Lone Parents: Parthenogenesis in Sharks

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The discovery that sharks can reproduce asexually means that mammals are the only jawed vertebrate lineage incapable of parthenogenesis. But can this surprising capacity make any difference to shark survival as their populations decline?

On an ordinary Midwestern morning in December 2001, the aquarium staff at Omaha's Henry Doorly Zoo began their regular morning rounds of feeding. What they discovered made their jaws drop. Inside a 45,000-liter aquarium containing three female bonnethead sharks was an 18-centimeter bonnethead pup.

Any shark birth is exciting, but in this case, it was a colossal mystery. The bonnetheads, a species of hammerhead (*Sphyrna tiburo*), had been collected in the Florida Keys as juveniles three years earlier, at just 33 centimeters in length, well before sexual maturity. None had been exposed to a male bonnethead in captivity. If by some odd chance one of the females had mated with another of the tank's species, which included wobbegongs and bamboo sharks, mating scars would have been evident. Could this pup be the world's first documented virgin shark birth?

Parthenogenesis, Greek for "virgin birth," occurs when an egg develops without fertilization by sperm. It does not include self-fertilization by hermaphrodites, which have both male and female parts within the same organism. Many invertebrate species reproduce parthenogenetically, including mites, bees, aphids, walking sticks, and parasitic wasps, but that is exceedingly rare among vertebrate species. Scientists have documented parthenogenesis in a handful of reptilian, amphibian, avian, and teleost (bony fish) species, but it was unheard of in cartilaginous fishes—sharks, rays, and chimaeras—or mammals.

Geneticists confirmed that the pup born to a female bonnethead shark at the Omaha Zoo had no father. Photograph courtesy of Omaha Zoo.

The pup did not survive long; the aquarists immediately sent the carcass for necropsy, where it was determined that a stingray in the tank had bitten the pup, leading to its death. The carcass was then handed over to the zoo's genetics department. Ed Louis, the zoo's head of genetics, had a hunch this would be a scientific breakthrough. "I thought it was parthenogenesis from the beginning," he says, despite scientific naysayers. "The potential to have sperm storage was almost impossible." It seemed unlikely that a juvenile shark would mate, and even if somehow it had, storing sperm for three years seemed a stretch. The chain catshark is the only species known by scientists to store sperm longer than a year.

No male required

Two different types of true parthenogenesis exist. In apomictic parthenogenesis, common in plants, the diploid germ line cells (gametocytes) do not undergo meiosis to create gametes but rather undergo regular cell division, or mitosis; offspring are clones of the mother. In automictic parthenogenesis, or automixis, the offspring are half clones of the mother.

Normally during meiosis, one diploid gametocyte replicates its chromosomes and splits into four gametes. In females, only one gamete becomes the ovum, or egg cell; this gamete receives the bulk of

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*Whitetip reef sharks (*Triaenodon obesus*) are common near coral reefs in tropical and warm temperate waters of the Pacific and Indian Oceans. Photograph: © 2008* www.marineencounters.com.au *John Rumney.*

the cytoplasm and nutrients, while the others become nonfunctioning polar bodies, which typically degenerate. In automictic parthenogenesis, one of the polar bodies fuses with the ovum, stimulating embryonic development. Because chromosomal crossing over—in which genetic material gets exchanged between the mother's paternal and maternal chromatids—occurs during the first meiotic cell division, each polar body differs genetically from the ovum. Each embryo resulting from this process has a unique genome, with half the genetic diversity of the mother. However, automictic parthenogenesis tends to increase homozygosity across most of the offspring's genome. Simply because of statistical probability related to chromosomal assortment and recombination within the same genome, the offspring ends up with two copies of the same allele for most genes. This also means that all offspring are the homogametic sex. Since sharks have an XY sex determination system, all parthenogenetically derived offspring are female, XX.

Louis started genetic analysis, first ruling out the possibility that another species in the tank had delivered a hybrid bonnethead offspring, since no one actually saw the pup being born. This was exceedingly unlikely since no male bonnethead was in the tank, and hybridization has never been documented in sharks. "It was obvious from the beginning that we could eliminate any other species from mitochondrial DNA. That was pretty simple," he explained. Next, they needed to identify which of the three bonnethead females was the mother and determine whether the pup had any unique genes that would have come from a father.

As it happened, Mahmood Shivji, director of the Guy Harvey Research Institute and Save Our Seas Shark Center at Nova Southeastern University in Florida, and Demian Chapman, his graduate student at the time, were in the process of developing DNA fingerprinting for wild bonnethead sharks as part of their research on mating systems. The zoo gave tissue samples to Chapman, who completed the analysis while working as a visiting scientist at Queens University in Belfast, in the lab of collaborator Paulo Prodohl.

"I was in Northern Ireland, and I bet a bunch of guys in the lab each a pint of Guinness that this shark was actually going to have a father," said Chapman, a spirited New Zealander and now an assistant professor and assistant director of science at Stony Brook University's Institute for Ocean Conservation Science. He used amplified fragment length polymorphism (AFLP) fingerprinting analysis to survey the shark pup's genome for paternal genes, a process very similar to a human paternity test. He also looked at four microsatellites. The analysis would reveal any unique alleles not present in the mother. Except it didn't.

"When I saw the DNA fingerprint, I about fell off the chair. None of the profile came from the father. Everything in the DNA profile you could see in the mother." Chapman's microsatellite and AFLP analyses confirmed that the bonnethead had not hybridized with another species, showed unambiguously which of the three bonnethead females was the mother, and revealed the pup was homozygous at each microsatellite locus tested, conclusively showing the pup was produced by automictic parthenogenesis.

Chapman describes the moment he saw the results as a watershed moment. "Up until then, we logically assumed male and female mate and they give birth," he says. "It was one of those just great eureka moments you have in science, when bam, you see this picture come out of nothing, when you see something that just completely surprises you."

"We were all dumbfounded and excited," said Julie Sommer, currently an evolutionary biology PhD student at the University of Nebraska-Lincoln, who worked in the zoo's genetics department and is coauthor of the study published in 2007. "The coolest part at the time was all the questions that were running through our heads, from an evolutionary, behavioral, and conservation standpoint."

There was no information on whether this occurred in other chondrichthyan species or in the wild. "The big question was, was this a fluke, or is this unique to hammerheads [a recently derived lineage of elasmobranchs]?" Chapman said. "As it turns out, not even a couple weeks after the first paper was released, there was a newspaper report of another shark potentially doing this, a blacktip. This time I didn't bet any Guinness on it."

Surprise pregnancy

In May 2007, staff at the Virginia Aquarium performed a routine annual exam on a nine-year-old shark, named Tidbit, that turned out to be anything but routine. Beth Firchau, the aquarium's curator of fishes, was in the shark's tank with the aquarium vet and two assistants. Using a pole syringe, they administered a sedative to Tidbit, which slowed the animal's reaction time just enough so they could restrain her and place her on a specially designed stretcher.

"Blacktips are carcharhinids," Fircham says. "They are very high-energy animals, and to restrain them takes a lot out of you and the animal." During the procedure they kept a constant check on Tidbit's vital signs, but the sedation put her under a bit too deeply, so they countered with a stimulant. "She started to come out, and she rolled off, swam out of the stretcher, bumped into the wall, and I was the next thing she was going to bump into. She bit me."

Firchau ended up with bites across her shin and calf. In the midst of the chaotic scene, in which ambulances were called and Firchau was swiftly taken out of the tank, others tried in vain to save the shark. "In the process of getting her stabilized, we unfortunately lost her," she said. What started as a routine exam ended with a hospitalized, shark-bitten aquarist and a dead shark, but the real surprise came at the necropsy. Despite the fact that Tidbit had been housed in the facility for nearly 10 years without exposure to a male blacktip, her uterus held a 30-centimeter-long pup. "When they called and told me she was pregnant, I was shocked," Firchau said. "You don't think of parthenogenesis as a possibility for vertebrates."

Blacktip sharks (*Carcharhinus limbatus*) are in the Carcharhinidae, or requiem shark family. Among this family's approximately 50 species are some of the most feared ocean predators: tiger sharks, blue sharks, and bull sharks. Like the bonnethead, this blacktip never had an opportunity to mate as an adult. "We brought the blacktips in from the [Florida] Keys. They were young of the year," says Firchau. Tidbit was transferred to the larger aquarium after three years, where she lived with nurse, sand tiger, and sandbar sharks.

*Demian Chapman subdues a night shark (*Carcharhinus signatus*) during research in Belize. Photograph: © Debra Abercrombie.*

Did Tidbit mate, but they missed it? They didn't think so. "If you've ever observed the process of breeding of sharks or its aftermath, it's pretty violent," Firchau explains. "The male will come up under the female, take her pectoral fin in his mouth, and hold onto the female while he mates with her, all the time biting." Male sharks have two penises—claspers, technically—which are extensions of the pectoral fins; they become rigid and insert sperm into the female during copulation. Only one is used at a time. "You can't miss it when a female's been bred. The female usually has many bites over her body," Firchau says. Nothing like that had ever been observed in the aquarium.

Chapman heard about the surprise pregnancy from news reports and immediately contacted the aquarium. He and Shivji knew that microsatellite DNA markers for blacktips were already available, so this would be another simple analysis. Once again, the pup's DNA showed the same pattern as the bonnethead: homozygosity across all five microsatellite loci tested. They published the result in 2008, the second confirmed case of virgin birth in sharks.

Professional and amateur aquarists have been on the lookout for parthenogenesis in other captive sharks since the news broke in 2001, particularly in egg-laying species. Doug Sweet, a curator at Detroit's Belle Isle Aquarium until it closed, started saving eggs of the small white-spotted bamboo sharks (*Chiloscyllium plagiosum*). This species is often kept in home aquaria. "Females actually will continuously lay eggs whether they have a male or not. Normally people would assume these are infertile eggs and throw them in the garbage," Chapman said. It appears eggs from this species seem to undergo parthenogenesis quite commonly, and at least two from the Detroit aquarium have grown into adults, now held at the Landry's aquarium restaurant in Detroit. "What actually happens is a female lays 20 to 30 eggs, and 1 to 3 develop," says Chapman, who is on the trail of genetically confirming parthenogenesis in these sharks.

In January 2008, news of yet another possible shark virgin birth surfaced. At Hungary's Nyiregyhaza Centre aquarium, a whitetip reef shark (*Triaenodon obesus*) gave birth to a pup. The pup looks almost toylike, and aquarists reportedly thought the pup was a joke when they saw it in the tank. Just like the other cases, aquarists had collected the mother as a juvenile, and she had never encountered a male whitetip shark. Shivji and Prodohl are conducting a genetic analysis to find out whether this is yet another virgin birth.

Is parthenogenesis good or bad?

With the rash of virgin births, it appears that shark parthenogenesis may be far more common than previously suspected. Shivji has worked on shark genetics and conservation for over a decade and, like others, is intrigued by the new revelation. "Our guess is that parthenogenesis is not unusual in sharks," he said.

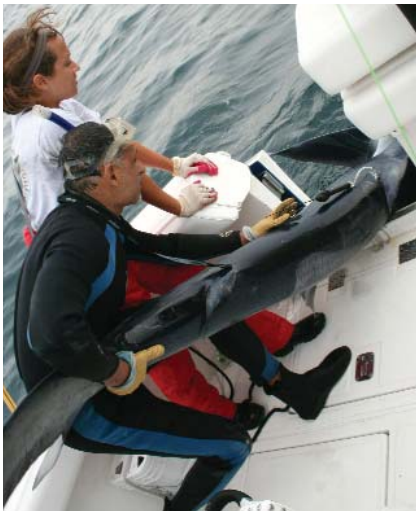
"What is unknown is how frequently that occurs in the wild." Scientists still don't know how many shark and ray species can reproduce by means of parthenogenesis, though suspected cases have now been confirmed in the above-mentioned four species, as well as in a zebra shark and a skate. Aquarists suspect they've missed more, since a larger shark could easily devour a newborn pup before anyone noticed it.

With most shark species in dramatic decline around the globe as a result of intense demand for shark fins, the ability of females to reproduce without a male may seem a good alternative reproductive strategy. "The obvious [thought] is, oh, this is cool because sharks can reproduce without mating. Well it's not that simple," Shivji explains. "If sharks are doing this in nature more commonly than people have known about, that would not be a good thing in term of the fitness of the population."

Studies show that sharks have declined anywhere from 50 to 90 percent from population levels just 20 years ago. Shivji has developed DNA fingerprint markers that can identify the species of shark whose fins have been intercepted by authorities. He and his colleagues also purchased shark fins in Hong Kong and Chilean fish markets and discovered that fins were often labeled incorrectly. In 2006, Shelley Clarke and Shivji published the first fishery-independent estimate of global shark mortalities: 73 million sharks killed annually, three to four times higher than reported shark catch figures.

Virgin birth, on the other hand, is akin to extreme inbreeding: Because the offspring are half clones of the mother, widespread parthenogenesis could substantially reduce genetic diversity in shark populations. "There are many cases in other wildlife where animals have a lot of inbreeding, so there's a lot of homozygosity," Shivji says. "As a result of this low diversity, they often have physiological and anatomical defects which make them less capable of functioning in the environment. The Florida panther is a good example." Besides the physical defect of deformed tails, Florida panthers also have reduced sperm counts and motility and, hence, reduced fecundity.

Richard Fitzpatrick captured a whitetip reef shark at Osprey Reef in the Coral Sea to implant a time-depth recorder to study sharks' movements around isolated atoll reefs outside Australia's Great Barrier Reef Marine Park. Photograph: © Wendee Holtcamp (www.wendeeholtcamp.com).



Mahmood Shivji and graduate student Lucy Howey release a blue shark (Prionace glauca) *after fitting it with an archival satellite tag in order to study its long-distance migrations. Fins from blue sharks make up the largest component of the global fin trade. Photograph: Brad Wetherbee.*

"The conventional paradigm says that you can get much more genetic diversity as a result of sexual reproduction. You've got genes from two different animals, but you have a lot of recombination going on. Crossing over in meiosis generates much more diversity, which produces a healthier, fitter population," Shivji explains. "Now, having said that, there are also organisms that seem to function extremely well with parthenogenesis."

Two factors increase the likelihood that sharks may experience difficulty finding mates as shark numbers decline worldwide. First, in most shark species, males and females do not hang out together except when they mate. "Sexual segregation in sharks is more the rule than the exception," Shivji says. Second, shark fisheries often intensively fish a particular location which, Shivji explains, also increases the chance that all of one sex could be wiped out in a particular region. Parthenogenesis has a further disadvantage for sharks: Through sexual reproduction, sharks can deliver up to 15 pups per litter; with parthenogenesis, in every case only one pup has been delivered. With egg-laying species, only a few develop from a clutch.

Parthenogenesis in sharks is ripe for further study. Since it's such a new discovery, no one has ever looked systematically through DNA databases for whether they might find evidence of parthenogenesis in wild populations. "Ordinarily in a big DNA database of a shark or any animal or plant, you don't look at every single genotype. You just have this big sea of numbers, DNA profiles," explains Chapman. "People should really look into whether they have these weird individuals...that have low genetic diversity and are significantly homozygous in every part of their genome." In addition, nothing is known about the mechanism by which the parthenogenesis occurs, or what stimulates it. Is it merely the absence of a male for a certain length of time? Are certain species more prone to it, or do certain environmental conditions make it more likely?

Genomic imprinting prevents parthenogenesis in mammals. In genomic imprinting, certain maternally inherited

*Mahmood Shivji surgically implants a tracking transmitter into a tiger shark (*Galeocerdo cuvier*) before releasing it. The researchers are studying how tiger sharks use coral reef habitats in the Caribbean. Photograph: Brad Wetherbee.*

genes get turned off, while the paternal copies of these genes remain active. Scientists believe this is due to an interesting intergenomic conflict in which the paternal version of the gene seeks to usurp as much nutrient from the mother as possible for the embryo, whereas the mother shuts down the maternal version to prevent that from happening. Most imprinted genes are involved in embryonic development, and since only the paternally inherited genes are active, a parthenogenetically produced female offspring fails to develop. Parthenogenesis has occurred in mammalian eggs, but the embryos die around day 10.

Yet the shark virgin birth discovery gives fair warning: in science, never say never. The Japanese research group led by Tokyo University of Agriculture professor Tomohiro Kono recently manipulated a mouse genome so that a parthenogenetic offspring grew into a healthy and reproductively viable mouse. The process required many steps and was anything but simple, but regardless, it surprised many by showing that under certain conditions, a healthy mammalian offspring can develop with no male genetic contribution.

"Many people think they know everything there is to know about nature, and every time you turn around it seems that nature pulls a fast one and teaches us how little we are and how little we do know," says Firchau, who is thrilled with the new discoveries, despite the traumas of the day, both her own injuries and the loss of Tidbit. "Whenever you lose an animal, you always try to find something positive that came from it. We are very fortunate that her death has given us a better understanding of her species and sharks all over the world."

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