bicarbonate helps to maintain pH levels in the water, which is lowered by the CO_2 released as the animals respire. The fact that the animals respire constantly means the water pH declines throughout the trip, making sodium bicarbonate a necessity for maintaining water quality. The solution? "Well, what's the common name for sodium bicarbonate? Baking soda! We went to the gas station and bought all of their baking soda," Snyder said. "We were like, 'We need all the baking soda you got!'" With the attendants gazing suspiciously at their box truck, Snyder explained, "We actually have fish back there …we need this, for the sake of the animals!"

Other times, problems pop up where the solutions are not so certain. Water is the most precious resource in transporting marine animals cross-country. "You just can't lose water," Snyder emphasized. Stopping for an animal care check one time, Snyder opened the back of the box truck to find that at the bottom of a rectangle tank, a boat-like plug meant to seal the water inside was broken. *Drip, drip, drip,* the tank had slowly been losing water during the transport. Even at this slow rate, it would be enough to drain the tank by the end of the trip.

"It's the type of malfunction we can't truly fix," Snyder said. "We can't replace the tank while we're on the road." Opting for the best possible fix, he wrapped it in garbage bag after garbage bag, securing it with zips ties and hose clamps. "We double-checked everything, then with a luck and a prayer we got back on the road," Snyder said. Amazingly, it held water for the remainder of the way.

"I love the challenge that it presents," Snyder said of transporting. And there's no doubt that Snyder was always up for a challenge. It wasn't uncommon for him to work 18-hour days, racking up 232 hours of work in a two-week period. "I loved it; I just couldn't get enough of it. I wanted to do more of it, I wanted to open up another aquarium!" It hardly seemed surprising that after about six months of working for OdySea, Snyder was promoted to Animal Care Specialist II. His commitment to the aquarium was incomparable, which Snyder believed was a huge factor in his promotion. "A lot of the other senior aquarists, they were just blown away and really impressed with my work ethic, the hours I was putting in, how I was so happy just to continue to do it."

Senior Aquarist Torres confirmed these beliefs. "He definitely proved himself during the opening process," she said. "He was very eager to jump in, to help out. He's very self-motivated." Snyder attributes much of his work ethic to his upbringing, where he was always taught the importance of earning things on his own. "My parents made me work for my money. And I still am really grateful for that work ethic I got." That work ethic translates into who he is now, and where he is today in his career.

After helping to set up the opening of the aquarium, receiving a promotion, and growing as an aquarist with OdySea, Snyder found himself looking for a new challenge, a chance to expand his knowledge. Since October of 2019, Snyder has been working as an aquarist and life support technician at Greensboro Science Center, North Carolina, where he maintains and supervises large life support systems while also providing animal husbandry for exhibits. Though he still gets to work with water chemistry, Snyder hopes to get a better grounding in the biology as well. That means for now, his transport days are done. "We went through some really interesting times opening up that aquarium. Really hard times, really amazing times." Snyder is grateful for the rare opportunity he had to experience the opening of an aquarium, and if the opportunity arises again, he'll jump at it.

The Girl With All the Questions

Sitting in a lecture room at the University of Illinois, Urbana-Champaign, in the 1980s, Karen Crawford was captivated by an intriguing man who gave a seminar on limb regeneration in salamanders. “He talked about amputating limbs and grafting tissues and mixing tissues together and I just thought, ‘That’s too cool if I could do that.’” Through her lab tech position at the university, Crawford was able to sit in on lectures like this. She decided to visit the man, Dr. David Stocum, at his office for career advice. Politely knocking on the door of the esteemed developmental biologist, Crawford recalls, “I said, ‘Excuse me Dr. Stocum, you don’t know me …’ and he goes, ‘sure I do.’” She was stunned, as she’d never spoken to the man before.

“You do?”

“‘Yes,’ she says he told her. “You’re the girl with all the questions.”

Even sitting in on a seminar class, Crawford stuck out as the girl with all the questions. Now a developmental biologist and professor of biology at St. Mary’s College of Maryland, Dr. Crawford has made a career out of asking questions through her research. She was recognized as an exceptional scientist, named a Whitman Center Fellow by the Marine Biological Laboratory (MBL) in both 2018 and 2019. In 2018, she peeled back an important layer with her research when she was able to successfully implement CRISPR technology to edit the DNA of cephalopods, the group that includes squid, octopus, and cuttlefish. Crawford continues to further explore effective, simplified methods for microinjections and embryonic gene editing of squid embryos at the MBL.

Crawford is incredibly personable, inviting anyone who's interested to "come take a look," as she fans her short-cropped blonde hair out of her face, looking up from her microscope and pushing her glasses up the bridge of her nose before rolling her chair aside to make room for you. She's someone who loves to chat, especially about her microinjection work. Typically, microinjections in cephalopods consist of researchers targeting early-stage cell clusters known as blastomeres. Targeting early stages in development ensures that as cells continue to divide, the material injected will be distributed consistently throughout the organism as tissues differentiate and grow. Most experimental injections use dextran, a red, fluorescent dye made of sugars, that allows researchers to track if the injection was taken up by the organism as it continues to grow. The need to inject at such early stages, not to mention the tedium of working with these techniques on a microscopic level, makes this process no easy feat. That's where Crawford comes in as she works toward streamlining these methods.

Recently, Crawford has been putting new techniques to the test. One thought she had was to try injecting into formed body tissues, rather than early stage cell clusters, to see if the dextran could be successfully taken up into the organism. She tried injecting into the tentacles, the mouth region, and the optic lobe of the brain. Each location caused a different pattern of dye spread. "If you inject in the brain, anything you inject goes immediately into the circulatory system. By being in the circulatory system it can go anywhere in the animal," Crawford explains. And once it is everywhere in the animal, the injected material can undergo local electroporation, a process that utilizes electric charge to make membranes more easily passable. Imagine a hotel in which every door will swing wide open with the push of a button at the front desk. That's what electroporation does—with a simple shock, every cell throughout the animal automatically becomes "leaky" and allows the dextran to seep inside. After using electroporation, injected material can be taken up more easily anywhere in the organism. This preliminary research demonstrates the potential for injection methods that target mature body tissues directly, a far easier method than targeting early stage cell division in embryos.

She's also tried injecting directly into the yolk of the hummingbird bobtail squid, *Euprymna berryi,* just to see if it would work. And it did. "There is a form of CRISPR cas9 that's linked to a yolk uptake protein that would allow us to more efficiently and effectively transform embryos," Crawford said of her progress. While this work is still ongoing, the initial findings are promising as she moves closer to her goal of simplifying injection techniques.

Crawford grew up on a farm in Indiana and always enjoyed caring for animals. She volunteered with a veterinarian when she was eight, walking dogs, looking after rabbits, and running errands. When visiting her grandparents' summer cottage in Little Lake Ipswich, she would spend her time exploring tide pools, both captivated and frightened by what the pools held.

"My mum used to call me her 'still-waters-run-deep' kid, because I would be staring off into the sky and looking at clouds and perfectly happy doing that," Crawford reflects. "What I think drew me to all of that was pattern, and discontinuities in patterns." Unevenness, like a crooked leaf on a tree, is something she's always noticed. "I like to see how things fit together … so it was really the change, but also the consistency in between patterns and organisms."

Crawford says that in essence, this is what being a developmental biologist is, studying how organisms go from egg to adult with all the parts in the right place. "I often talk about how most folks go to a football game and watch football, I go to a football game and I think, 'Wow look at all that normal development.'" To her point, we all tend to grow up to have a nose positioned centrally on our face, two eyes, two ears, ten fingers and ten toes. "That's all due to cell communication during development that sets up the right pattern that allows that to form."

After completing her undergraduate degree, Crawford spent the following two years working as a lab technician until she felt confident she could take on her own research. This was around the time Crawford sat in on Stocum's class. Never before had she been exposed to developing organisms and the idea of regeneration. Walking into his office after lecture that day, she recalls seeking Stocum as a mentor for her intended master's degree in cell and developmental biology. Instead, Stocum heavily influenced Crawford to pursue a PhD in anatomy and apply for a fellowship to do so.

More than 30 years have passed since that initial interaction between the two. Crawford speaks of Stocum as a father figure, her "scientific father," a label which Stocum happily recognizes. And the picture of those whom they've collectively mentored, or with whom they have collaborated, weaves an intricate scientific family tree. Stocum asserts that this sense of belonging and connection to Crawford and their associated scientific community has always been an important part of his life.

Pursuing zoology and anatomy has led Crawford to a career in understanding the embryology, development, and genetics of large-brained invertebrates: cephalopods. Crawford was first exposed to the sucker-covered creatures during the embryology course hosted annually at the MBL in 1983. She returned to the MBL in '84 and '85, developing an invitro fertilization and culture method for the local squid, *Doryteuthis pealeii.* For more than 30 years, Crawford has looked specifically at this coastal, pelagic squid species, commonly known as the longfin inshore squid. This species was named a model organism for human diseases when it was discovered that they had large axons, which had huge implications for the field of neuroscience. The squid giant axon is instrumental in helping us to understand what changes in disease like Parkinson's and Alzheimer's mean for basic function of neurons in the human body.

Crawford hints that this is the big picture behind it all: translational research and the potential to better understand the human condition. But then there's also "science for the sake of science." Crawford points out there's so much still to learn about these animals, and that they could have more tricks behind their suckers than we can imagine.

Signs of this appeared with research on the genetics of pigment in the bobtail squid and longfin inshore squid. Scientists at the MBL have successfully "knocked out" genes—which means they blocked the responsible DNA sections—for producing pigment in both species. Researchers working closely with the bobtail squid have had success in knocking out pigment on only half of the organism, and then found that after the squid hatched and began to grow, pigment was somehow reintroduced to tissues where, genetically speaking, it should have been absent.

While they've been puzzled by that, Crawford's methods have proven successful. "I have managed to get two guides working together into embryos early enough that I have clear, knocked-out embryos. That's in *Doryteuthis*." We're talking total knockouts, squid with a complete lack of pigment. This early work demonstrates that microinjections and gene editing technology do, in fact, have the ability to completely remove genes from an organism, which may prove useful one day if anyone wants to knock out genes that cause human disease. The next step? Trying to design a way to introduce new genes into an organism. "We're at least conceptually developing that, just to see if we can," Crawford said. "And I'm sure we can."

Though she has held the position of university professor for almost three decades now, Crawford still thinks of herself as a student. And sometimes the people she learns from are those she's been asked to teach—the students enrolled in her class, or those who work in her lab. "I'll make a connection because of some basic principle I just taught them. And then all the sudden click, click, click, and the parts of a question I'm trying to wrestle with in my research comes together," Crawford says. This tends to happen in courses that she doesn't regularly teach, which occurs when you're a liberal arts professor and teach across disciplines. For Crawford, this just says that if you're wrestling with a question, it may just be a good idea to step outside of your realm.

Queen of the Metas

"A good amount of the job is observational," Taylor Sakmar said as he crouched in front of a tank, peering in at the multicolored, unusual creature staring back at him. It was early September and my first day as a cephalopod operations intern at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts. Sakmar stood up and took a slight step aside so I could begin to make some observations of my own. Peering back at me through a w-shaped pupil was a *Metasepia pfefferi,* commonly known as the flamboyant cuttlefish, a name inspired by its vibrant color palette, with eight arms ranging from shades of pink and yellow to orange and brown waving gently with the flow of the water. Its mantle, the main body cavity, flashed bands of brown and white. Like a cool breeze pricks goosebumps across human skin, soft tissue known as papillae mocked horns all over the creature's body as it bobbed close to the water surface.

"So, with these guys, it's actually not a good sign to see them swimming around. It usually means they're stressed," Sakmar said. *Metasepia pfefferi,* often referred to in the lab simply as "metas," are the smallest species of cuttlefish, at their largest growing to about the size of a credit card. They spend much of their time on the ground, trudging slowly across the sand and nestling under rocks or shells. Sakmar, the senior cephalopod culture specialist at MBL, relayed a list of other behaviors to look out for and left me to begin distributing snacks of live grass shrimp to the colorful creatures.

From that point forward, I would be the point-woman for this species (Queen of the Metas, as one co-worker joked). Everything from feedings to cleaning the intake tubes that supplied water to their tanks would fall under the umbrella of my responsibility. But my ultimate goal was to "close the lifecycle"— in other words, get them to reproduce. Successful reproduction and subsequent maintenance of ideal growth conditions is referred to as culturing. The cephalopod lab aims to culture a variety of species as we work to define and maintain the standard of care for cephalopods in lab and aquarium settings.

Being crowned Queen of the Metas was not a title to take lightly. Nicknamed the "divas of the sea," these cuttlefish are easily stressed and difficult to care for in captivity. Although a few others had attempted the feat, my supervisor Bret Grasse was the first to successfully have this species lay eggs and raise those hatchlings to adulthood in captivity at the Monterey Bay Aquarium in California in 2010.

"It was very nerve-racking," Grasse, manager of cephalopod operations, recalled. Cephalopods, the class that includes squid, octopus, and the chambered nautilus alongside cuttlefish, are sensitive creatures in general. "They don't handle stress very well." That means that even obtaining the species and acclimating them into human care was difficult. From there, the challenges only continued.

Grasse was starting from scratch. Prior to his work in culturing the flamboyant cuttlefish, very little was known about the species. The only notes that existed were from divers or researchers observing the cuttlefish in their natural habitat in the waters of the Indo-Pacific, meaning there was no knowledge of keeping them in lab or aquarium settings. He had to figure out what prey items they prefer, what kind of substrate and rock formations they liked, and where they prefer to lay eggs. "Once you finally are successful and get them to lay eggs, that's where the real challenge starts," Grasse added.

"They [have to] hatch out away from any adults or any aggressive flow so they can begin the first stages, the most sensitive stages, of their lives in the most optimal conditions possible." Once these optimal conditions were obtained and baby *Metasepia* began to hatch, Grasse felt a large sense of relief. He had accomplished his goal, with seven *Metasepia* hatchlings surviving through adulthood, but he didn't stop there. He had learned a great deal from raising his initial population and the next generation was exponentially more successful, resulting in 100 hatchlings raised through adulthood.

"It was honestly like a dream come true," Grasse said. "They were the catalyst that allowed for the creation of the world's first large scale public cephalopod public display, Tentacles." The opening of the exhibit at the Monterey Bay Aquarium was a huge moment in Grasse's career. He believes this pioneer work paved the way for a deeper scientific understanding of cephalopods. Since then, they've become integral in aquariums worldwide and are now being investigated as model organisms for human disease research.

Research investigating cephalopods as model organisms fits into the larger focus of the MBL, which is to inform the human condition through the use of aquatic model organisms. Today, the cephalopod operations team works to produce multiple generations of various cephalopod species with the goal of breeding and raising organisms for aquarium displays or for human disease modeling, such as anesthetic testing, limb regeneration studies, and genetic testing. Captive culturing programs such as MBL's help to reduce the strain on wild populations and maintain nature's biodiverse habitats, making this practice sustainable.

Sakmar wasn't kidding when he said most of the job was observational. I would spend most of the next month trying to determine which cuttlefish were male and which were female, simply from watching their behavior. Flamboyant cuttlefish show little sexual dimorphism, meaning there are no physical external characteristics to determine their gender. The only physical characteristic to look for is size, as females tend to get slightly bigger. But that can be a tricky, and not always reliable way, to distinguish them. Instead, behavior unveils their sex.

One day I hopped up on a step stool to drop a few shrimps into a tank at feeding time. I came eye level with two metas having a face off. Both metas had their arms outstretched; they were light in color except the tips of their arms, which had turned to black. They were wiggling back and forth, dusting each other with their outstretched arms. I watched for a moment before turning and calling out to Sakmar, “I think I just made an observation.” He walked over to where I was standing with my eyes glued to the tank. “Oh yeah. That’s what we call paint brushing.”

As I had observed it, paint brushing is a form of male-versus-male aggression. The more dominant male, which in this case was the larger male, wins. Males will also exhibit this behavior when mating to determine if a fellow flamboyant is male or female. As I had learned, males will return aggression, while females will be submissive, usually a sign they’re willing to mate. But other times, as I often observed, the females simply try to get away.

Cephalopods, sometimes referred to as the shape-shifters of the sea, are able to create dazzling color displays and achieve a range of body patterning by manipulating a combination of factors. Behind their ability to create such displays are an important group of cells known as chromatophores. Chromatophores are pigment-containing cells attached to muscles. Imagine how spokes look on a bicycle, that’s how the muscles surround these sacs of pigment. The muscles expand and contract the cells, allowing for the variety of colors a cephalopod can put on display. Body postures, like the one termed paint brushing, can also be made up of textural components, when papillae extend outward to form small, soft peaks on the animal, as well as locomotor and posturing components.

There are other behaviors aside from paint brushing that help aquarists determine the gender of metas. Males, for example, typically seek higher ground while females will spend more time under rocks and shells. But metas rarely make things easy and their tricky tactics can only add to the confusion. Some males will be more submissive, making them appear to be females to caretakers. In other populations there may be dominant females that display male behaviors.

As they get older and closer to sexual maturity, the act is eventually dropped, and distinguishing sex differences comes easily. While males still react slowly and feed meticulously, female *Metasepia* begin to take out shrimp with gusto. Females quickly lock their w-shaped pupils with their prey and aim their feeding tentacles with great accuracy. Previously eating one shrimp per meal, sexually mature females will take down three to five times more food as they put vast amounts of energy into ova production. Metas are often slow-moving creatures, so when they began to eagerly attack prey, that's a good sign for reproductive health. As the days passed and the observations continued, I began to notice that the metas were getting hungrier, the females were getting larger, and the males were paint brushing more and more. We had to be close to getting eggs, I was sure of it.

Spot cleaning one of the tanks with a turkey baster on an afternoon at the end of October, I noticed a small white blob bouncing along the sand. Eyes widened and heart racing, I ditched the turkey baster and begin to look under rocks and shells. Sure enough, on the underside of one large scallop shell clung seven small sacs of tissue. Eggs. EGGS. We had eggs! I clambered around the lab excitedly looking for Sakmar. “We have eggs!” Sakmar did a spin, punched the air excitedly, and practically shouted, “Yes!”

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“How many coolers did you grab? Just one?” Grasse called to me from where he was parked next to a dumpster, chucking away trash from his white Honda Civic so we could all fit inside. “Go ahead and grab another, I’m feeling ambitious.” I turned to head back to grab a second cooler, the last thing we’d need before packing up the car and heading to the beach.

The tires turned on the gravel, kicking up small rocks and dust as we pulled into the Woodneck Beach parking lot. Grasse shifted his car into park and stepped out. A tall guy with fine, light-brown hair and a bright, toothy smile, he stood in brown neoprene overalls with size twelve rain boots sewn to the suit just above the ankle. He reached into the trunk and turned to me, a net in one hand and a bucket in the other, revealing that bright smile. “Let’s go get some shrimp!” he said.

We were going shrimping to provide food for our growing cephalopod empire, which consisted of hundreds of specimens representing eight different species of octopus, squid, and cuttlefish. Cephalopods are a type of mollusk, closely related to slugs, sea snails, and clams. This highly intelligent group of animals is often identified by their many arms and suckers. Everything from the California two-spot octopus and Hawaiian bobtail squid to the Malaysian stumpy cuttlefish could be found living under our expertise and care.

Grasse takes his cephalopod work seriously, but not too seriously. He's the kind of guy who aspires to put a Lego Star Wars figure in a tank with baby octopuses, just for the humor of a ton of tiny octopuses climbing all over it. In our weekly meeting, I glanced over and happened to notice the tall socks peeking out from his pant legs where the face of Chewbacca was staring back at me.

"I got my sense of humor from *Seinfeld,*" he once shared with me.

Not to be fooled by his goofy and personable demeanor, Grasse is an innovator and an intellectual. He was the first to culture the striped pyjama squid, *Sepioloidea lineolata* and the flamboyant cuttlefish in captivity. More recently, he was also the first to culture *Octopus chierchiae,* the pygmy zebra striped octopus, through three successful generations. With confidence that his methods are the right ones, Grasse sharply defends his dogma when it comes to running the cephalopod operations. He doesn't hesitate to rebut a bad idea or shape it to sound more like his own. He's most excited about his work when it's hands-on, building or fixing system set ups or interacting directly with the animals. "I've always been intrigued and passionate about exploring the unknown and coming up with solutions to problems that are challenging, but you know, obtainable," Grasse said.

Grasse astutely dials up his charisma when discussing his work and its potential to donors and scientists alike. At the 9th Annual Aquatic Models for Human Disease Conference, he presented his research in culturing cephalopods alongside some clean-cut, rather dry scientists. Several of the presenters seemed tired of their own research as they sighed, spoke in monotone voices, and interlaced their factual sentences with an "uh" or "um." But Grasse had a breath of enthusiasm for his work, evident with each word he spoke. Unlike the other scientists, his PowerPoint graphics (a "live free die young" poster illustrating the short lifespan of cephalopods) and colorful analogies kept the audience engaged. He boasted of pioneering, innovating, and experimenting; everything science is supposed to be.

The manager of operations and a supervisor, Grasse is often tucked away in his office, far away from the hands-on work that he likes most about his job. As he climbed higher in the ranks, Grasse found himself further removed from the pioneer work he's so passionate about. But he's still quick to come to solutions when there are issues in the lab. Once, while changing out a filter situated in a high-up location during routine maintenance, a screw snapped and the filter opened up a cascade of seawater. After a frantic but failed attempt to reposition the filter and stop the water flow, I knew I'd have to seek out help and own up to the damage. Grasse was far from upset once I told him. As we walked back to the lab to fix the filter fiasco Grasse said, "That's what I love about this job, the constant troubleshooting. Nothing's ever a routine because we're always answering new questions and finding new fixes."

Grasse's innovative approach to his own work inspired me to approach mine in the same way. With his influence, I now looked around the lab with a keen eye, wondering how the care of the metas could be improved with new methods or new ideas. The cephalopod operations team as a whole tends to be in this mindset, looking for ways to improve and define the standard of culturing and care provided to these animals. Grasse is confident that in the future, cephalopod culturing will be highly influential in human disease modeling. People hadn't realized it quite yet, but the future of medicine could be in the hands—or, well, the tentacles— of cephalopods.

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From the end of October through December, I practiced techniques in harvesting and incubating *Metasepia* eggs. Carefully lifting low-hanging rocks and checking under the smooth, vaulted surfaces of shells, I'd find clutches of a dozen eggs, give or take a few, delicately waving with the undulations of the water in the tank. When ready to lay their eggs, female *Metasepia* seek out low, vaulted surfaces, as these locations offer the best protection for their eggs and themselves as they go through the process of laying. In the wild they've been seen laying eggs into coconut cells, but in our lab, they often lay them under shells. Lifting their arms up into the selected surface, they carefully deposit eggs one by one, tacking them to the chosen surface with a biological glue.

At the MBL, our mission is to work toward creating and providing the standard of care for cephalopod species. This standard of care includes optimizing the animals' hatching success. This is why we opt to practice techniques in artificial incubation. Artificial incubation, instead of keeping the eggs in the tank with their mothers and other co-habituating adults, allows for close observation of embryos, prevents bio-fouling, and ensures proper oxygenation of embryos. Cuttlefish mothers provide little to no maternal care and removing eggs from their habitat allows the females to lay additional viable eggs. This method is also safer for hatchlings who may find their parent's environment harsh, with high water flow that could push them around and the possibility of being mistaken for prey by adults. In the end, artificial incubation allows aquarists and researchers to create the most optimal conditions for hatching.

Taking sharp-tipped forceps, I gently scraped away the biological adhesive that glued the transparent eggs to the shells, delicately plucking them off and easing them into a bucket of seawater, one by one. The importance of the task was not lost on me. Every dime-sized egg felt valuable, a small promise of life within. Each step I took during harvesting and incubating could affect the possibility of that small promise reaching reality. Although just an intern, I felt I had been given a great task, and with it, the inherent pressure of succeeding in nurturing each little life.

The next step before housing the eggs in an incubator is a ReVive bath, which is essentially a rinsing of the eggs with a plant-based surface cleaning extract. Typically used for corals, ReVive irritates copepods and ciliates, tiny organisms that live in seawater that crawl around and eat away at the external egg casings. Artificial incubation involves using the soda bottle method, an innovative design by Grasse. The incubation device is constructed using two empty plastic bottles, cut to size, as the incubator shell and a mesh partition between the two bottle halves. The upward-facing cap is removed, and an airline inserted to allow a discharge point for air bubbles and exiting seawater. The bottom-facing cap is partially cut out and replaced with a mesh screen to allow for fresh seawater supply. Developed in 2013, this effective incubation method turned out to be a rather dazzling display, the eggs springing around in the soda bottles like balloons being tossed into the air.

As the days passed and the embryos bounced and bubbled around inside their soda bottles, the transparency of the egg casing welcomed me into their tiny worlds. I watched as their beady red eyespots developed, their heads and arms began to form and wrap around the yolk. Their mantles began protruding, followed by the formation of chromatophores, the pigment cells that allow for their color-changing abilities. Right before my own eyes, I watched tiny replicates of adult *Metasepia* form. I began to feel attached to the growing embryos, as if I had laid the eggs myself. I was a meta mother, a meta mother of many.

By January, hatchlings had taken over the lab. Still leading the care of the adults and now their 220 offspring, I would dote on the tiny cuttlefish, carefully conducting counts each morning, then feeding them. Feeding would take an hour and a half of my morning or longer, as I meticulously assessed the number of miniature mysid shrimp to place into the tank. Mindful not to startle them, I would bend down and peer into the tank with an angled flashlight to watch them feed. They'd turn slowly, bounding around on mantle papillae projections known as glutapods, a term that quite literally means "butt feet." Their tiny movements were miniscule, meticulous, and slow.

Over countless hours of feeding and caring for these delicate animals, I began creating my own narrative with the critters. I'd chide the chunky ones for taking more food than their fair share, although regardless I was happy to see them eating and growing, as any mother would be. Just like a litter of puppies, there tended to be a "runt" in each group. I'd keep a close eye on the little guys during feeding to make sure they got at least one mysid each. I practiced my patience, not wanting to leave behind unwanted prey items that would rot and could cause the metas stress, and potentially death. Time consuming as it was, it was time that never felt wasted. The early stages of life are the most sensitive and most critical.

As I astutely cared for the delicate divas, a new idea was forming. I had now spent around four months observing and caring for these creatures. The pressure to succeed in raising the cuttlefish kept me working at my highest caliber. I felt that my understanding of their care was not quite perfected, but reached a professional level. My notes had begun to pile up, my observations numerous. I wanted to dig deeper, there was much to still be discovered about these animals beyond their care and keeping.

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In the world of behavioral science, there is something called an ethogram. An ethogram categorizes all the behaviors of a given species, an extremely large undertaking for any animal. Ethograms are important for any animal caretaker as they provide the foundational knowledge necessary to interpret and understand animal behavior, and thus take better care of an individual or group of species. With all my own behavioral observations, from hatchlings to adults, I began to look into what ethograms existed out there for the *Metasepia.*

Flipping through the literature, it almost immediately struck me that I had seen behaviors that were not yet described here. The ethogram I had on hand was the only one that existed. Publishing new observations felt well within my realm, as Sakmar told me from day one that my job would primarily involve watching the tanks. So, I made a plan and typed up a proposal. I figured with some camera equipment and analysis tools, a few weeks of video recordings and jotting down more notes from personal observations, all in combination with the existing literature, I could compile and begin to understand the different behavior patterns of *Metasepia*.

Grasse and Sakmar approved my proposal, agreeing that the existing information felt incomplete. Early on I recognized that conducting an experiment to create an ethogram would be no easy feat. It would involve describing color, body position, locomotor components, and textural components of each behavior. I hardly knew where to start. Also, as a female scientist, I couldn't help but note that thus far my research had been guided solely by men. I began having meetings with senior scientists such as Dr. Roger Hanlon and Dr. Josh Rosenthaul for advice on collecting behavioral data and experimental design.

My nerves spiked beforehand, feelings of imposter syndrome and intimidation set in, especially as Grasse and Sakmar always insisted they accompany me to these meetings. Though always insightful, I often felt small during these meetings, “talked at,” instead of “talked with.” But I began to keep a journal that was strictly for jotting down their advice and my own experimental notes, a place for all my questions and inquiries to take shape.

After months of thinking, researching, designing, and building, it was time to start the real science. Few things can truly prepare you for stepping into the world of research, especially as this was the first experiment I designed and conducted on my own. Just as I had fostered the growth of the cuttlefish themselves, I would now be nurturing my own research project. It was the end of March when everything for my experiment was finally up and running.

I proudly perused my system, looking at the mounted mirrors, carefully hung cameras, and properly angled infrared lighting for nighttime observations. The pressure to succeed once again washed over me. Time and other resources had been invested into this project— *my project*, my idea, my experiment. I peered into the tanks with a sense of gratification, looking at the growing *Metasepia* that I had once watched tumble in a soda bottle. They seemed huge now, healthy and happy—at least, far as one could call cuttlefish happy. I clicked off the LED strip lights overhead the experimental set-up before walking away for the weekend. Next week would be the first week of April, a new month seemed the perfect time to begin collecting data.

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Mornings at the lab are the busiest time of the day. Rounds need to be completed to ensure water is flowing in and out, all the animals need to be fed, and on some days, we have routine maintenance to perform. These duties for the day are all hashed out in our morning huddle, a casual debrief that includes college students, interns, post docs, and our supervisors.

I had taken a long weekend and returned to the lab on a Tuesday. It was April 2, and I came in with a good idea of what my plan would be for the next 8 hours. I was ready to announce that I would begin data collection. Then, Sakmar turned to me, "Hannah, today we'll have you rinsing off some tanks…" His words may have trailed off, I don't remember. But something in that simple phrase gave it all away.

"What happened?" I said, looking around the circle, certain my eyes were wide. "No one told you …" one of the students said quietly, glancing at everyone else, then down to her feet. What had happened over the weekend? I searched my colleagues faces frantically, my eyes landing on Travis Snyder, a cephalopod culture operations specialist who had been my ally in system design and set-up of my experiment, then again back on Sakmar. "There was a malfunction."

Cephalopods are short-lived animals. Even the Giant Pacific Octopus is known to live for only around four years. Grasse always warned against getting attached to the animals for this reason, which is why we tend to avoid naming them. But this was different. To me, we hadn't just lost animals.

I had little will to continue with my study. All of those months' worth of observations, research, and preparation felt wasted. In the background of my work, caring for the cuttlefish and through nurturing my research, a fear of failure had lurked, a fear that was now reality. To say I felt discouraged is an understatement.

Still, I knew what had happened was not my fault. Quite honestly, we were lucky to have such an astute team that noticed something was amiss in the first place. Our system set-ups use a combination of artificial seawater and natural seawater that comes directly from Great Harbor, which opens up to the Vineyard Sound. Cephalopods require warm water, standards that Massachusetts seawater doesn't meet year-round. This is why we use submersible heaters, safe in water, set with sensors that prevent them from getting too warm, and even equipped with a fail-safe system that sends alerts to our supervisors' cellphones.

But even with all the cautionary measures in place, a portion of one submerged heater became exposed in the water. Typically, the fail-safe system would cause the circuit to trip, but that didn't happen in this case. Ultimately, the exposed portion of the heater was injecting the seawater with copper, a heavy metal that's toxic to cephalopods. In the same way humans die when carbon monoxide molecules bind with and suffocate our blood cells, cephalopods die from heavy metals.

Our team caught it early, the slightest sense of an unusual sound and smell had set them off on an investigation, leading to the discovery of the exposed heater. But we had already lost three of the animals. The remaining eight *Metasepia* involved in my experiment were relocated, where they proved to be in good health, but the entire system was poisoned. We began the process of breaking down and sanitizing the system, cutting off waterflow and draining every tank, taking down every camera and LED light strip, scrubbing everything and circulating bleach through the system, followed by a neutralizer known as sodium thio. We had to start over, entirely from the ground up. It would take about a week to sanitize everything, followed by rebuilding the experimental apparatus and reinstalling the cameras.

The entire MBL cephalopod team felt the pain of this blow. It was out of our control, there was nothing the team could have done differently. Yet, Grasse felt that perhaps the *Metasepia* were too sensitive of a species for this experiment, and I understood his trepidations.

Grasse suggested I work collaboratively with another cephalopod researcher, a Nobel laureate, taking my same experimental design but instead cataloguing the behavior of the stumpy cuttlefish, *Sepia bandensis*, a hardier species with the potential for broader research applications compared with the *Metasepia*. This new project could ultimately use an ethogram as a component of the research. It was undeniably a great opportunity to be a part of something bigger, so I agreed.

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By now it was summer time, and Woods Hole had evolved into a different town. Once quaint and quiet, it had transformed into a bustling beachside hot spot. I often describe the change from the winter to the summer as a human migration. Thousands of people flock to the Cape, everyone from vacationers to summer researchers that filled up the campus and bring a whole new life to the MBL.

There was a new breath of life for my research, too. The experiment began running rather quickly this time, especially because this was my second round of setting it up. By June I was successfully collecting data, a step I had never gotten to with the metas. And things were going smoothly, something the scientist inside of me once didn't believe could happen. I now spent my days with an entirely different species, the "stumpies," understanding entirely different behaviors. Despite my new direction and focus, I was still leading feeds and care of *Metasepia*, observing behaviors daily as I had before, but perhaps with less gusto.

By end of June, early July, I was wrapping up data collection and starting to tackle data analysis. The opportunity to pick up on an old interest resurfaced when the metas began laying eggs again. As I harvested and incubated the transparent orbs of life, I was once again welcomed into their tiny world, watching them hit developmental milestones safe inside their eggs, yet right before my eyes.

Understanding the embryonic development of cephalopods has long been a high priority at the MBL. Research on the embryogenesis of the local longfin inshore squid species, *Doryteuthis pelaeii*, and the classification of this squid as a model organism helped pave the way for an entire team that now dedicates their days to understanding the embryonic development of the hummingbird bobtail squid, *Euprymna berryi,* and the Hawaiian bobtail squid, *Euprymna scolopes.* These teams can implement CRISPR-cas9 technology into the developing embryos of these species, successfully inserting and deleting gene material into the animals. This type of research could have human disease implications, with the thought that *Euprymna* species could be a model organism.

Despite all the research that's ongoing and already published about cephalopod embryonic development, I once again found the literature lacking for the *Metasepia.* What little I did find included records from an early 1900s Swiss zoologist Adolf Naef, whom I came to simply refer to as "the Naef." I wrote up a proposal and once again Grasse and

Sakmar approved. The opportunity to dive back into research with the species I cared for most brought me a renewed sense of assurance and motivation in my abilities as a scientist. And, even though I was far behind what many of the senior scientists at the MBL had accomplished, working through the failure of my first experiment and succeeding with my second had galvanized my confidence.

My new experimental design was engineered to look at and mark the growth of *Metasepia.* Each week, the plan was to photograph a set of embryos via microscope imaging. As days passed, I would note at what point in gestation key anatomical features began to form. Despite my preliminary understanding of background information, I felt like I didn't know the first thing about embryonic development in cephalopod species. What was I doing? I began to feel the heavy weight of imposter's syndrome again. I'm not qualified for the scope of this research. My knowledge of these animals was almost entirely from observation, supplemented by Grasse and Sakmar's knowledge and a few scientific papers that I read here and there. Part of me knew I could do this, but part of me feared I'd be inadequate, that failure would once again wash over my work with the *Metasepia.*

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I was stepping outside of my realm of comfort. Knowing very little about embryonic development or developmental staging, I took a leap of faith. With a delicate hand I'd manipulate the position of the growing *Metasepia* under the microscope, trying to get the best angle for an image. I photographed them in this manner three times a week, and I did it for three weeks, watching as they developed key morphological features such as eyes, arms, cuttlebones, and chromatophores.

There's a lot that happens on the cellular level during embryogenesis. You'll hear developmental biologists throwing out words like "blastulation" and "gastrulation." These are all markers of early cell division, something that's key in being able to inject CRISPR-cas9, a gene-editing molecule that researchers at MBL were utilizing in the Hawaiian bobtail squid and the local longfin inshore squid. But I was more interested in the organ primordia, the cell networks where major organs and body features first begin to appear.

Looking through the clear outer egg casing at the yolk, the first sign of primary organ development are dark spots forming on the yolk's surface. Two half-moon spots rest above a central, circular ring. The half-moons will quickly form into eye stalks, and the central ring protrudes to become the cuttlefish's main body cavity, the mantle. Below the ring are eight dots that will soon develop into arms. As the arms develop, they wrap around the yolk such that the animal appears to be grasping its source of sustenance. The animal grows and the yolk shrinks, and a distinct head forms, which the eye stalks shrink into as they shorten. The arms and the mantle elongate, and after 20 days the cuttlebone—the internal hard structure that aids in buoyancy control in cuttlefish— and the color-shifting chromatophores have formed.

The animal inside the egg is a tiny replica of an adult flamboyant cuttlefish, fully equipped with all its body parts, even able to camouflage and dispense ink inside its egg sac. However, because inking inside of the egg is dangerous for the young animal, high stress at this stage can cause pre-hatching mortality. This is why once the cuttlebone had formed, my experiment was set up to stop imaging and switch to a more passive form of incubation, one that would protect the hatching success of the embryo.

This cohort of *Metasepia* took about 29 days of gestation, measured from when the egg was laid to the time of hatching. The imaging process went by quickly, and before we knew it, tanks throughout the lab were once again populated with the tiny colorful creatures, bounding around on their glutapods and flashing their vibrant bands of color.

It was time again for me to switch over to data analysis. Many people picture a glamorous life of the marine biologist, working with animals, snorkeling and scuba diving into the vast underwater world in the name of research and discovery. What they don't realize is that a marine biologist is primarily bound to her office for most of the year, crunching numbers and analyzing data. It's all fun and games until your data collection must be turned into numbers.

After running through the records and looking for significant milestones, I found that the embryos developed consistently, with only one individual lagging behind, and only by a day, at that. I was able to mark at what day, over weeks of development, key anatomical features could be expected to develop. These initial insights are a prerequisite to further understanding the development and evolution of *Metasepia,* which in turn allows aquarists and researchers to track normal growth in captive held metas. With a better base knowledge of embryonic development, *Metasepia* could also be considered as a model organism candidate for human disease modeling.

As I considered these implications, my imposter syndrome sank away. I had real results; I may have just done real research. A world of scientists and aquarists could potentially benefit from this. I could even publish this, and publications are the currency of science. For me, this publication would be the ultimate validation of the trials and triumphs I had experienced over my year and a half with the cephalopod program.

Throughout my time at the MBL, I had sought the advice and guidance of a number of researchers. I worked alongside some of the greatest minds in the world of marine biology. I had begun to form a scientific family of my own, between the mentorship of Bret Grasse and Taylor Sakmar, the hands-on help provided by Travis Snyder, and the career insights from Dr. Jen Morgan and Dr. Karen Crawford, two successful female scientists. I felt like I was a part of something larger than my own endeavors.

I also realized that despite the fact I had been mentored almost exclusively by male faculty members, it was my own attention to detail and nurturing qualities, which one might label "feminine," that had led to my success. I often found myself doubting my own intrinsic abilities, but those inherent qualities were ultimately the key to my success. On the very last day of my internship at MBL, I sat down with another great mind, Dr. Carrie Albertin, an embryologist known for her work sequencing the genome of the California two-spot octopus. Grasse believed that Albertin would provide expert guidance as I went through the publication process. As I sat in her office, I found myself apologizing for my research, stating that it wasn't quite on the level of what she does, but it could still have value to researchers … couldn't it? Albertin stopped me in my tracks. "What you've done here is real research. And there's no doubt you can publish this. I would be happy to look at it for you and point you in the best direction I can."

As two women sitting across from each other, I felt she must have a depth of understanding to my doubts that one could only sympathize with through their own similar experiences. My gratitude for her confidence in me was an overwhelming feeling, possibly as overwhelming as the amount of thank yous that poured out of me as we talked. “Go be excellent,” Albertin said as I left her office. Washed over with emotion I turned and smiled back, feeling heat rise in my face and my throat constrict. I finally saw that I have what it takes to be a scientist. I don’t need to fulfill a male-dominated “scientist” archetype; I just need to follow my sense of curiosity and practice skills of discipline and observation, qualities that were within me all along.

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Author's Biographical Sketch

Hannah Knighton was born in Cleveland, Ohio, but moved around the East Coast throughout her childhood. She started working in aquariums at the age of 16 and from there her interest was piqued. She earned a B.A in English and a B.S in Marine Science from Jacksonville University. Her research experience includes studying coral reef and nearshore hard-bottom ecosystems in the Florida Keys. More recently, she studied flamboyant cuttlefish behavior and embryology as a cephalopod operations interns in Woods Hole, MA. She has since identified a passion for communicating her interests in wildlife and environmental science topics. Her stories have been published with Smithsonian Magazine, Motif Magazine, and the Well. Currently, Knighton is a science writing intern for the Smithsonian Institute's ocean education platform, the Ocean Portal based in Washington D.C.