Vol. 45: 85–98, 2021
https://doi.org/10.3354/esr01122

ENDANGERED SPECIES RESEARCH
Endang Species Res

Published June 3

Female philopatry in smalltooth sawfish *Pristis pectinata*: conservation and management implications

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ABSTRACT: Populations are more effectively managed with information on breeding and dispersal behavior, making the evaluation of these characteristics essential for effective conservation of a species. In the USA, 2 critical habitat units were designated in 2009 for the federally endangered smalltooth sawfish *Pristis pectinata*. Previous research in the Charlotte Harbor Estuary Unit (CHEU) of critical habitat shows that female smalltooth sawfish are polyandrous and highly philopatric to nursery grounds. However, these characteristics have not yet been examined in a larger area of designated critical habitat: the Ten Thousand Islands/Everglades Unit (TTIEU). We used microsatellite genotypes from 214 juvenile smalltooth sawfish to examine mating and dispersal behavior via sibship analyses and reconstruction of parental genotypes with the program COLONY. Parental reconstruction yielded 71 female and 117 male genotypes. Many females returned to the same region within TTIEU for parturition on a biennial cycle; however, at least 1 female switched parturition sites within TTIEU and at least 2 females produced litters in both TTIEU and CHEU over the study period. The maximum number of pups genetically assigned to 1 female was 12, a number that is consistent with that found for CHEU (8 pups), and within the litter size range reported (7 to 14) for the species. Confirmation of these mating behaviors and reproductive characteristics is important for understanding how the present population uses these protected habitats at different life stages, and for determining future habitat protection and population expansion strategies to restore smalltooth sawfish to previously occupied areas of their range.

KEY WORDS: Philopatry · Polyandry · Reconstructed genotypes · Critical habitat · Elasmobranch

1. INTRODUCTION

Mating and dispersal behaviors are critical drivers of genetic variation and its distribution across the landscape. Consequently, information about these key behaviors is needed to implement effective management strategies for species conservation (Waples 1991, Fraser & Bernatchez 2001). Movement, migration, or sex-biased philopatry and dispersal are particularly well-known aspects of behavior that can govern gene flow, making management actions based on these behaviors influential for evolutionary processes (Slatkin 1985, Allendorf et al. 1987, Garant et al. 2007, Dionne et al. 2009). For instance, in a species that exhibits polyandry and female philopatry, gene flow between populations depends mostly on

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male dispersal. Thus, any threats that negatively affect dispersive males and/or areas where females give birth would also affect genetic diversity in the species. On a broader scale, this means that threats imposed on the dispersing sex may prevent gene flow into adjacent populations, increasing differentiation, which the mating strategy may not be able to balance. As such, an evaluation of mating strategy and sex differences in dispersal behavior is essential for effective management, mitigation of threats, and conservation of genetic diversity necessary for adaptation.

For elasmobranchs (sharks, skates, and rays), populations are often categorized as stocks (Booke 1981), local populations (Andrewartha & Birch 1984), or in cases of species listed on the United States Endangered Species Act (ESA), distinct population segments (National Marine Fisheries Service [NMFS] 1996, 61 FR 4722). Support for these categories is often based on movement studies (e.g. Morrissey & Gruber 1993) or habitat definitions (i.e. Heupel et al. 2007), in combination with genetic research to identify elasmobranch mating behaviors and gene flow (Portnoy & Heist 2012). Many studies have demonstrated that sharks exhibit: female philopatry and male-biased dispersal (Feldheim et al. 2002, Hueter et al. 2004, Speed et al. 2010, Chapman et al. 2015); polyandry resulting in litters with multiple paternity (e.g. Saville et al. 2002, Chapman et al. 2004, Feldheim et al. 2004, Daly-Engel et al. 2007, Portnoy et al. 2007, Lage et al. 2008, Byrne & Avise 2012); and sperm storage in females (Bernal et al. 2015), which can be a mechanism for post-copulatory female choice (Birkhead 1998). However, there are far fewer studies (e.g. Roycroft et al. 2019) that investigate mating strategies and dispersal behaviors for rays, including natal philopatry (i.e. individuals reproducing in their exact birthplace) and regional philopatry (i.e. individuals reproducing in the same general area of their birth) as defined by Chapman et al. (2015; but see philopatry of rays reviewed in Flowers et al. 2016). Interestingly, recent research on sawfishes (Family Pristidae) has shown that this group of rays exhibits many of the same mating strategies as sharks, including female regional and natal philopatry to nursery grounds, male-biased dispersal, and multiple paternity (Phillips et al. 2011, 2017, Feutry et al. 2015, Feldheim et al. 2017, Green et al. 2018), informing the management strategies of these populations to preserve genetic variation. Since all 5 species of sawfish are considered Endangered or Critically Endangered by the International Union for the Conservation of Nature Red List of Threatened Species (IUCN Red List; Dulvy et al. 2016), it is important to understand their mating and reproductive strategies to identify populations, develop management actions, and conserve each species.

In the USA, one of the last global strongholds for the smalltooth sawfish *Pristis pectinata*, a single distinct population segment (DPS) was defined for the species when it was listed on the ESA in 2003 (68 FR 15674). Shortly after, in 2009, the National Marine Fisheries Service identified, designated, and protected 2 areas of critical habitat for juveniles: Charlotte Harbor Estuary Unit (CHEU) and Ten Thousand Islands/Everglades Unit (TTIEU; NMFS 2009, Norton et al. 2012). Both TTIEU and CHEU are located within the current core range of the species in southwestern Florida and are approximately 100 km apart (Fig. 1). Natural, coastal habitat in this part of Florida consists of mature mangrove forests (including red mangrove *Rhizophora mangle*, black mangrove *Avicennia germinans*, and white mangrove *Laguncularia racemosa*), oyster and seagrass beds, and sandy mangrove islands with shallow (<3 m) freshwater rivers, tidal creeks, and brackish bays. TTIEU is the larger of the 2 units (2505 km^2) and includes waters within Everglades National Park (ENP; including Florida Bay), Ten Thousand Islands Aquatic Preserve/National Wildlife Refuge (TTINWR), and a portion of Rookery Bay Aquatic Preserve (RBAP). Because this area is largely held in public ownership by the US Department of the Interior and is remote, TTIEU has very little anthropogenic habitat modification. In contrast, CHEU is flanked by the cities of Fort Myers and Charlotte Harbor, resulting in both modified habitat (e.g. seawalls, docks, and deep channels for shipping) and anthropogenic influence (e.g. heavy boat traffic, runoff, recreational activities). Nevertheless, CHEU contains 896 km^2 of essential nursery habitat for juvenile smalltooth sawfish, including the Caloosahatchee and Peace Rivers as well as the waters within Sanibel Island, Pine Island Sound, and Gasparilla Sound. Since listing, research has largely focused on juvenile smalltooth sawfish and their habitats in both CHEU and TTIEU (Brame et al. 2019), as adults are large, highly mobile, and difficult to capture, track, or observe returning to specific sites for mating or parturition. Nevertheless, researchers have been able to make some inferences about adults based on what has been learned from juveniles. For instance, Feldheim et al. (2017) reconstructed parental genotypes from juveniles captured in CHEU to determine mating strategy, litter size, and evidence of regional philopatry in adult female smalltooth sawfish. The authors concluded that female smalltooth sawfish mate with multiple males

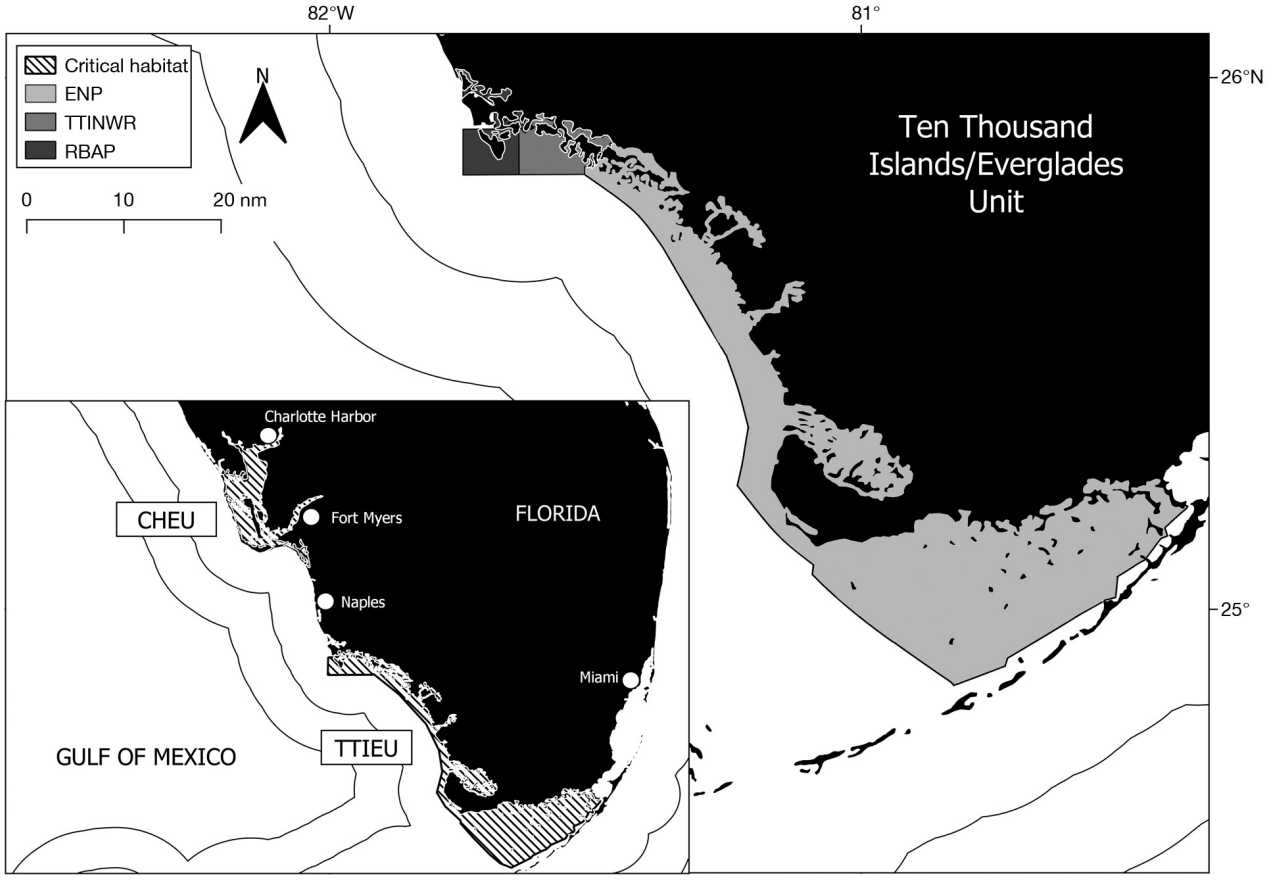

Fig. 1. Location of subareas within the Ten Thousand Island/Everglades Unit (TTIEU) of smalltooth sawfish critical habitat, including Everglades National Park (ENP), Ten Thousand Islands National Wildlife Refuge (TTINWR), and Rookery Bay Aquatic Preserve (RBAP). Inset shows TTIEU in relation to the Charlotte Harbor Estuary Unit (CHEU) in southwestern Florida

and return biennially to the same area in CHEU to give birth to pups (maximum of 8 pups assigned to a single female; Feldheim et al. 2017). However, these characteristics have not yet been examined in the second, larger area of designated critical habitat: the TTIEU.

Evaluating how female smalltooth sawfish use TTIEU is critical for confirming mating strategy, philopatry, and pups per female, and important for future studies estimating population size or regional gene flow, all of which are vital factors for expansion and recovery of this species. Therefore, we reconstructed parental genotypes from juvenile smalltooth sawfish captured in the TTIEU of designated critical habitat to: (1) determine frequency of parturition, mating behaviors, and number of pups associated with adult female smalltooth sawfish using TTIEU; (2) compare our findings with those in the CHEU; and (3) provide baseline information useful for future studies on recruitment and population structure as the population continues to expand and recover.

2. MATERIALS AND METHODS

2.1. Data collection

Within TTIEU, abundance surveys targeting smalltooth sawfish occurred during 2000–2015. In the early years of the survey (2000–2007), sampling occurred opportunistically throughout the year and in all areas of TTIEU (see Wiley & Simpfendorfer 2007). During 2008–2009, sampling occurred monthly in the northern parts of TTIEU, including TTINWR, RBAP, and northern parts of ENP near Chokoloskee Island in the spring and early summer (January to June; Fig. 1; Bethea et al. 2010). By 2010, sampling was occurring monthly (February to October) in all areas of TTIEU (Bethea et al. 2015). Sampling took place for 1 wk per month, but specific nursery sites were only sampled once per trip (e.g. in 2011, Grocery Creek, RBAP was sampled once per month from February to October). Over the years, the number of specific nursery sites sampled increased as we gained

knowledge of juvenile smalltooth sawfish habitat use, particularly in ENP — the largest area within TTIEU. For example, a tip from a local guide revealed that juvenile smalltooth sawfish were common on the west side of Chokoloskee Island (ENP), a site that had not been sampled prior to 2010. Over the course of 16 yr, we sampled random sites throughout the region plus as many as 2 specific nursery sites in RBAP, 4 in TTINWR, and 12 distributed throughout ENP for most months every year (February to October). Most often, individuals were captured with monofilament gillnets, but longlines or rod and reel baited with striped mullet *Mugil cephalus* were also used (Wiley & Simpfendorfer 2007, Bethea et al. 2015). Up to 4 gillnets were used per site, set perpendicular to the shoreline, and allowed to soak for 0.5 h with constant monitoring (Bethea et al. 2015). Because of the gear employed, areas sampled, and behavior of smalltooth sawfish, this survey design selects for juvenile smalltooth sawfish (<250 cm stretched total length; STL).

Upon capture, each individual was tagged internally and externally as well as sexed and measured (cm) following Bethea et al. (2015). The birth year for each juvenile captured was determined by comparing the STL at the month/year of capture to von Bertalanffy growth rates estimated from tag-recaptured juveniles ($K = 0.140^{-1}$; D. Bethea & J. Carlson unpubl. data) and aged vertebrae ($K = 0.219^{-1}$; Scharer et al. 2012). Lastly, a small tissue sample (<2 g) from the trailing edge of the second dorsal fin was removed for genetic analysis. Before release, gear was removed from the water to avoid immediate recapture, since released smalltooth sawfish tend to remain in the vicinity after capture (Simpfendorfer et al. 2010, Hollensead et al. 2016, 2018). We suspected that groups of similarly sized juvenile smalltooth sawfish caught at the same site within the same month would be littermates, since individuals <250 cm STL have small activity spaces (0.08 to 0.68 km^2 based on 95% kernel density estimates) and tend to remain in nursery areas for up to 3 yr (Hollensead et al. 2016, 2018). Based on the finding of female regional philopatry at CHEU (Feldheim et al. 2017), we also assumed that related individuals would be present at sites consistently harboring juveniles over the years (i.e. maternally related half-siblings across years).

2.2. Genetic analyses

Tissue samples were stored in 95% ethanol until DNA extraction, which followed manufacturer protocols in the DNeasy Blood and Tissue kit (Qiagen). We used 17 polymorphic microsatellite loci (Feldheim et al. 2010, Fields et al. 2015) using cycling parameters and genotyping methods developed in previous studies (Feldheim et al. 2010, Maddox & Feldheim 2014, Fields et al. 2015). Duplicate genotypes indicating a genetic recapture were removed from the dataset, and the remaining genotypes were evaluated for allelic dropout, stuttering, and null alleles with Micro-Checker v. 2.2.3 (van Oosterhout et al. 2004). We did not assess genetic diversity here because others have evaluated it for the DPS (see Chapman et al. 2011) and for CHEU (Poulakis et al. 2014, Feldheim et al. 2017).

Putative siblings were determined using COLONY v.2.0 (Wang 2004, Jones & Wang 2010) and our approach followed that taken by Feldheim et al. (2017). Briefly, 3 COLONY runs were made in total: one assuming male monogamy and female polyandry, a second replicate run of the first to verify sibling relationships, and a final run with both sexes being polygamous that could refute the first and second runs. All runs assumed no clones in the dataset and a 0.5% genotyping error rate. Pairwise comparisons of juvenile genotypes were used to determine sibship: a set of full siblings had to have a maximum of 4 alleles in common across all loci, while a set of half-siblings had to have 5 or more alleles in common for 3 or more loci (Feldheim et al. 2004). While some relationships are uncertain with this method, we assumed any half-siblings would be maternally related because (1) female *Pristis pectinata* in CHEU display a polyandrous mating strategy (Feldheim et al. 2017); (2) female sawfishes tend to display regional philopatry in other locations (i.e. Phillips et al. 2011, 2017, Feutry et al. 2015, Feldheim et al. 2017); and (3) female smalltooth sawfish have a biennial reproductive cycle resulting in related half-siblings every other year (Feldheim et al. 2017). These assumptions do not preclude encountering paternally related half-sibs (see following paragraph). Without complete sampling of each litter (e.g. sacrificing a pregnant female, witnessing live birth in its entirety), it is difficult to determine parental and sibling relationships with complete certainty.

Once sibling relationships were established, parental genotypes were reconstructed, first based on the COLONY output, and if necessary, by hand, following Feldheim et al. (2004). For each reconstructed female, we recorded year(s) of parturition, the number of pups per litter, and the number of sires contributing to each litter. We assumed pup capture location to be proximal to the parturition location,

since juvenile sawfish tend to have a limited home range (Hollensead et al. 2016). Due to these small home ranges and low likelihood of pups moving between parturition sites (Hollensead et al. 2016, 2018), we also assumed that any half-siblings born in the same year at different parturition locations (e.g. RBAP and ENP) were paternally related (i.e. the same male mated with 2 different females that gave birth in 2 different locations). To determine if individuals were using both CHEU and TTIEU, we compared both male and female reconstructed genotypes to the reconstructed parental genotypes from Feldheim et al. (2017). Lastly, we removed any reconstructed parental genotypes based on the assignment of only 1 or 2 pups, since parental genotypes and half-sibship are difficult to assess with only 2 pups.

3. RESULTS

Over 16 yr, tissue samples were collected from 310 juvenile smalltooth sawfish throughout TTIEU: n = 59 (2000–2007); n = 40 (2008–2009); and n = 211 (2010–2015). Most samples were collected from ENP (>250), especially after 2011, while roughly 25 individuals were sampled in both TTINWR and RBAP. Because juveniles remain in nursery areas for up to 3 yr and have the ability to overwinter in TTIEU (Hollensead et al. 2018), individuals that were similar in size were often caught together. We discovered 3 duplicate genotypes in the dataset from individuals that had been recaptured and removed them from further analysis, leaving 307 juvenile genotypes. After removing family groups with ≤2 pups, a total of 214 genotypes were used to reconstruct parental genotypes; 4 of these 214 genotypes were discovered (*a posteriori*) to be pups born and caught in CHEU, which we left in subsequent analyses as a negative test for regional philopatry (i.e. if pups caught in TTIEU have regionally philopatric mothers, then they should not be maternally related to any pups from CHEU; Table 1). For the majority of the reconstructed family groups (74%; n = 37), all 3 runs were in agreement, and in the remaining cases (n = 13), the first and second runs agreed (i.e. male monogamy and female polyandry). Comparisons of all results to those of Feldheim et al. (2017) are summarized in Table 2.

The 50 reconstructed family groups were comprised of 71 female and 117 male reconstructed genotypes and 138 litters. Of the 71 females using TTIEU during the study period (2000–2015), we detected 44 that gave birth once, 24 that gave birth more than once exhibiting regional philopatry, and 3 that gave birth more than once but changed parturition locations (Female#31, Female#89, and Female#9). Overall, regionally philopatric females returned to the same area within TTIEU for parturition, and in some cases, to the same mud flat. Female#66, for instance, gave birth in 2013 to at least 5 pups in Chokoloskee Bay (ENP) and returned to the same bay in 2015 to give birth to at least 1 pup. Within TTIEU, we detected 21 females that exclusively used ENP for par-

Table 1. Number of juvenile smalltooth sawfish sampled and genotyped in each area of the Ten Thousand Islands/Everglades Unit of critical habitat during 2000–2015: Everglades National Park (ENP), Ten Thousand Islands National Wildlife Refuge (TTINWR), and Rookery Bay Aquatic Preserve (RBAP). Note the additional 4 genotypes from the Charlotte Harbor Estuary Unit (CHEU) from 2005. Gaps indicate no data

Year	ENP	TTINWR	RBAP	CHEU	Total
2000	2				2
2001	3				3
2002	3				3
2003		5			5
2004	2				2
2005	5	4		4	13
2006	5		5		10
2007	13	1			14
2008	17		4		21
2009	4	1			5
2010	4	2	1		7
2011	32	3	6		41
2012	28	2			30
2013	16	1			17
2014	28	1			29
2015	9	3			12
Total	171	23	16	4	214

Table 2. Comparison of results from this study and Feldheim et al. (2017), including numbers of samples used in COLONY, numbers of reconstructed males and females, number of litters, maximum number of pups assigned to a single female, mating strategy, parturition frequency, and evidence of philopatry to nursery areas

	Feldheim et al. (2017)	This study
Tissue samples collected	349	307
Reconstructed females	55	71
Reconstructed males	192	117
Number of litters	142	138
Max. no. of pups	8	12
Mating strategy	Polyandry	Polyandry
Parturition frequency	Biennial	Biennial
Regional philopatry?	Yes	Yes

turition (Fig. 2), some as many as 4 times over the 16 yr study period, including 3 females that were loyal to TTINWR and none to RBAP. However, 1 female changed parturition areas within TTIEU in sequential breeding cycles: Female#31 used ENP in 2011, but gave birth in TTINWR in 2013. Two females were found to use both TTIEU and CHEU for parturition. In 2003, Female#89 gave birth to 3 pups in Faka Union Bay (TTIEU), but in her next reproductive cycle (2005) gave birth to at least 1 pup in the Peace River in CHEU. Similarly, Female#9 gave birth in TTIEU in 1999 to at least 1 pup, but in 2005 gave birth in CHEU to 2 pups. In addition to these 2 females, we detected 5 males that sired litters in both TTIEU and CHEU.

Regardless of philopatric behavior, most females used ENP for parturition (n = 51, 72%), while fewer used TTINWR (n = 11, 15%) and RBAP (n = 5, 7%), producing litters from 1998–2015 (Fig. 3). Within the bounds of ENP, we detected 9 females using the area for parturition in 2011 (29 pups total), 10 females in 2012 (28 pups total), and 18 females in 2014 (28 pups total). The number of pups genetically assigned to a single litter ranged from 1 to 12, but the majority of litters (n = 96) had 1 to 3 pups genetically assigned. Most of the identified litters were produced by females that mated with a single male within a breeding cycle (n = 44, 62% of females). Fifteen females exhibited serial monogamy across years, mating with a different male each breeding cycle, producing full-siblings within years and maternal half-siblings across years. The remaining females (n = 12) exhibited polyandry within a year, mating with multiple males that resulted in maternal half-siblings within a litter. This polyandrous behavior was further confirmed by 2 male genotypes associated with multiple

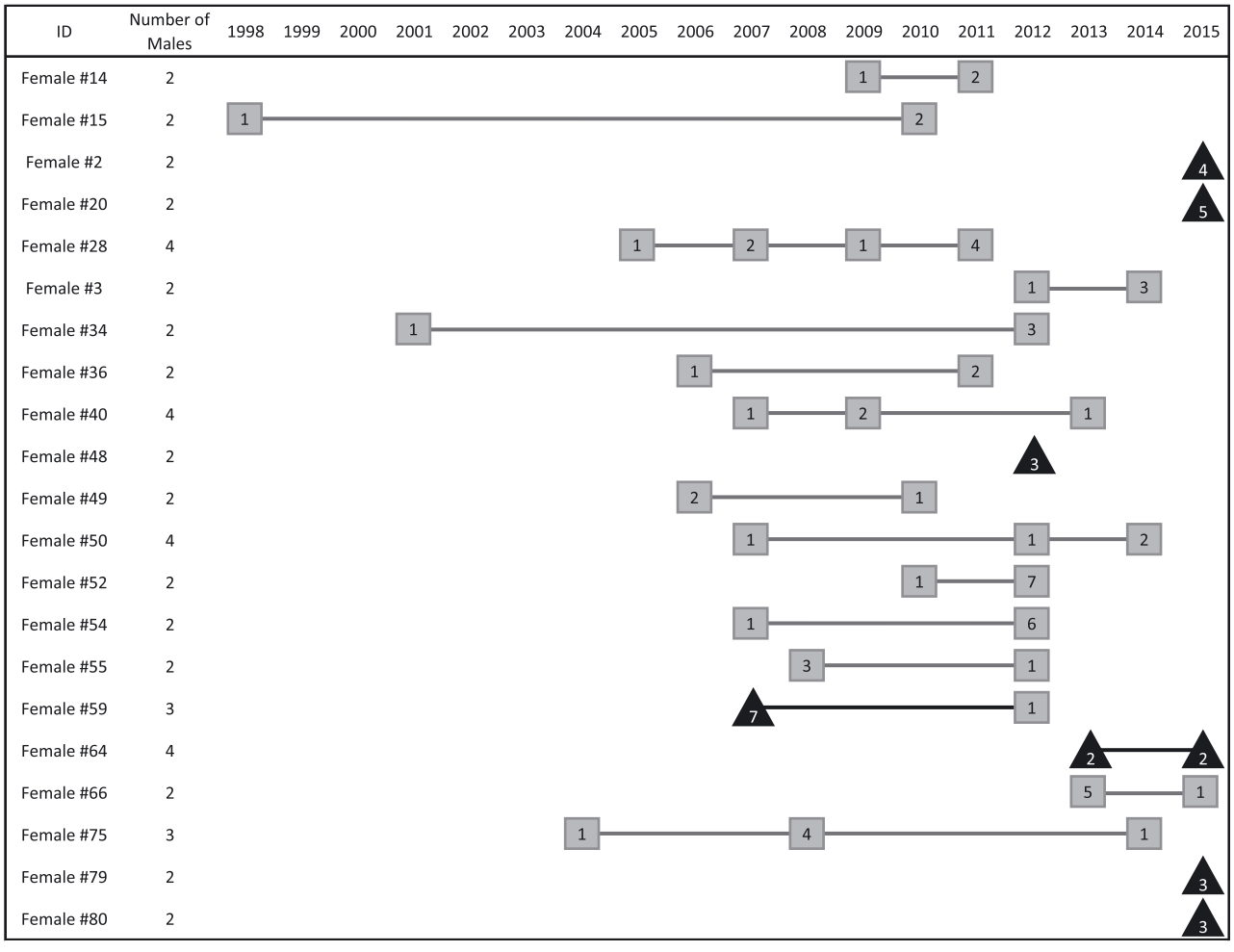

Fig. 2. Number of female smalltooth sawfish (n = 21) exclusively using ENP for parturition during 1998–2015. The number of pups produced by females mating with different males between years (■) or displaying polyandry within years (▲) is given inside each symbol

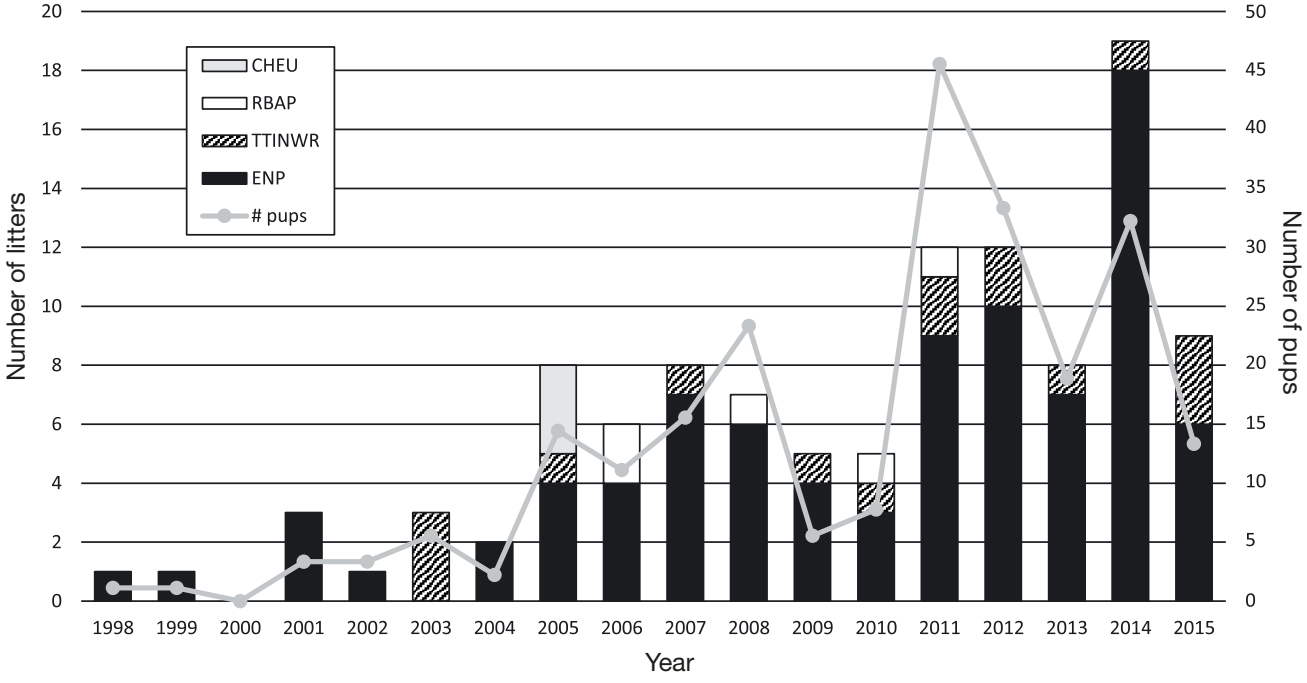

Fig. 3. Number of litters and pups produced by 71 female smalltooth sawfish using TTIEU for parturition from 1998–2015. Bars represent the number of litters detected (left axis) each year by area within TTIEU: ENP, TTINWR, and RBAP. An additional 3 litters were detected from CHEU in 2005. The grey line (right axis) represents the number of pups detected from those litters in each year. In the most recent year (2015) we detected 13 pups, representing 9 litters from 2 areas (ENP and TTINWR). See Fig. 1 legend for definition of abbreviations

litters from different females. For example, Male#111 mated with Female#48 in 2012 to produce a litter and later mated with Female#64 in 2015 to produce another litter. There was 1 case of a female mating with the same male across breeding seasons: Female#75 mated with Male#106 in 2008 and again in 2014.

In the course of examining COLONY results, 13 reconstructed genotypes, originally identified as female, were re-classified as males based on recorded year(s) and locations of parturition and species' life history information. For smalltooth sawfish, it would be atypical for a single female to give birth to 2 separate litters of full siblings in different locations sired by different males in the same year simply due to energy costs and logistics. The more parsimonious explanation is that a single male mated with multiple females at a mating aggregation site, and those females gave birth in different areas within the same year. Given this logic, these 13 parental genotypes were assigned as male and the siblings were considered paternally related. These 13 males each mated with 2 to 3 different females in a single year or over successive years, resulting in 49 pups. One male had a 10 yr gap between litters, mating with Female#46 in 2005 and Female#115 in 2015. Most of the females associated with these males gave birth in ENP (n = 22), but 1 male mated with 3 different females that gave birth in 3 different areas (ENP, TTINWR, and CHEU).

4. DISCUSSION

4.1. Biological findings

4.1.1. Regional philopatry

We found that female smalltooth sawfish (n = 24 reconstructed genotypes) were regionally philopatric to the TTIEU for parturition, returning to the same location (e.g. Chokoloskee Bay) or area (e.g. ENP) to give birth, a result consistent with CHEU smalltooth sawfish (Feldheim et al. 2017). However, unlike CHEU smalltooth sawfish, which were equally philopatric to 2 main areas (Feldheim et al. 2017), we found that females using TTIEU for parturition only consistently used areas within the bounds of ENP, despite suitable habitat availability in TTINWR and RBAP. While ENP is the largest of the 3 areas within TTIEU and probably supports more pups (as suggested by the high number of samples we collected from ENP vs.

TTINWR or RBAP), it is unclear why a female smalltooth sawfish would preferentially give birth in one area and not the other given that boundary lines are arbitrary and habitat is similar. Instead, we suggest that unequal sampling effort or lack of knowledge about specific parturition sites between the 3 areas (i.e. several parturition sites are known in ENP, but only one is known in RBAP; Table 1) could be causing the differences observed in female philopatry. Furthermore, identifying philopatric behavior with these methods is dependent upon half-siblings being identified across years, so if fewer pups are sampled in TTINWR or RBAP, it limits verifiable evidence of the behavior in those areas. Additionally, sibship analyses can demonstrate deviations in philopatric behavior: we identified 1 female that shifted between specific nursery areas within TTIEU to give birth (i.e. ENP to TTINWR), something that <4% (2 of 55) of smalltooth sawfish also did in CHEU (Feldheim et al. 2017). The reasons for switching sites are only speculative, but could include some combination of favorable environmental conditions in a particular year or the larger amount of suitable nursery habitat found in TTIEU (2505 km^2) versus CHEU (896 km^2).

Based on sibship, we found evidence of 2 female smalltooth sawfish that used both CHEU and TTIEU for parturition. While 1 of these females was a genetic match between the 2 critical habitat units, the reconstructed genotype of the other female was not. This second female may not have genetically matched for a number of reasons: (1) there were unidentified errors in pup genotyping and/or in reconstruction for this female preventing an exact match (although the first female matched with the same methods); (2) this female could be a misidentified male (as described in Section 3.); (3) there could be a misidentification in the full and half-sibling relationships which would hinder accurate genotype reconstruction (although all 3 COLONY runs agreed on these relationships); or (4) this particular female has no parturition habitat preference and may not have displayed philopatric behavior. Unfortunately, with no samples from pups in subsequent years (i.e. after 2005), it is difficult to know with certainty the habits of this second female. However, the documentation of the potential for females to shift between multiple parturition sites is an important biological factor to consider, especially because movement between the 2 areas by larger adults is possible (Carlson et al. 2014).

Most philopatry studies for elasmobranchs are focused on shark species and there are far fewer studies for batoids, though philopatric and seasonal residency research for sawfishes may be the exception (Flowers et al. 2016). For example, female largetooth sawfish *Pristis pristis* are at least regionally philopatric (i.e. return to their general birthplace region for parturition; Chapman et al. 2015), based on analysis of mtDNA and population structure (Phillips et al. 2011), but will return to their natal rivers to give birth if there are no barriers (i.e. natally philopatric; Chapman et al. 2015, Feutry et al. 2015). Results from the present study and Feldheim et al. (2017) show regional philopatry by female smalltooth sawfish, but determining if the species is natally philopatric will require considerable sampling effort of all life stages (tag-recapture, acoustic monitoring, genetic recapture, etc.) over at least a decade based on current estimates of maturity (Brame et al. 2019). Ultimately, philopatric behavior, whether natal or regional, stems from the increased juvenile survival gained from giving birth in an area with known resources (e.g. protection from predators and high prey availability; Cortés 2002, Heupel et al. 2007), a strategy that will play a role in the recovery of this elasmobranch.

4.1.2. Reproductive cycle

We also found evidence of a biennial reproductive cycle in female smalltooth sawfish, as reported by Poulakis et al. (2014) and Feldheim et al. (2017). Although we could not assign pups to every female every reproductive cycle, we were able to identify many females that did follow this pattern, including 1 female that had litters in 2005, 2007, 2009, and 2011 (Fig. 2). Determining parturition frequency with sibship analysis is largely dependent on estimated birth year from captured juveniles; while it is more difficult to estimate birth year for older individuals (R. Scharer pers. comm.) due to the paucity of growth rate data to make estimates (Scharer et al. 2012, Brame et al. 2019), all but 4 of the smalltooth sawfish sampled herein were <250 cm STL, a size and age for which there is the most growth data (Simpfendorfer et al. 2008, Scharer et al. 2012, D. Bethea & J. Carlson unpubl. data). Biennial reproductive cycles presumably allow the female sufficient time to go through vitellogenesis, egg maturation, and ovulation before the next mating event and subsequent gestation (Pratt & Carrier 2001, Carrier et al. 2004). However, under favorable environmental conditions, it is possible that some female smalltooth sawfish are capable of or have even evolved annual reproduction, given that this has been observed in other elasmobranchs. For example, the leopard shark *Triakis semifasciata*

exhibits an annual reproductive cycle, which may be influenced by warm, shallow bays where mature females aggregate throughout the year (Smith & Abramson 1990, Hight & Lowe 2007). Similarly, captive smalltooth sawfish are physiologically capable of annual reproduction (Flowers et al. 2020); however, additional data and analyses would be required to confirm this in wild individuals.

The maximum number of pups genetically assigned to 1 litter in TTIEU was 12, a number that is consistent with that found for CHEU (8 pups) using the same methods, and within the range of litter sizes reported (7 to 14 pups per litter; Feldheim et al. 2017, Brame et al. 2019, J. Gelsleichter & G. Poulakis pers. comm.). No gravid females or birthing events were observed during sample collection, and only a few juveniles were observed with a sheath on their rostrum or open umbilical scar (indicating a neonate), thus we do not assume to have sampled all potential littermates. Rather, we report general trends in the reproductive cycle and number of pups per female, features that are consistent with other aplacental viviparous (embryos nourished internally by a yolk) elasmobranchs (Carrier et al. 2004).

4.1.3. Mating strategy

Some TTIEU smalltooth sawfish exhibited a polyandrous mating strategy that is typical of many elasmobranchs (Pratt & Carrier 2001, Carrier et al. 2004). Polyandry was evident when we assumed half-siblings were maternally related since most females were assigned multiple males for their litters. Additionally, we rarely identified the same male genotypes over the 16 yr study period, a finding that is consistent with that of CHEU smalltooth sawfish (Feldheim et al. 2017). As with many elasmobranchs, mating has not been observed for this species, so evidence of genetic polyandry (i.e. litters sired by multiple males) is important for determining how mating strategy affects other factors like genetic variation. For instance, females may be able to increase the genetic diversity of their offspring by mating with multiple males (but see DiBattista et al. 2008); however, more studies are needed to determine whether or not this potential benefit outweighs the potential risks (e.g. exposure to parasites, sexually transmitted diseases) associated with polyandry. In general, none of the few studies that have tried to determine the benefits of polyandry in elasmobranchs (Portnoy et al. 2007, DiBattista et al. 2008) have identified direct or indirect benefits to females. As a result, convenience polyandry, where females allow mating attempts by multiple aggressive males, is often invoked to explain polyandry in elasmobranchs (Griffiths et al. 2012, Marino et al. 2015, Barker et al. 2019).

Overall, monogamy is rare in elasmobranchs, as most species studied exhibit polygamous mating strategies (Pratt & Carrier 2001), but we found relatively few incidents of polyandry in TTIEU compared to CHEU (Feldheim et al. 2017). The seemingly monogamous pairings detected in TTIEU could be caused by one of several scenarios. First, any incomplete sampling of litters resulting in a lack of evidence for polyandry does not confirm monogamy, especially since complete sampling of elasmobranch litters is rare and difficult without sacrificing pregnant females (e.g. Lyons et al. 2017). Second, it is possible that females would only have the opportunity to mate with 1 male in a particular reproductive cycle if there were a paucity of mature males present in the population, a hypothesis that has also been proposed for the parthenogenesis observed in the species (Fields et al. 2015). Third, it could be that females are actually mating with multiple males, but because of sperm competition or cryptic female choice, only a single male ends up siring all the offspring in a litter (Andersson & Simmons 2006, Portnoy & Heist 2012, Lyons et al. 2017).

4.1.4. COLONY caveats and reconstructed parental genotype outliers

Although COLONY tends to inflate the number of parents, particularly males, reconstructed from offspring genotypes, we mitigated this by using 17 polymorphic loci (Neff & Pitcher 2002, Sefc & Koblmuller 2009, Wang 2018). In addition, the relatively large litter sizes of smalltooth sawfish compared to other rays allow for better detection of multiple sires (Jones et al. 2010, Portnoy & Heist 2012, Wang 2018). We identified fewer males than Feldheim et al. (2017) despite having similar juvenile sample sizes (Table 2). This could be because we removed almost a third of the pup genotypes and their associated reconstructed parental genotypes from analysis due to high uncertainty in the parental reconstructed genotypes and half-sibship relationships. While the removal of these family groups does directly reduce the number of reconstructed male genotypes, we hypothesize that incomplete sampling of litters (i.e. only finding 1 or 2 pups from a litter) is more common in TTIEU due to a larger number of potential parturition sites (TTIEU

is 3 times the size of CHEU). Because of this difference in area and potential for incomplete sampling, far more samples are removed, which artificially lowers the number of males associated with these nursery areas. Since the number of reconstructed parental genotypes is dependent on analysis of juvenile genotypes (>3 related pups needed to reliably reconstruct parental genotypes), it is important that juvenile monitoring, including the collection of genetic data, continues in TTIEU to further understand these relationships (e.g. the number of males siring each litter).

We identified 13 reconstructed parental genotypes that were incorrectly assigned sexes by COLONY and were inconsistent with the original assumption that any half-siblings would be maternally related. These corrections were made possible based on our knowledge of the biology of female smalltooth sawfish, including polyandry, philopatry, and biennial reproduction. We determined that half-siblings could not be maternally related based on estimated birth year and parturition location of the pups. For instance, in the original COLONY output, 'Female#50' mated with 2 different males to produce a litter in RBAP and ENP all within the spring of 2006, but this scenario is inconsistent with the patterns observed and family groups constructed in both this study and Feldheim et al. (2017). In this example, it would be more likely that the pups from these litters are paternally related and that 'Female#50' is actually a male mating with multiple females within the same year, thus this individual was reassigned to Male#50 in our pedigree. If a paternal relationship did not make biological sense, and there were no user errors in operating COLONY, then these 13 relationships would have been more dubious. However, we were confident in switching the sexes of the parental genotypes in these 13 cases because the adult genotypes and their associated litters were consistent with the patterns observed in the remaining reconstructed female genotypes (n = 71) and other studies (i.e. Feldheim et al. 2017). In addition, we are confident about the accuracy of the remaining reconstructed female genotypes (n = 71) because of the high agreement between COLONY runs assuming polyandry. Because reconstructed parental genotypes are based solely on sampled offspring genotypes, more complete sampling of litters would verify these relationships.

Overall, the sampling design for the system and the number of polymorphic loci used in this study make it ideal for reconstructing parental genotypes (Jones et al. 2010); however, samples from adults will lend further insight regarding the frequency of parturition, any age-associated effects, or number of males mating with each female, since offspring genotypes could be directly assigned to specific adults based on their genotypes. Ultimately, inclusion of genotypes from adults captured in the field will be useful for confirming mating strategy and behaviors found in both critical habitat areas.

4.2. Conservation and management implications

This study contributes to the growing evidence that female smalltooth sawfish are regionally philopatric (as defined by Chapman et al. 2015) and reproduce biennially, while male smalltooth sawfish, like other sawfishes (e.g. *P. pristis*, Phillips et al. 2017; *Anoxypristis cuspidata*, Green et al. 2018), tend to disperse and are responsible for gene flow across a broader region. Philopatric behavior has important management implications, specifically, the nursery areas within the 2 critical habitat units will maintain the most individuals and genetic diversity for the population if they are managed separately, since specific females use each unit exclusively. This exclusivity is a behavior that was not considered when the DPS was originally defined (68 FR 15674) or when the DPS was evaluated for genetic structure in subsequent studies (i.e. Chapman et al. 2011, Poulakis et al. 2014), which assumed equal gene flow by both sexes. Since males are likely facilitating the gene flow (and therefore lack of structure) in this DPS, we suggest that future studies account for female regional philopatry in any evaluation of population structure for this species.

Given that males are likely responsible for most of the gene flow in this DPS, it is also critical that mating aggregation sites be identified and protected. Mating aggregation events, depending on where and what time of year they occur, could disproportionally put larger individuals at risk for fishing mortality, potentially resulting in long-lasting effects on genetic diversity, effective population size, and population growth. Effective management of mating aggregation sites may include reducing or eliminating fishing either seasonally, geographically, or both. Future sibship or mtDNA (i.e. female-mediated gene flow) analyses that examine additional genotypes from multiple geographic areas simultaneously will help to clarify how often and to what extent female smalltooth sawfish adjust their parturition locations and how genes are contributed to each area.

Differences in environmental cues from each area could be influencing behaviors among individual females, since CHEU and TTIEU are disparate in both geographical size and anthropogenic influence. For instance, the flow of the Caloosahatchee River in CHEU is highly managed by the Southwest Florida Water Management District (Stoker 1992), and physicochemical changes in the water affect juvenile smalltooth sawfish movement in the river (Poulakis et al. 2013). This water management may also explain why parturition peaks from April to May in CHEU (increased freshwater flow from Lake Okeechobee; Poulakis et al. 2011, 2013), but occurs over a longer period within TTIEU (March to July; Bethea et al. 2015), perhaps due to a more natural flow regime in the system. Continued research throughout TTIEU is needed to fully understand under what conditions females are using this habitat: this should take the form of both long-term surveys in ENP that track known parturition sites and expanded efforts in TTINWR and RBAP that identify other parturition sites. As with philopatric behavior, these physical differences may necessitate different management and conservation strategies to preserve unique individuals in each unit, and consequently, the genetic diversity in the population as a whole.

The results from this study can be applied to 2 goals outlined in the Smalltooth Sawfish Recovery Plan by NMFS (2009). One of these goals is to protect and restore habitat, especially for juveniles. With this study, we confirm that habitat within TTIEU should continue to be protected for both juvenile and adult life stages, as juveniles use the area for growth and shelter, while females move in and out of the area to give birth. Restoration of habitat between the 2 units could be important for preserving any genetic connection between them, since we show that adult females move at least occasionally between these 2 areas. In addition, any mature individuals (male or female) exiting these nursery areas en route to a mating aggregation site (e.g. Florida Bay; Papastamatiou et al. 2015) may benefit from habitat restoration between the 2 units.

A second Recovery Plan goal is to restore the species to previously occupied areas. In this case, our study further clarifies how adult female smalltooth sawfish use coastal areas, which informs if and how adjacent areas might be accessible for reoccupation. Since females are regionally philopatric, expansion of the population by males will not necessarily result in the successful establishment of the species in previously occupied areas. Instead, population expansion may depend on other factors: one possibility may be population density, if females disperse to new parturition sites when currently occupied areas reach carrying capacity. This study did find evidence of a few females changing parturition sites (although philopatric behavior was most common), which demonstrates that the dispersal of females to new areas for parturition is possible and may further indicate that protected areas like TTIEU and CHEU are close to carrying capacity, though more formal studies are required. Given the utility of juvenile genotypes in answering questions about adult life stages, this study reflects the need for continued juvenile monitoring within the critical habitat units and identification of any other areas occupied by juveniles (e.g. Indian River Lagoon or Tampa Bay), since juvenile survivorship is ultimately responsible for species persistence.

This study provides much needed complementary information for a second, larger area of designated critical habitat for smalltooth sawfish in southwestern Florida, which is not only useful for understanding the current DPS, but also for providing baseline information critical for evaluation of other potential DPSs should natural population expansion occur. Characteristics uncovered by this study and Feldheim et al. (2017), including mating strategy, philopatry, and number of pups genetically assigned per female, can be combined with other information on genetic diversity, effective population size, and juvenile survivorship to provide the foundation for understanding how an imperiled population can recover and expand effectively (Simpfendorfer 2000, Cortés 2002, Frankham 2005). Monitoring these aspects in conjunction and over time will be necessary to determine whether the US DPS of smalltooth sawfish is capable of growth in abundance and/or spatially and how best to facilitate an expansion that will result in long-lasting effects.

Acknowledgements. The authors thank C. Simpfendorfer for his foundational research on smalltooth sawfish in southwestern Florida. Immense gratitude is due to the multitude of interns and volunteers who supported this project over 16+ yr; your hard work did not go unnoticed. This project would not be possible without the dedication and fearless leadership of D.M. Bethea — thank you for your continued mentorship. Thank you to R.M. Scharer and G.R. Poulakis for help with determining birth year for tricky samples and B.M. Deacy for edits and helpful conversations. Thank you to T. Iandimarino, A. Terry, and all of the park rangers at the Everglades National Park Gulf Coast Visitors Center in Everglades City, FL and the Flamingo Visitor Center in Homestead, FL. This research was performed under National Marine Fisheries Service Endangered Species Act permit numbers 1352, 13330-01, 13330-02, and 17787; and Everglades National Park Scientific Research and Collect-

ing permit numbers 1999-130, 2001-0001, 2002-0006, 2003-0018, 2004-0030, 2005-0032, 2007-0039, 2009-0002, 2010-0007, 2011-0010, 2012-0008, 2013-0008, 2014-0009, and 2015-0002. The scientific results and conclusions, as well as any views or opinions expressed herein, are those of the authors and do not necessarily reflect those of their institutions. Special thanks to A. Brame and S. Norton at NOAA SERO for providing funding over the years. This material is based upon work that is supported by the National Institute of Food and Agriculture, US Department of Agriculture, McIntire Stennis program.

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Editorial responsibility: Brendan Godley, University of Exeter, Cornwall Campus, UK
Reviewed by: 5 anonymous referees

Submitted: July 30, 2020
Accepted: March 1, 2021
Proofs received from author(s): May 13, 2021