Vol. 50: 81–91, 2023
https://doi.org/10.3354/esr01224

ENDANGERED SPECIES RESEARCH
Endang Species Res

Published February 16

Costs of parthenogenesis on growth and longevity in *ex situ* zebra sharks *Stegostoma tigrinum*

Lance Adams[1,*], Kady Lyons[2], Janet Monday[1], Elizabeth Larkin[1], Jennifer Wyffels[3,4]

[1]Aquarium of the Pacific, 100 Aquarium Way, Long Beach, CA 90802, USA
[2]Georgia Aquarium, 225 Baker Street, Atlanta, GA 30313, USA
[3]Delaware Biotechnology Institute, University of Delaware, 15 Innovation Way, Newark, DE 19711, USA
[4]Ripley's Aquariums, 7576 Kingspointe Parkway, Suite 188, Orlando, FL 32819, USA

ABSTRACT: The zebra shark *Stegostoma tigrinum*, a popular aquarium fish, is an endangered species that is known to readily reproduce both sexually and through facultative parthenogenesis while in human care. Artificial insemination trials that took place between 2011 and 2013 resulted in the hatching of 2 sexually produced (herein heterozygotes) and 10 parthenogenetic sharks that allowed for a retrospective comparison of growth, feeding and longevity between offspring produced from 2 distinct reproductive modes. Parthenogenetic offspring were generally smaller at hatch than their heterozygous counterparts and, after the first several months post-hatch, failed to increase in mass and length at the same rate as heterozygotes. Parthenogenetic offspring exhibited non-normal swimming behaviors such as spiraling, spy hopping and head standing, which may have been correlated with a gradual decline in the ability of some sharks to properly suction feed. Median lifespan for the parthenotes was 1.05 yr (range: 0.27–6.64 yr); one of the heterozygotes lived to 2.37 yr of age, and the other was alive at the time of this writing in August 2022 and had reached reproductive maturity. By contrast, the 2 longest surviving parthenotes perished just prior to reaching sexual maturity (~5.5 and ~6.5 yr). Parthenogenesis has been documented among *ex situ S. tigrinum* maintained in aquariums across the globe, and this study demonstrates substantial negative costs to fitness in parthenogenetic offspring compared with their heterozygous siblings. The reduced fitness of parthenotes has implications for managing populations in human care as well as for *in situ* conservation efforts.

KEY WORDS: Reproduction · Allometry · Survivorship · Food consumption · Deformities

1. INTRODUCTION

Successful reproduction is fundamental for species propagation and is particularly important for threatened or imperiled species and the management of their recovery. Success, in this context, is dependent not only on adults breeding but also on their offspring surviving to reach sexual maturity and subsequently reproducing themselves. Barriers to achieving this reproductive success can occur at many stages, and identifying the cause of the issue(s) is important for implementing effective management and conservation strategies.

While reproductive barriers may vary, maintaining genetic diversity via sexual reproduction is often a concern for threatened species due to their limited population numbers (Saccheri et al. 1998, Spielman et al. 2004). To further complicate the reproductive landscape, select species have the ability to reproduce both sexually and via parthenogenesis, the latter of which may exacerbate challenges to maintain genetic diversity. In particular, the most commonly proposed parthenogenetic mechanism used by many

*Corresponding author: ladams@lbaop.org

Publisher: Inter-Research · www.int-res.com

vertebrates is terminal fusion automixis (Robinson et al. 2011, Booth & Schuett 2016), a process whereby diploidy is restored through the fusion of the egg nucleus with its second polar body (Lampert 2008). This mechanism tends to result in high levels of offspring homozygosity when either DNA microsatellites (few loci) or next-generation sequencing (thousands of loci) is employed to determine parentage (Feldheim et al. 2010, Card et al. 2021, Ryder et al. 2021, Wyffels et al. 2021). Despite this, next-generation sequencing methods have demonstrated that parthenotes produced by terminal fusion automixis do retain some degree of heterozygosity, which is rarely detected when using microsatellite markers (Card et al. 2021). Nevertheless, parthenogenetic offspring are genetically less diverse than their mothers (Pearcy et al. 2006, Hedrick 2007, Card et al. 2021). Furthermore, the outcome of offspring produced by parthenogenesis as well as fitness of those offspring is not well studied (Reynolds et al. 2012, Moreira et al. 2021), and the impact parthenogenesis may have for success and recovery of threatened populations *in situ* is unknown.

Documentation of facultative parthenogenesis in the natural environment is rare (Booth et al. 2012, Fields et al. 2015), except in squamate reptiles, where facultative or obligate parthenogenesis occurs as part of their normal reproductive life history (Avise 2008, Kearney et al. 2009). For most other vertebrates, observations of parthenogenesis have been documented repeatedly among vertebrate species held in human care across academic, private hobbyist, and zoo and aquarium collections (Booth & Schuett 2016, Ryder et al. 2021). For elasmobranchs (sharks, skates and rays), parthenogenesis has almost exclusively been observed for animals in human care and when females are maintained in single-sex groups (Feldheim et al. 2022), with the exception of 1 observation in a wild smalltooth sawfish *Pristis pectinata* (Fields et al. 2015). The unexpected birth of a shark or ray in the absence of a male prompts a reproductive investigation, and genetics are used to confirm parentage. Parthenogenesis among elasmobranchs is hypothesized to occur via terminal fusion automixis, producing female-only offspring that are homozygous when genotyped by microsatellites (Chapman et al. 2007, Feldheim et al. 2017, Wyffels et al. 2021). Parthenogenesis in the endangered zebra shark *Stegostoma tigrinum* has been observed at multiple institutions across the world (Robinson et al. 2011, Dudgeon et al. 2017, Adams et al. 2022). *S. tigrinum* is popular in aquarium collections, with a preference for keeping female-only populations due to the risk of injury from male aggression, factors that may lead to an increased probability of observing parthenogenesis in this species compared with other elasmobranchs. However, *S. tigrinum* are documented to reproduce parthenogenetically despite co-habitation with a male (Adams et al. 2022), suggesting this phenomenon may be more widespread than initially thought.

Parthenogenesis represents a hurdle for maximizing genetic diversity of species held in human care (Watts et al. 2006). When breeding is desired for maintaining a sustainable *ex situ* population among member aquariums, the Association of Zoos and Aquariums maintains a Species Survival Plan for *S. tigrinum* that manages animal transfers and breeding plans via a studbook (Villaverde et al. 2020). Because all documented *S. tigrinum* parthenotes are homozygous to date, parthenogenesis challenges the ability of zoos and aquariums to maintain genetically diverse *ex situ* populations in the long term. Likewise, parthenogenesis may negatively impact current efforts to develop a reinforcement program for *S. tigrinum* in Indonesia (Traylor-Holzer 2021) — a region where this species is functionally and regionally extinct — if the founder population for re-introduction originates from parents held in aquariums that include parthenotes. Besides decreasing genetic diversity, parthenotes may also increase the overall mortality rate of the population if they are less fit compared with their sexually produced counterparts. However, knowledge of the long-term implications of parthenogenetic reproduction is limited in elasmobranchs (Straube et al. 2016) but necessary to inform managed breeding and predict the efficacy of reinforcement efforts.

Due to the failure of a mature mixed-sex population (2 females and 1 male) of *S. tigrinum* maintained at the Aquarium of the Pacific (AoP) to produce offspring, artificial insemination (AI) trials were conducted in 2011 and 2013 on a third singleton female to explore the use of this technique to overcome reproductive barriers (Adams et al. 2022). Egg-laying activities were monitored for the 2 females (Yin and Yang) housed with the male (Carlbe) and in the artificially inseminated singleton female (Fern). As a result of these efforts, 12 *S. tigrinum*, representing a mix of sexually and parthenogenetically produced offspring, were hatched and reared. Historic hatching, food consumption, growth and mortality data were leveraged for this retrospective examination of hatchling growth and survivorship to understand the potential costs of parthenogenesis for *S. tigrinum*. The specific aims of this study were to compare (1) incubation time and shark morpho-

metrics at hatch, and (2) post-hatch food consumption, growth and longevity between sexually and parthenogenetically produced offspring.

2. MATERIALS AND METHODS

2.1. Study system

Egg cases were collected as part of a companion study reporting successful use of AI in the zebra shark *Stegostoma tigrinum* (Adams et al. 2022). The population was comprised of 2 females (Yin and Yang) housed with an adult male (Carlbe) and 1 isolated female (Fern). AI procedures were approved by the AoP Research Advisory Committee. Husbandry of all animals was in accordance with industry standards for animal care through the Association of Zoos and Aquariums, and procedures were performed under the supervision of the attending veterinarian (L. Adams).

Egg cases laid by all 3 females were visually monitored by candling eggs underwater weekly to detect and track embryo development over the course of incubation. Eggs with deteriorating yolks or those that resulted in embryonic death were removed from the study. Egg cases were suspended on a plastic mesh shelf mid-water column in a closed system comprised of 3 round (1100 l) interconnected tanks. Temperature was maintained at 23.8 to 25°C, and a submersed spray bar was used to provide or direct constant water flow over the eggs.

2.2. Hatchling husbandry and monitoring

Upon hatching, sharks were removed from the incubation system and placed in 1 of 2 interconnected tanks on the same closed system and monitored daily. Hatchlings were weighed (total mass) and measured (total length [TL], straight-line distance from the tip of the snout to the tip of the tail) within 1 wk of hatching and subsequently examined weekly for 2.5 mo or more until the shark expired or was transferred to another aquarium. At approximately 45 cm TL, sharks outgrew the hatchery system and were moved to various mixed tropical species exhibit tanks (>19 000 l). A 12 h light:12 h dark cycle was maintained with artificial lighting throughout the duration of the study and for all systems.

At AoP, sharks were offered a mixture of shrimp, clam and squid or a mixture of teleost prey items (Table S1 in the Supplement at www.int-res.com/articles/suppl/n050p081_supp.pdf) daily at ~3% of their body weight. For a subset of animals, a daily food log was kept, detailing days until first meal after hatch, food items offered and quantities consumed. Daily food logs were used to calculate percent weekly food consumption (i.e. Σ g food offered each day for 1 wk/Σ g food recommended each day for 1 wk), with 0% representing fasting and 100% representing full consumption of an individual's allotted amount. Percent weekly food consumption served as a metric to determine appetite.

Neonatal and juvenile sharks were monitored daily for changes in swimming behavior, demeanor and navigation patterns within the exhibit that might be correlated with a failure to thrive. Radiographs were taken during physical examination of the first shark that exhibited symptoms and were subsequently performed on a majority of sharks to determine frequency of spinal abnormalities and co-occurrence with other observed clinical problems. Radiographs were analyzed for number of spinal abnormalities (e.g. malformed vertebrae, hemi-vertebrae, fused vertebrae) and their placement along the spinal column. For sharks that were transferred to other institutions, historic morphometrics and necropsy information, including date, were obtained if available.

2.3. Data analyses

Sexually produced offspring were limited to 2 individuals (herein heterozygotes); therefore, descriptive statistics were used to compare incubation time, hatching length and hatching mass for parthenotes and heterozygotes, and data were visually inspected for differences.

Detailed food consumption was compared between heterozygotes and a subset of parthenogenetic offspring. Percent weekly food consumption was visually inspected across time between heterozygotes and parthenotes and overall central tendency (i.e. mean ± SD) for each group calculated. For each shark, percent weekly food consumption was paired with growth morphometrics by week to assess the relationship between food consumption and growth.

Growth was examined from several perspectives and compared between heterozygotes and parthenotes. Basic allometry (i.e. relationship between total mass and length) was evaluated using a mixed-effects model with shark ID as the random term and parentage as a fixed effect for measurements taken up to 25 wk post-hatch. Absolute growth over time

was visualized through monthly comparisons (30 ± 3 d) of length or mass between groups across 1 yr post-hatch.

Changes in growth during early life (i.e. first 25 wk) between heterozygotes and parthenotes were also examined. First, growth rate per week (i.e. gain or loss in cm or g per week) was calculated for each individual as the difference between sequential measurements divided by the number of days per week between those measurements. For example, if a shark's TL was 34 cm 28 d post-hatch and 36 cm 35 d post-hatch, then its growth rate would be 2 cm wk^{-1} (i.e. 36–34 cm/[35–28 d/7 d wk^{-1}]). This growth metric calculation was reiterated over the course of the study using weekly measurements. A linear model was then used to evaluate the relationship between the growth per day (i.e. rate of change in length or mass per week) and the week of measurement. Slopes from linear models were visually compared between parthenotes and heterozygotes to determine if rate of growth was different between groups.

Presence or absence of external or internal deformities or non-normal behavior was noted to determine its prevalence for each group. Lifespan (median, range) was descriptively compared between heterozygotes and parthenotes, and cumulative survivorship curves were constructed as the percent of living offspring through 2022.

3. RESULTS

3.1. Hatching

Twelve individuals hatched between 2012 and 2015. Paternity testing confirmed that 8 hatchlings were parthenotes and 2 were heterozygotes, produced from sexual fertilization as a result of AI (Adams et al. 2022). Two hatchlings (P278 and P357) were not genotyped because access to genetic testing was not readily available at the time, and tissues were not saved for retrospective testing. These individuals were provisionally classified as parthenotes (hereafter denoted with an asterisk when specifically referenced) because they hatched from eggs laid 23 and 329 d after the last confirmed heterozygote (i.e. oviposition occurred 56 and 362 d post-AI for suspected parthenotes versus oviposition of 18 and 33 d post-AI for heterozygotes) and beyond the timeframe documented for sperm storage in this species (Adams et al. 2022); however, sperm storage can be lengthy in some elasmobranchs (Jordan et al. 2021), and exceptions are possible. At least 1 parthenote was genetically confirmed from each female, with 6 produced by Fern, 1 by Yin and 1 by Yang (Table S2). All offspring, parthenotes and heterozygotes, were female.

Incubation period (time from oviposition to hatch) was similar between the 2 groups, with heterozygote time to hatch (143 and 145 d) falling within the range of parthenotes (median, range: 146, 138–158 d). With the exception of the longest hatchling (33.5 cm TL), heterozygotes (29.5 and 30 cm TL) were longer than parthenotes at hatching (median, range: 27.75, 26.0–33.5 cm TL). By contrast, heterozygote total mass at hatching (103 and 104 g) fell within the range of parthenote total mass (105, 80–114 g).

3.2. Food consumption

Daily food consumption logs were recorded for 5 animals (3 parthenotes and 2 heterozygotes) for a minimum of 9 wk (P357*) and up to 22 wk (H442, heterozygote), with most recorded for 17 wk (P113, P200, H440). Parthenotes began eating after hatch sooner than heterozygotes, 6 to 7 d versus 11 to 12 d post-hatch, respectively. However, once hatchlings commenced feeding, percent weekly food consumption was similar between parthenotes (93 ± 21%) and heterozygotes (92 ± 23%), although parthenote consumption varied widely (Fig. S1). Parthenote P113 was the only shark to show a continual decline in food consumption starting at 13 wk. While records of daily food logs end at 17 wk for this animal, it survived for 1 yr (~53 wk) post-hatch. No relationship was observed between weekly food consumption and weekly changes in length or mass.

3.3. Young-of-year growth

There was a positive relationship between length and mass for both parthenotes and heterozygotes during the first 25 wk of life ($p < 0.0001$); however, there was a statistically significant interaction between length and genetic status ($p = 0.001$). At comparable lengths, parthenotes were heavier than heterozygotes ($\log_{10}$ model slope, 95% CI: p = 2.31, 2.24–2.39; $H = 2.53$, 2.43–2.63) (Fig. S2).

Parthenotes were distinguishable from heterozygotes for overall absolute growth, growth rate per week and rate of change in growth (i.e. acceleration). Heterozygotes were longer than parthenotes at hatch, and parthenotes did not achieve comparable lengths even by

6 mo (Fig. 1a). With respect to body mass, parthenotes were comparable to heterozygotes during the first few months post-hatch; however, by 5 mo post-hatch, heterozygotes were consistently heavier (Fig. 1b).

TL increase per week was generally faster for heterozygotes (mean ± SD: 1.87 ± 0.92 cm wk^{-1}) compared to parthenotes (1.20 ± 1.01 cm wk^{-1}); however, rate of change in growth per week (i.e. acceleration) was not statistically significant for either of the heterozygotes and 5 of the 8 parthenotes (i.e. sharks lengthened at a constant rate; Table S3). Three parthenotes (P278*, P357*, P385) demonstrated a slowing of growth per week after approximately 5 wk post-hatch until they ceased to lengthen at all between sequential handlings and ultimately perished (Fig. S3).

Differences between heterozygotes and parthenotes for both absolute mass increase per week and rate of change (i.e. acceleration) were more pro-

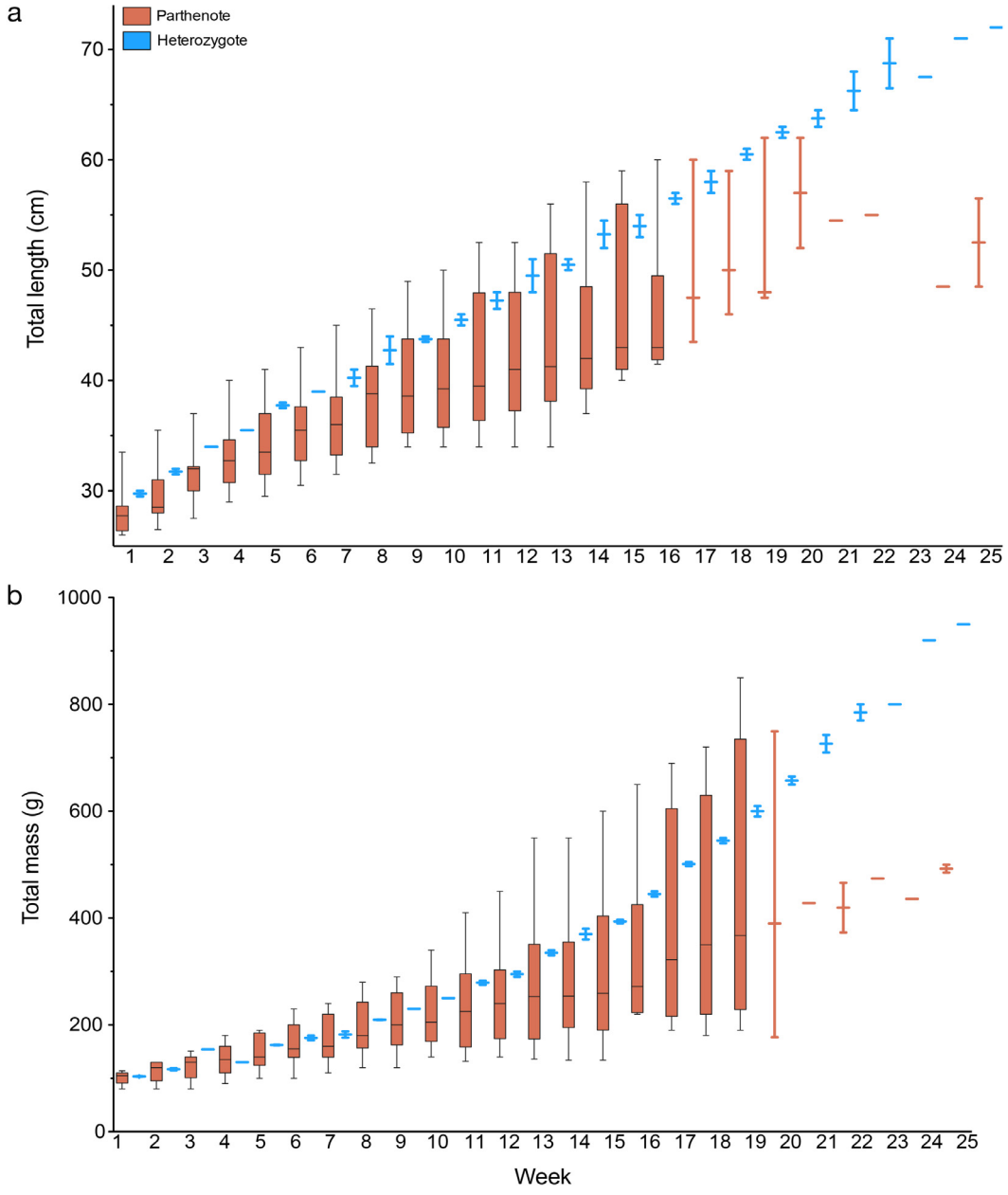

Fig. 1. (a) Total length and (b) total mass taken weekly over 6 mo (25 wk) for parthenotes (red) and heterozygotes (blue). Error bars represent minimum and maximum values with median values and 25th and 75th quartiles shown in box plots. Red box plots represent weeks where measurements were available for 3 or more individuals, line plots represent measurements for only 2 individuals and single horizontal lines represent data from only 1 individual

nounced than those for length (Fig. S4). Mass increase per week was generally faster for heterozygotes (35.6 ± 24.0 g wk^{-1}) than for parthenotes (14.3 ± 23.1 g wk^{-1}), which was highly variable among individuals. Among parthenotes, 2 individuals (P200 and P277) had mean mass increases per week that were comparable to heterozygotes; however, their rate of weight gain increase per week was not (Table S3). Other parthenotes either gained mass at a relatively constant rate (P113, P255, P278*) or gained more slowly over time (P357*, P385, P503).

3.4. Behavioral and physical abnormalities

Many parthenotes gradually demonstrated a reduced ability to suction feed over time, to the point where many of them could not swallow food even when placed directly in their mouths (n = 5). At feeding time, sharks were noted to approach and circle food items, demonstrating clear interest; however, as they lost the ability to suction feed, they would perform unusual behaviors (e.g. head standing and nose pressing on food items), seemingly in an effort to apprehend food. Among these 5 parthenotes, no other concurrent neurological deficits were noted (i.e. assessment of other cranial nerve activity and spinal nerve function appeared normal), and jaws exhibited usual range of motion when manipulated. As these sharks became less able to feed unassisted, several medical interventions were pursued, including antibiotics and tube feeding. However, medical treatment did not resolve feeding or behavioral issues, and continued weight loss necessitated humane euthanasia. Three of 10 parthenotes also exhibited symptoms associated with neurological issues such as episodes of abnormal swimming behaviors (spiraling and spy hopping).

Radiographs revealed vertebral abnormalities along the spine in all but one shark (P113), presenting as one or more malformed vertebrae that were hemivertebrae (1), fused vertebrae (6) or shortened vertebrae (5) as well as mild kyphosis, lordosis or scoliosis associated with the malformation (Fig. 2a,b; Table S4; Fig. S5). There was no apparent relationship between number of spinal deformities (which may shorten the length of the spinal column) and length at hatch. As most abnormalities occurred in the tail, they were not suspected to contribute to the inability to assist with prehension of food by parthenotes, and any potential concurrent defects of the skull that could have contributed to neurological deficits in the jaw would have been too small to identify radiographically. Each of the heterozygotes had 1 incidence of malformed vertebrae in the spinal peduncle, while most parthenotes had 1 to 3 incidences. Vertebral abnormalities manifested externally as a zigzag in the tail of the parthenote with the most malformed vertebrae (P200; n = 12; Fig. 2c). One parthenote (P113) had a cleft in the dental lamina of the palatoquadrate cartilage and had several instances of gastric distention (Fig. 2d).

3.5. Longevity and survivorship

Half of the hatched offspring were transferred to other institutions within the first 2 yr of life. Four parthenotes (P255, P505, P503 and P200) were transferred to Ripley's Aquarium of Myrtle Beach, South Carolina, USA, at >37 wk post-hatch, and 1 parthenote (P542) was transferred to Wonders of Wildlife, Springfield, Missouri, USA, at 16 wk post-hatch. One heterozygote (H440) was sent to Newport Aquarium, Newport, Kentucky, USA, at 60 wk post-hatch. Remaining sharks were maintained at AoP until their time of death, except the second heterozygous shark (H442) that was still alive at the time of this writing.

More sexually produced offspring (50%) have survived to present day (i.e. 2022) than parthenotes (0% survivorship; Fig. 3). One heterozygote lived to 2.37 yr (H440), and the other heterozygote (H442) is currently thriving at AoP at approximately 8 yr of age at the time of this publication. Median lifespan of parthenotes was ~1.05 yr (range: 0.27–6.64 yr), with the 2 provisionally assigned parthenotes having among the shortest lifespans observed (0.29 and 0.36 yr). The 2 longest surviving parthenotes lived to 5.39 yr at Wonders of Wildlife (P542) and to 6.64 yr at Ripley's Aquarium of Myrtle Beach (P503). Neither P542 nor P503 achieved either the same length or mass at their time of death compared with their heterozygous sister, H442 (Fig. 4). Both parthenotes were noted as failing to thrive (i.e. experiencing substantial weight loss) towards the end of their life despite veterinary intervention. Necropsy information revealed internal organ issues related to a ruptured cystic ovary and egg yolk peritonitis present (P542) and ascites with bile-tinged intracoelomic fluid present accompanied by an enlarged gall bladder with immature follicles in the ovary (P503).

4. DISCUSSION

In a small population of zebra sharks *Stegostoma tigrinum*, parthenogenetically produced offspring

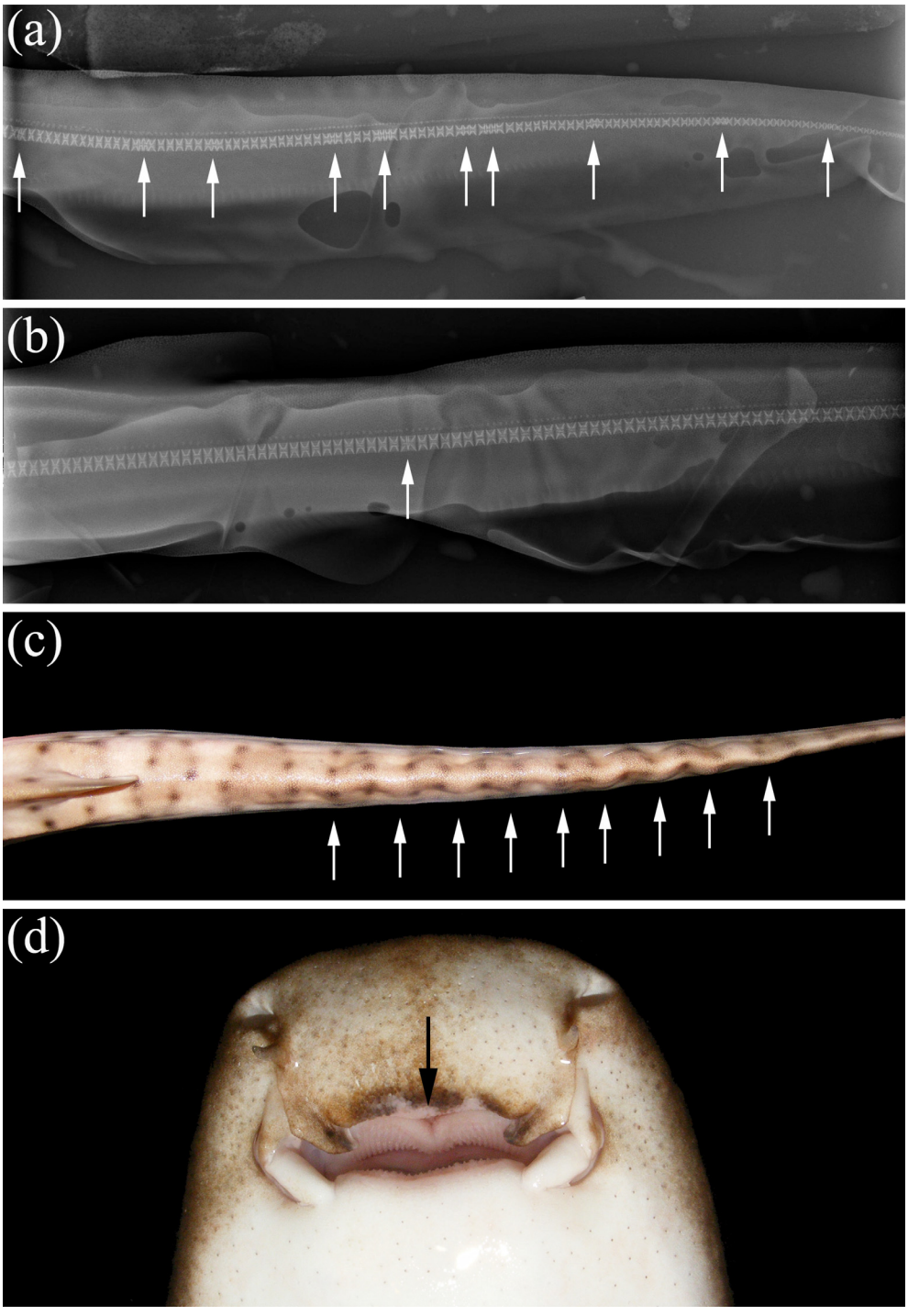

Fig. 2. Radiograph of lateral right-side view of the caudal spinal column along the tail for (a) a parthenogenetic offspring (P200) and (b) a heterozygous offspring (H440). (c) Externally, vertebral deformities in P200 were observed as kinks or bumps in the tail. Only 1 parthenogenetic shark (P113; d) had a cleft in the upper jaw. Abnormalities are denoted by arrows

failed to thrive, with 50% of individuals deceased within a year of hatch. Despite sample sizes being unbalanced and limited for heterozygous offspring, this retrospective study provides compelling evidence that there is a cost to reproducing parthenogenetically. For *S. tigrinum*, this cost may provide an explanation for why early-life mortality of this species is high in human care (Watson & Janse 2017, Adams et al. 2022). Furthermore, the curious association of physical deformities and neurological disor-

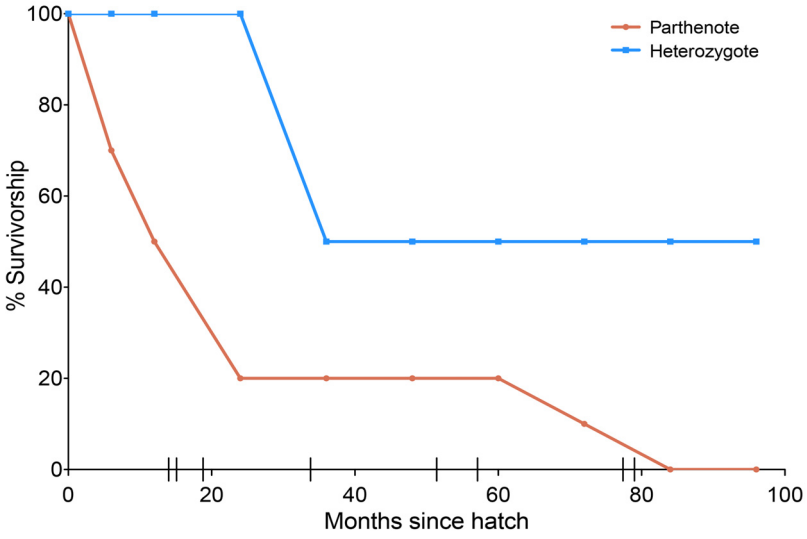

Fig. 3. Survivorship over the course of the study shown for heterozygotes (n = 2) and parthenotes (n = 10). Longer hash marks along *x*-axis indicate the month since hatch in which individuals perished (these may represent 1 or more offspring)

ders with parthenogenesis warrants further investigation into the mechanisms by which these phenotypes manifest. Reproduction via parthenogenesis has been observed repeatedly in this species, and understanding the prevalence of this phenomenon in *S. tigrinum* across institutions is needed to inform long-term sustainability of the population in human care and *in situ* conservation efforts of this endangered species.

Upon hatching, no clear and apparent physical differences existed between parthenogenetic and heterozygous offspring. The incubation period for heterozygotes was within the range of parthenotes; however, length at birth for heterozygotes was generally longer than for parthenotes although birth mass was similar. While greater variation in mass and length was observed for parthenote early-life metrics, it is difficult to empirically determine if an actual difference existed between groups or if differences observed were an artifact of low sample size. Therefore, more detailed studies with greater sample sizes are needed to determine if *in ovo* development was substantially affected in parthenogenetic offspring compared with their heterozygous counterparts.

Despite similar allometric baselines at hatch, stunted or impaired growth in parthenotes was apparent by the end of the first year of life. Divergence in growth trajectories may not have been obvious in the first few months after hatch because parthenotes were generally heavier for their TL compared with heterozygotes, which could have obscured differences in absolute growth of parthenotes versus heterozygotes. Nevertheless, parthenotes were unable to match heterozygote mean growth per week, which ultimately contributed to parthenotes being noticeably smaller and lighter than heterozygotes by ~5 mo post-hatch. Among parthenotes, differences in total mass per week over time grouped into 3 categories: initially similar with a later decline, stagnant or declining. Increase in shark length per week was more variable than mass, but regardless, mean rate of increase in length per week for heterozygotes was approximately double that of parthenotes. Growth disparities between heterozygotes and parthenotes remained consistent over time, such that the longest living parthenotes never achieved TLs or mass comparable to the longest surviving heterozygote. This suggests that even if parthenotes were to survive longer, they may not grow to compa-

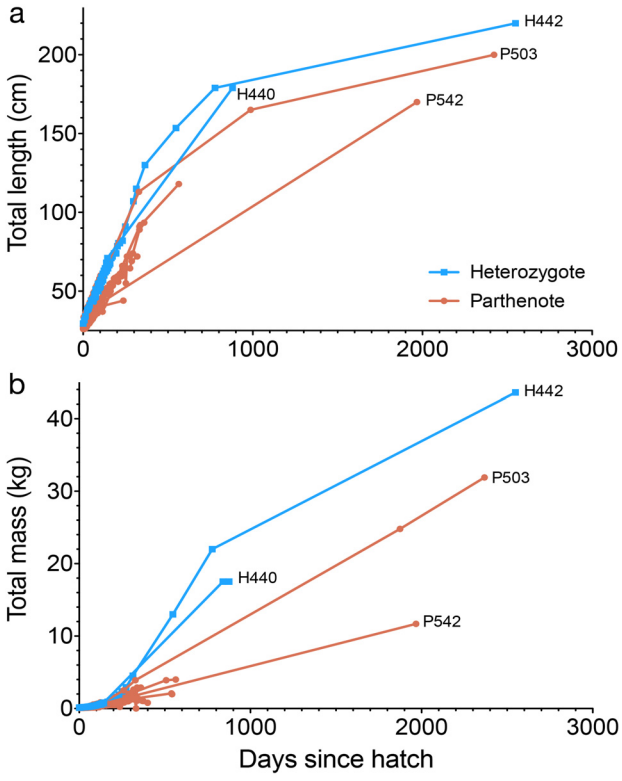

Fig. 4. (a) Total length and (b) total mass for both parthenogenetic and heterozygous offspring at their last available measurement (i.e. either at necropsy or at most recent physical examination). ID numbers of offspring surviving longer than 2 yr are denoted

rable lengths, which could have implications for *in situ* survival.

Impaired growth of parthenotes was not due to a difference in quantity of food offered or quality in nutrition compared with heterozygotes. In general, parthenotes commenced regular feeding a few days earlier than heterozygotes; however, both groups showed a robust ability. Considering that *S. tigrinum* reproductive life history likely leverages a strategy of producing many young at smaller sizes like other oviparous species, it is not surprising that neonatal and young-of-year animals are particularly voracious, as there is a biological imperative to assimilate nutrients to grow as quickly as possible. However, many parthenogenetic offspring appeared to lose the ability to properly suction feed several months post-hatch, which was attributed to a neurologic disorder, possibly with a genetic basis, after physical factors were ruled out. Once anorexia manifested, parthenogenetic sharks never recovered even with medical intervention and gradually failed to thrive, with most humanely euthanized. Interestingly, many of the abnormal swimming patterns noted in this study have been documented at other public aquariums rearing *S. tigrinum* hatchlings but were attributed to nutritional deficiency. However, parentage was not genetically confirmed in those hatchlings and staff may have been unaware of the possibility that the offspring were actually parthenogenetic. Identifying the presence of parthenogenetic offspring in a cohort could be important for managing their care and determining more effective solutions if or when they fail to thrive.

Besides differences in growth, survivorship was starkly divergent between heterozygotes and parthenotes. At the time of this publication, all parthenogenetic offspring had perished compared with half of the heterozygous offspring. Notably, by the end of their first year, 50% of parthenotes had died, and by the end of the second year, 80% of parthenotes had died. In the aquarium industry, *S. tigrinum* early-life mortality is recognized as being relatively high (Watson & Janse 2017). However, linking early-life death to parthenogenesis is difficult, as genetic testing of all hatchlings is not commonplace and reserved for situations when young result where males are not present. Rather, institutions that hold both males and females assume offspring are sexually produced, even when evidence demonstrates that parthenogenesis can be the more prevalent mode of reproduction even in the presence of semen in this species (Adams et al. 2022). Furthermore, knowledge of natural mortality *in situ* is severely lacking, so it is impossible to know if the observed mortality rate is comparable to the *in situ* population or an artifact of human care. Parthenogenesis may occur more often than it is empirically documented and could be a contributing factor to why early-life mortality appears to be prevalent in this species when held *ex situ*.

Longevity was another key biological trait that fundamentally differed between heterozygotes and parthenotes. The 2 longest surviving parthenotes (~5.5 and ~6.5 yr post-hatch) died near the time when *S. tigrinum* females become sexually mature, 6 to 7 yr of age (Watson & Janse 2017). H442, the only surviving animal from this study, laid her first eggs at ~6.5 yr old and shares the same maternal lineage as the 2 longest living parthenotes previously mentioned. For reference, heterozygous animals collected from the wild as juveniles have been maintained in human care for over 25 yr (L. Watson pers. comm.). It is possible that inbreeding depression, resulting from being homozygous as a parthenote, may cause the expression of certain deleterious genes as animals are transitioning from juveniles to adults, at least for the animals in this study. However, in the whitespotted bamboo shark *Chiloscyllium plagiosum*, a first-generation parthenote female was able to reproduce herself (although unisexually), suggesting that parthenote survival and subsequent sexual maturity in an elasmobranch species is possible (Straube et al. 2016). In reptiles with ZW sex determination, first-generation parthenotes (males) were observed to have physically normal hemipenes and testes, raising no concerns that these animals were incapable of physically reproducing; however, condition and presence of spermatozoa was inconclusive, as these animals were examined post-mortem after being frozen (Reynolds et al. 2012).

As previously noted, parthenogenesis was the predominant reproductive mode exhibited by females in the AI study (Adams et al. 2022), even though clear negative downstream outcomes are associated with being a parthenote (i.e. internal/external deformities, slower growth, lower survivorship, shorter longevity). In other species, similar negative outcomes have been observed among parthenogenetic littermates, even when some individual parthenotes do survive to reach sexual maturity. For example, relatively high litter mortality early in life or stillborn offspring are noted along with developmental deformities in a variety of snake species reproducing parthenogenetically in human care (Reynolds et al. 2012, Booth & Schuett 2016) and even in other elasmobranchs (Straube et al. 2016). Understanding the degree to which these observations are overrepresented in *ex*

situ parthenogenetic litters compared with situations where the species would reproduce sexually remains open ended and difficult to answer for threatened or imperiled species where limited *in situ* information is available. Nevertheless, the occurrence of deformed littermates, stillborns and other abnormalities among parthenogenetic litters may be attributed to their homozygosity, where deleterious recessive alleles have a higher probability of being expressed that may ultimately contribute to developmental deficiencies, similar to highly inbred sexually reproducing wild populations. For example, in the Glanville fritillary butterfly *Melitaea cinxia*, highly inbred populations had lower larvae survival, shorter longevity and decreased rates of egg hatching (Saccheri et al. 1998), consequences not dissimilar to outcomes of some parthenogenetic litters noted earlier, suggesting that the lack of genetic diversity may lead to poorer overall outcomes as predicted by evolutionary theory on the benefits of sexual reproduction.

S. tigrinum appear to be especially prone to reproducing via parthenogenesis when in human care settings (Robinson et al. 2011, Dudgeon et al. 2017, Adams et al. 2022). Whether this biological phenomenon is more frequently noted in *S. tigrinum* because this species is more charismatic compared with other oviparous elasmobranchs also commonly held in human care (e.g. *Chiloscyllium* spp.) or because this species has a biological propensity to use this mode of reproduction is unknown. Nevertheless, understanding the long-term implications of parthenogenesis on individual health and fitness has population-level consequences for management and conservation both *in situ* and *ex situ*. While born with comparable body conditions, heterozygous offspring quickly diverged from their parthenogenetic sisters, the latter of which were never able to catch up. Furthermore, parthenotes in this study had generally short life spans, and the 2 longest living animals never reached reproductive age. Taken together, parthenotes appear to be less fit than their heterozygous counterparts, and this raises questions about how conservation organizations should invest their resources if parthenogenetic offspring are included in population management and recovery programs. This is the first study to monitor long-term outcomes of parthenogenesis in an elasmobranch species and demonstrates there is a cost associated with parthenogenetic reproduction.

Acknowledgements. Thanks to Shara Seals for veterinary technician assistance during AI procedures and AoP husbandry staff, who assisted with handling during procedures and care of the sharks in this study. The authors also thank Piper Rackley, Allyson Stiles, Amiya Tucker and Mackenzie Lee for assistance in transcribing data into electronic form. Thanks to Stacia White at Ripley's Aquarium at Myrtle Beach, Frances (Betsy) Mackie at Wonders of Wildlife and Jolene Hanna and Jen Hazeres at Newport Aquarium for providing necropsy reports and available morphometric data while animals were in their care. The authors declare no conflict of interest. This study was financially supported by AoP.

LITERATURE CITED

Adams L, Lyons K, Larkin E, Leier N and others (2022) Artificial insemination and parthenogenesis in the zebra shark *Stegostoma tigrinum*. Front Mar Sci 9:886616

Avise JC (2008) Clonality: the genetics, ecology, and evolution of sexual abstinence in vertebrate animals. Oxford University Press, New York, NY

Booth W, Schuett GW (2016) The emerging phylogenetic pattern of parthenogenesis in snakes. Biol J Linn Soc 118:172–186

Booth W, Smith CF, Eskridge PH, Hoss SK, Mendelson JR, Schuett GW (2012) Facultative parthenogenesis discovered in wild vertebrates. Biol Lett 8:983–985

Card DC, Vonk FJ, Smalbrugge S, Casewell NR and others (2021) Genome-wide data implicate terminal fusion automixis in king cobra facultative parthenogenesis. Sci Rep 11:7271

Chapman DD, Shivji MS, Louis E, Sommer J, Fletcher H, Prodöhl PA (2007) Virgin birth in a hammerhead shark. Biol Lett 3:425–427

Dudgeon CL, Coulton L, Bone R, Ovenden JR, Thomas S (2017) Switch from sexual to parthenogenetic reproduction in a zebra shark. Sci Rep 7:40537

Feldheim KA, Chapman DD, Sweet D, Fitzpatrick S, Prodöhl PA, Shivji MS, Snowden B (2010) Shark virgin birth produces multiple, viable offspring. J Hered 101:374–377

Feldheim KA, Clews A, Henningsen A, Todorov L and others (2017) Multiple births by a captive swellshark *Cephaloscyllium ventriosum* via facultative parthenogenesis. J Fish Biol 90:1047–1053

Feldheim KA, Wyffels J, Lyons K (2022) The role of aquaria in the advancement of elasmobranch reproductive biology. Front Mar Sci 9:963542

Fields AT, Feldheim KA, Poulakis GR, Chapman DD (2015) Facultative parthenogenesis in a critically endangered wild vertebrate. Curr Biol 25:R446–R447

Hedrick PW (2007) Virgin birth, genetic variation and inbreeding. Biol Lett 3:715–716

Jordan RP, Graham CT, Minto C, Henderson AC (2021) Assessment of sperm storage across different reproductive modes in the elasmobranch fishes. Environ Biol Fishes 104:27–39

Kearney M, Fujita MK, Ridenour J (2009) Lost sex in the reptiles: constraints and correlations. In: Schön I, Martens K, van Dijk P (eds) Lost sex: the evolutionary biology of parthenogenesis. Springer, Dordrecht, p 447–474

Lampert KP (2008) Facultative parthenogenesis in vertebrates: reproductive error or chance? Sex Dev 2:290–301

Moreira MO, Fonseca C, Rojas D (2021) Parthenogenesis is self-destructive for scaled reptiles. Biol Lett 17:20210006

Pearcy M, Hardy O, Aron S (2006) Thelytokous parthenogenesis and its consequences on inbreeding in an ant. Heredity 96:377–382

Reynolds RG, Booth W, Schuett GW, Fitzpatrick BM, Burg-

hardt GM (2012) Successive virgin births of viable male progeny in the checkered gartersnake, *Thamnophis marcianus*. Biol J Linn Soc 107:566–572

Robinson DP, Baverstock W, Al-Jaru A, Hyland K, Khazanehdari KA (2011) Annually recurring parthenogenesis in a zebra shark *Stegostoma fasciatum*. J Fish Biol 79: 1376–1382

Ryder OA, Thomas S, Judson JM, Romanov MN and others (2021) Facultative parthenogenesis in California condors. J Hered 112:569–574

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proc Natl Acad Sci USA 101:15261–15264

Straube N, Lampert KP, Geiger MF, Weiß JD, Kirchhauser JX (2016) First record of second-generation facultative parthenogenesis in a vertebrate species, the whitespotted bambooshark *Chiloscyllium plagiosum*. J Fish Biol 88:668–675

Traylor-Holzer K (2021) Population viability analysis (PVA) report for population augmentation of zebra sharks (*Stegostoma tigrinum*) in Raja Ampat, Indonesia. UCN SSC Conservation Planning Specialist Group, Apple Valley, MN

Villaverde R, Levans J, Lawless A (2020) Population analysis & breeding and transfer plan: zebra shark (*Stegostoma fasciatum*), AZA Species Survival Plan Yellow Program. AZA report (13 January 2020)

Watson L, Janse M (2017) Reproduction and husbandry of zebra sharks, *Stegostoma fasciatum*, in aquaria. In: Smith M, Warmolts D, Thoney D, Hueter R, Murray M, Ezcurra J (eds) The elasmobranch husbandry manual II: recent advances in the care of sharks, rays and their relatives. Ohio Biological Survey Spec Publ, Columbus, OH, p 421–432

Watts PC, Buley KR, Sanderson S, Boardman W, Ciofi C, Gibson R (2006) Parthenogenesis in Komodo dragons. Nature 444:1021–1022

Wyffels JT, Adams LM, Bulman F, Fustukjian A, Hyatt MW, Feldheim, KA, Penfold LM (2021) Artificial insemination and parthenogenesis in the whitespotted bamboo shark *Chiloscyllium plagiosum*. Sci Rep 11:9966

Editorial responsibility: Austin Gallagher, Herndon, Virginia, USA
Reviewed by: W. Booth and 2 anonymous referees

Submitted: August 28, 2022
Accepted: December 20, 2022
Proofs received from author(s): February 12, 2023