LIFE HISTORY ECOLOGY OF THE SHARKSUCKER, ECHENEIS NAUCRATES, IN THE GULF OF MEXICO

by

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Bachelor of Science
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DEDICATION

Dedicated to René.

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ABSTRACT

LIFE HISTORY ECOLOGY OF THE SHARKSUCKER, ECHENEIS NAUCRATES, IN THE GULF OF MEXICO

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The purpose of this investigation was to understand how symbiotic sharksucker-host interactions may have shaped life history characteristics of the symbiont. Here, I examined growth, trophic ecology, and reproduction of Sharksucker, *Echeneis naucrates*, in the northeastern Gulf of Mexico. Age was determined from otoliths, and growth (in standard length, SL) was modeled as a von Bertalanffy function. Males and females grew at similar rates (K = 0.54 and 0.51 year^{-1}, respectively), but asymptotic length was significantly higher in females (L_∞ = 514 mm) than males (L_∞ = 445 mm). Diet varied by size group. Ectoparasitic copepods and other small crustaceans were the most frequently occurring item (78%) in the stomachs of small (<249 mm SL) sharksuckers, and fish was the second most frequent item (46%). Large sharksuckers consumed crustaceans less frequently (31%) and fish more frequently (60%). Crustaceans comprised a higher mean proportion of volume (MV) of small sharksucker diet (54% vs.

13%), and MV of fish was lower (15% vs. 32%). Sharksucker stable isotope N and C values exhibited significant trends with body size by location. Small (<249 mm SL) sharksuckers were enriched in $\delta^{13}C$ and depleted in $\delta^{15}N$. Further, those from the Big Bend area were depleted in $\delta^{13}C$ and $\delta^{15}N$ relative to samples from the Florida Keys. Gonad histology indicated that sharksucker are indeterminate batch spawners with a peak reproductive period in the late summer in the northeast Gulf of Mexico. Gonadosomatic indices peaked for both male and female sharksuckers in August. Mean relative batch fecundities estimated from hydrated oocytes was 42.0 hydrated oocytes per gram ovary-free body weight (OFBW). Due to unique life history adaptations, this symbiotic species appears particularly vulnerable to host availability during critical life stages. Thus, the conservation status of host species (i.e., sharks), which are in decline in many regions, is intimately tied to the population status of the sharksucker.

INTRODUCTION

The sharksucker, *Echeneis naucrates,* is a wide-ranging, moderately sized remora with a cosmopolitan distribution that inhabits predominantly shallow temperate and warm waters. Remoras engage in interspecific symbiotic relationships with other vertebrates through physical attachment to a host by means of a cephalic sucking disc. The cephalic disc is a unique adaptation of the Echeneidae and is presumed to supply multiple fitness benefits such as transportation, access to novel food sources, exposure to reproductive partners, and protection from predation. The sucking disc and lack of an air bladder exhibited by the members of the Echeneidae are believed to be adaptations towards enabling both facultative and obligate symbiotic life histories. Echeneid phylogeny is currently under review and of particular interest is the question of whether obligate or facultative symbioses are the more derived character. Clarification of the interspecific impacts of symbiotic echeneids adds to the understanding of elasmobranch ecology. It is unknown whether population declines in elasmobranch species impact echeneid symbiont populations and if so, in what way. A deeper understanding of the life history of echeneids and of the nature of the sharksucker-carcharhinid relationship will help in clarifying the level of interdependence of these taxa.

Many elasmobranch species are heavily exploited with several stocks classified as overfished. These fishing removals may affect symbiotic species that are not direct

targets of the fishery (Musick et al. 2000; Cortes et al. 2002; Brewster-Geisz 2005). Key issues for echeneids are:

(1) A need to understand the nature of the symbiosis to know how harvesting of elasmobranch species may affect sharksuckers and in turn how this may affect elasmobranch population ecology. It is uncertain whether sharksucker-elasmobranch symbioses are mutualistic, commensal, or parasitic.

(2) The unique hitchhiking relationship between echeneids and host species suggests multiple possible constraints on sharksucker life history, including limited mating opportunities, restricted spawning times and locations, rapid development and ontogenetic growth, host availability bottlenecks, and size limits for effective hitchhiking.

Phylogeny

The sharksucker is a wide ranging teleost of predominantly shallow temperate and warm waters in the remora family Echeneidae (Cressey and Lachner 1970; O'Toole 2002). Echeneidae is a family of fishes with a shared characteristic of a first dorsal fin that has been modified into a sucking disc. This allows them to attach to other organisms such as elasmobranchs, cetaceans, sea turtles, and bony fishes in order to gain transportation, probable access to novel food supplies, and possible protection from predation (Cressey and Lachner 1970; O'Toole 2002; Gray et al. 2009). The *Echeneis* genus contains *Echeneis naucrates* and *E. neucratoides*, although there remains some question as to whether *E. naucrates* and *E. neucratoides* truly represent separate species or are simply conspecifics that display phenotypic variations (Gray et al. 2009).

Phtheirichthys is composed solely of *Phtheirichthys lineatus*. Together the *Echeneis* species and *P. lineatus* make up the Echeneiinae (O'Toole 2002). *Remora albescens* (previously *Remorina albescens*), *Remora australis*, *Remora brachyptera*, *Remora osteochir*, and *Remora remora* comprise the *Remora* genus and make up the Remorinae. Echeneidae is a subset of Echeneoidea that also contains the families Rachycentridae and Coryphaenidae (O'Toole 2002; Gray 2005; Gray et al. 2009). Evolutionary relationships among the three families and within Echeneidae itself are incompletely resolved; however, the fishes in Rachycentridae and Coryphaenidae are considered the sharksuckers closest non-echeneid relatives (O'Toole 2002; Gray et al. 2009). Cobia (*Rachycentron canadum*) is the sole species in the Rachycentridae while Coryphaenidae consists of the two species of dolphinfish, *Coryphaena equiselis* (Pompano Dolphin) and *Coryphaena hippurus* (Dolphinfish or Mahi-mahi) (O'Toole 2002; Rocha-Olivares and Chávez-González 2008). Cobia and the dolphinfishes are large piscivores with rapid growth rates, adaptations that are considered incompatible with the development of symbiotic life histories like those displayed by their close echeneid relatives. However, cobia are frequently found in close association with skates and rays; though the dolphinfishes are not known to associate with elasmobranchs, they are commonly attracted to floating objects (O'Toole 2002; Gray et al. 2009). It has been suggested that these behaviors may illustrate the steps toward developing eventual symbiotic relationships (Gray et al. 2009). Ecological constraints on organisms engaged in symbiotic relationships likely contribute to life-history variations in the ecology of echeneids versus cobia and the dolphinfishes. Obvious morphological adaptations of the

echeneids related to their symbiotic lifestyle, such as the sucking disc, lack of a swim bladder, and smaller overall sizes, are likely to be accompanied by other significant life history adaptations in growth, diet, and reproduction.

Symbioses

Echeneids engage in symbiotic relationships to various degrees from facultative to obligate. Interspecific relationships such as symbioses often have far-reaching ecological consequences, which may have widespread impacts across multiple taxa (Sazima et al. 2010). For example, Bshary found that the addition or removal of cleaning fish in coral reef environments significantly impacted local fish distribution patterns, even of species that did not usually associate with cleaner fish (Bshary 2003). Understanding life-history adaptations is a critical component for the development of ecologically sound management approaches especially in the case of symbiotic relationships in which the attributes of one species have immediate and intertwining consequences on other organisms.

The species in *Echeneis* and *Phtheirichthys* display generalized host choice and predominantly facultative symbioses and occupy warm, shallow waters, whereas the pelagic *Remora* species range from moderate generalists to specialists with obligate symbionts (Gray et al. 2009). Echeneids show marked morphological differences in body shape and cephalic disc size related to degree of facultative or obligate symbioses. The more specialized obligate echeneids in the *Remora* genus have smaller bodies with larger relative disc sizes than those of the facultative echeneids in *Echeneis* and *Phtheirichthys* (Cressey and Lachner 1970). Smaller bodies are most likely a symbiotic-based adaptation

to the need for a hitch-hiking species to stay smaller than its host species while also reducing potentially detrimental hydrodynamic drag effects. Larger disc sizes probably allow for more secure attachment to fast moving pelagic host species in the remoras but could be a hydrodynamic impediment in facultative species that are often found free-swimming in shallow waters (Cressey and Lachner 1970).

Despite their widespread distribution and tendency to congregate with commercially fished species such as sharks and billfish, sharksucker life-history patterns have not been studied. Compared to some other members of the family Echeneidae, especially those fishes in the genus *Remora*, sharksuckers are not well represented in the literature. *E. naucrates* and *E.neucratoides* differ from several other members of the Echeneidae in that they are not host specific, do not require a host species and are often found free-living (Cressey and Lachner 1970; O'Toole 2002). Because sharksuckers are adapted to both free-living and symbiont life histories they offer potential insight into how obligate symbioses may have developed from free-living ancestors. It is unclear what determines sharksucker host selection and how strong those attachments remain.

While widely believed to at least occasionally act as cleaner fish, preying on the parasites of their host animals, actual published accounts of echeneid dietary preferences are often conflicting. For example, remoras have been variously reported as feeding on the parasites of their hosts (Cressey and Lachner 1970, Sazima et al. 1999), or scavenging on scraps of their hosts' prey (Strasburg 1959), or on the feces of their hosts (Williams et al. 2003), as well as preying on free living prey such as small fishes and plankton (Cressey and Lachner 1970). Which of these hypotheses are most accurate and whether

the sharksucker engages in commensal, mutualistic, or parasitic symbioses (and to what degree) is currently unknown.

The echeneid-host relationship has been variously characterized as the mutualistic relationship of a cleaner fish to its host (Sazima et al. 1999); as a commensal relationship which provides efficient transportation, food, and protection from predators while the host receives no benefit and suffers no harm (O'Toole 2002); and as a potentially parasitic relationship in which the host is harmed by the increased hydrodynamic drag or by skin irritation caused by the attachment of the symbiont via the sucking disc (Schwartz 1992; Brunnschweiler 2006). Recent papers on the methods used by both sharks and dolphins to remove attached echeneids appear to supply some support for the parasite hypothesis at least in certain cases (Brunnschweiler 2006; Weihs et al. 2007). Other species such as *Remora remora* have been shown to act as mutualistic cleaner fish which feed on the parasites of their hosts (Cressey and Lachner 1970). These relationships have been demonstrated to supply mutual benefits to both the cleaner and the host (Cressey and Lachner 1970; Sazima et al. 2010). Little published evidence for adult sharksuckers substantially or primarily feeding on the parasites of their host exists; however, at least one account of juvenile sharksuckers acting as station-based cleaners for reef fish has been published (Sazima et al. 1999). This implies that some juvenile sharksuckers may well act as cleaner fish at least in coral reef habitats and that host parasites may form a larger part of the juvenile diet than the adult diet. If mature sharksuckers do not in fact prey on their host's parasites then they may not offer any concrete benefits to their hosts and instead may actually harm them through physical damage or increased bioenergetic

costs. There has been some support for the hypothesis that a sharksucker's attachment to a host organism causes physical irritation and/or possible hydrodynamic drag (Schwartz 1992; Brunnschweiler 2006; Weihs et al. 2007). Although they are considered generalists in terms of host selection, sharksuckers seem to frequently associate with elasmobranch hosts such as sharks and rays (Cressey and Lachner 1970).

Sharksucker symbioses highlight the need for better understanding of the potential ecological- and conservation-related impacts of the sharksucker-elasmobranch relationship. This is particularly important in light of the continuing decline of numerous elasmobranch populations and the desire for ecologically sound shark fisheries management practices (Burgess et al. 2005). Many species of elasmobranch are especially vulnerable to exploitation due to a combination of being slow to reach maturity, low rates of fecundity, and slow rates of population growth (Musick et al. 2000; Cortes et al. 2002). Many of the shark species echeneids are known to associate with, including numerous carcharhinid and sphyrnid species common to the Gulf of Mexico, are currently considered to be overfished or at risk of overfishing by the National Marine Fisheries Service (Cortes et al. 2002; Brewster-Geisz 2005). There is insufficient information to evaluate risk of sharksucker population impact due to exploitation of hosts.

Ontogeny

It seems likely that the relationship between sharksucker and host depends on the developmental stage of the sharksucker. Echeneids may well proceed ontogenetically from obligate to facultative symbiont. Sharksuckers develop the cephalic disc on

metamorphosing from free-living pelagic larvae to the juvenile form. Larval sharksuckers in captivity begin to develop the cephalic disc as early as 9 days post-hatching and at least in one case began attaching to the glass tank walls at around 35 days of age, (55 mm standard length (SL)) (Nakajima et al. 1987). Other researchers have estimated that juvenile echeneids begin obligate attachment to hosts when they reach between 40 and 80 mm SL (Strasburg 1964). Newly transformed juveniles in potentially inhospitable habitats (i.e., pelagic ocean environments) are unlikely to be able to migrate on their own and presumably need to be able to attach to a host immediately to be transported to more appropriate habitats, such as highly structured coral reefs or coastal environments where cover and forage are readily available to smaller fish. Juveniles failing to attach to an appropriate host may be subject to hostile environments with unsuitably low temperatures or they may be unable to find food, leading to starvation. These vulnerable juveniles likely receive direct benefits from associating with a host species at an early age. Rapid ontological development of the cephalic disc likely allows juvenile echeneids to attach to a host while they are still small and especially vulnerable to predation. In addition to protection from predators, attached juveniles would gain access to far more potential food items than unattached juveniles. Host ectoparasites may supply an immediate food source. Foraging on the remains of the host's meals or on prey disrupted by the host's hunting activities would likely offer a larger array of food choices than could otherwise be acquired by free-living juvenile echeneids. This situation provides fitness advantages that are counterbalanced by a trade-off for sharksucker recruitment in the form of a limited supply of hosts.

Apparent obligate symbiosis of juvenile echeneids appears to become a facultative relationship for older and larger fish. Adult sharksuckers are often found free-swimming in suitably warm and shallow environments such as coral reefs (Cressey and Lachner 1970; O'Toole 2002). Adult fish are less vulnerable to predation and are more adept foragers, able to live independently for short periods of time. However, adult sharksuckers frequently continue to attach to host species and likely continue to gain many of the benefits of this attachment as the juveniles do, with the potentially added benefit of being brought into contact with potential mates as their host comes into contact with other echeneid-bearing hosts. This facultative symbiosis of adult echeneids may simply be a result of natural host size limitations where the number of available potential hosts of appropriate size (such as large elasmobranchs) is lower than the number of adult echeneids seeking hosts.

The need to attach to a host constrains sharksucker body size at every life stage. Smaller echeneids potentially have access to a wider range of host sizes and can successfully share hosts. Smaller body size is likely to have increased trade-offs between predation risk and fecundity. By comparison, closely related, large-bodied, fast-moving cobia and dolphinfishes are not subjected to this size-selective pressure. The size constraints placed on echeneids, where smaller sizes equal greater access to potential hosts, likely causes sharksuckers to exhibit less rapid growth rates and smaller overall body sizes than cobia and dolphinfish. Echeneids that reach sexual maturity at these smaller body sizes receive a benefit in increased host availability, which must be balanced against a trade-off in size-related fecundity.

Diet

Few studies have examined sharksucker diet but the wide variety of prey items reported in the literature are in concordance with the opportunistic foraging expected of a facultative symbiotic lifestyle. Previous stomach content analyses for echeneid species have reported multiple food items including planktonic organisms, such as amphipods, copepods, and decapods; ectoparasitic copepods; fishes, mollusks and crustaceans; and fecal matter from host organisms (Strasburg 1962; Cressey and Lachner 1970; E. H. Williams et al. 2003). Echeneids display varying amounts of dietary specialization, with the Echeneiinae exhibiting generalist feeding preferences, whereas many of the remora species are specialized feeders. Because sharksuckers display facultative symbiosis as opposed to the more specialized obligate symbionts of the remora family, their dietary preferences are likely to be more generalized and thus a wide variety of food items are expected to be found. Independent, free-living sharksuckers presumably forage for multiple prey items while those living with hosts may feed on the hosts' feces and/or the remnants of the hosts prey in addition to foraging for their own prey. Parasitic copepods are predicted to make up a small but integral part of the sharksucker diet, particularly at the juvenile stage, while the rest of their food items are probably obtained opportunistically (Cressey and Lachner 1970). Echeneids are likely to consume many of the same food items as their hosts, including potentially scavenging any scraps left behind by their hosts. Echeneids are also likely to venture short distances away from their hosts to forage independently.

Reproduction

Little is currently known regarding sharksucker reproduction. Echeneid-elasmobranch relationships presumably complicate sharksucker reproductive histories, particularly with regard to mate selection and to spawning locations and times. Spawning times and locations are likely constrained by the sharksuckers' need to remain in close proximity to the host. Given echeneid morphology and observed swimming performance, it is unlikely that sharksuckers that abandon an elasmobranch host for long periods of time would be able to regain association with that particular individual. Unattached echeneids are at risk of being stranded in an inhospitable environment unless they are able to quickly find a replacement host. Echeneid spawning periodicity and duration probably cue on the migratory patterns of their hosts. Cobia are migratory, multiple-batch spawners with a spawning period spanning several spring and summer months (Brown-Peterson et al. 2001). Coryphaenids are also migratory and year-round multiple batch spawners in the warmest parts of their range (Ditty et al. 1994). An echeneid without strong swimming ability and linked to a host must depend on the host's travels to reach a suitable spawning site. This may limit mate selection in addition to affecting length of access to appropriate spawning sites.

Objectives

For this study I examined several aspects of sharksucker life history to elucidate the nature of their symbiotic relationship to elasmobranchs (facultative versus obligate, and mutualistic versus parasitic) and to understand what life history adaptations are correlated with the evolution of this group, particularly in regard to the evolution of symbiotic relationships. Gray et. al. (2009a) demonstrated significant similarities in host

dispersal and phylogeography in the echeneid *R. osteochir* and their host species, which supports the idea that both population structure and evolutionary patterns among symbionts may ultimately depend on host ecology. Host-sharksucker interaction is likely to impact all aspects of echeneid life history, from reproductive adaptations to ontogenetic development to dietary preferences. These basic characteristics of sharksucker life history are poorly known. A better understanding of these factors allows for a comparison of echeneid life history to cobia and dolphinfish that gives further insight into the evolution of symbiotic life histories. This project aimed to address these knowledge gaps in the life history of *Echeneis naucrates*. Objectives of this study were: (1) to develop size-at-age information to model life-time growth, (2) to characterize diet composition in juvenile and adult sharksuckers, (3) to quantify trophic position and source of primary production for sharksuckers and compare this across age groups, and (4) to describe reproductive mode, seasonality, fecundity, and maturation schedule.

MATERIALS AND METHODS

Study Site: Gulf of Mexico Coast of Florida

Sharksuckers were collected from ongoing coastal shark surveys conducted by Florida State University in the northeastern Gulf of Mexico. The northeastern coastal Gulf of Mexico ranges from subtropical to warm temperate. Within the gulf a broad continental provides a large area of low-energy shallow water to the Big Bend area of the Florida coastline (Dawes et al. 2004). Nearshore substrates provide substantial seagrass habitats broken up by sand banks or occasional hard bottom. Strong seasonal temperature fluctuations allow for a multitude of temperature regimes and vegetative growth. These seasonally highly productive environments support a diverse population of shark species and provide an important habitat range for sharksuckers during the warmer months. While data on optimal temperatures for sharksuckers are lacking, captive sharksuckers have been observed to cease spawning activity at water temperatures below 25 °C (Nakajima et al. 1987). A study on cold-water effects on multiple fish species included a single sharksucker, which died at a water temperature of 11.7 °C (Schwartz 1964). Based upon what is known about temperature tolerance, coarse range maps, and limited habitat descriptions in taxonomic guides, it is unlikely that sharksuckers would be able to tolerate winter conditions in the northeastern Gulf of Mexico. While those echeneids that occur in warmer coral reef environments may stay free-living year round, the echeneids in

temperate habits likely depend on host species for migration. For example, female blacktip sharks (*Carcharhinus limbatus*), a common host for sharksuckers, move northward in the gulf in May to give birth and then migrate 100 nautical miles south to overwinter in waters with surface temperatures >20 °C (Keeney et al. 2005, Hueter et al. 2005). Thermal constraints increase pressures on echeneids to find suitable reproductive habitats during warmer months while still remaining able to find a host prior to or during the fall elasmobranch migrations to reach favorable winter ranges.

Sample Collection

Sharksuckers (n = 338) were obtained opportunistically from surveys of coastal shark species in the Gulf of Mexico between the months of April and October in 2010, 2011 and 2012. Fish were collected in the Big Bend area of Florida in the northeastern Gulf of Mexico (n = 182) and from the Florida Keys (n = 156). A small number of fish collected in October 2009 for feasibility study were also incorporated into the dataset. The surveyed elasmobranchs were captured with gill nets, trawls, and longlines. As the sharks were brought on board, associated sharksuckers were captured either by dip net or by the use of baited hooks on hand-held fishing poles. Sharksuckers caught on the long-line hooks or entangled in the gill nets were also noted and retained for analysis. A biopsy punch was used to remove a dorsal plug of white muscle to be submitted for stable isotope analysis from fish larger than approximately 180 mm (standard length, SL). Specimens were frozen whole as soon as possible after capture for subsequent laboratory processing. A subset of fish (n = 33) were placed on ice for subsequent weight measurements and extraction of gonads for histology. Excised gonads were weighed to

the nearest 0.001 grams (g) and then immediately immersed in Bouin's solution. Gonads were moved from Bouin's into 70% ethanol within 24 to 36 hours (h). Once gonads were removed, the body of the fish (with the digestive tract) was frozen.

Initial Processing

For initial processing of frozen specimens, weight (to the nearest 0.01 g) and length were measured, and a second plug of white muscle for stable isotope analysis was extracted from the dorsolateral region of the right side. Comparison of weight between fresh and frozen fish revealed that freezing resulted in a 4% loss of mass, therefore weights of fresh specimens were used for all analyses except where noted.

Growth

Otolith Preparation

To develop size-at-age information to model lifetime growth otolith thin sections were used to age juvenile and adult sharksuckers. Preliminary examination of sharksucker otoliths revealed a structure that is very similar to Rachycentridae; therefore, identification of annuli to determine age followed Franks et al. (1999). To prepare otolith thin sections, pairs of sagittal otoliths were removed, cleaned with water and placed in polycarbonate vials to dry for a minimum of two weeks. A single sagittal otolith per specimen was embedded in resin and sectioned transversely to create a thin section through the core.

Larger otoliths from fish >200 mm SL were embedded in EpoFix resin and then sectioned on a Bueller IsoMet^{TM} diamond saw. Embedded otoliths were sanded on a series of increasingly finer abrasive papers, starting with 800 grit and proceeding through

1200 to 1400 grit before being polished on a lapping cloth sprinkled with alumina powder. After polishing, otoliths were visually examined and photographed at magnifications between 50X and 400X on a Leica DM 2500 microscope. Otolith annuli were counted independently by two people to determine yearly ages. Disagreements between estimated ages were resolved by mutual reexamination of otoliths.

Smaller otoliths from fish less than 200 mm SL were placed on a glass slide and mounted in thermoplastic glue (Crystal Bond) on glass slides. The Crystal Bond embedded otoliths were allowed to cool overnight before being polished using the same procedure as the sectioned otoliths.

Although otoliths of fish between 100 and 200 mm SL were prepared for daily annuli counts, counts far exceeded 100 days and it was determined that daily age estimates with light microscopy would be unreliable.

Currently there are no validation studies of mark formation periodicity on otoliths of sharksuckers. One frequently used validation method is marginal increment analysis (MIA), in which the measurement and composition (opaque vs. translucent) of the marginal increment is compared to the preceding increments to determine rate and timing of increment deposition. A variation on this idea is quantifying the binomial presence/absence of an opaque zone on the margin of the otolith. Opaque zones in temperate fishes generally accumulate during the spring and summer (Hyndes et al. 1992). The presence of opaque margins on fish captured during summer months combined with a decreased presence of opaque margins on fish sampled in other months supports an annual trend of otolith deposition. Both of these methods were applied to

sharksucker otoliths, however inferences are limited due to the lack of samples during colder months of fall and winter. Annual ages were assigned to fish on the basis of counts of translucent annuli under reflected light and after taking into account month of capture. Translucent marginal increments were considered incomplete and so were not counted. A birthdate of June 1 was assigned to all fish after taking into account the histology, GSI and MIA results.

Age-length keys were tabulated by sex and von Bertalanffy growth in the sharksucker was modeled using ages determined from otoliths similar to Franks et al. (1999) for cobia. The von Bertalanffy growth model predicts length (SL) at age (t) as a function of three parameters: a theoretical asymptotic length (L_∞, mm SL), theoretical time at which the length was zero (t_0, years), and the rate ate which length increases toward the asymptote (k, per year):

Equation 1: von Bertalanffy theoretical growth equation (von Bertalanffy 1957)

$$(SL\mathrm{t} = L_\infty(1 - \exp\,[-K(t - t_0)])$$

Growth was modeled by sex and for the pooled data.

Each of the von Bertalanffy growth models was anchored using a mean hatch length of 7 mm SL observed for newly hatched larval sharksuckers in captivity (Akazaki et al. 1976, Nakajima et al. 1987). This ensured that differences between sexes or comparisons with other species were not biased by the low sample size of age-0 fish in these data.

Log_{10} total weights (WT) were compared to Log_{10} standard lengths (SL) using the linear regression formula:

Equation 2: Log_{10} Weight - log_{10} standard length

$$\log_{10} WT = a + b(\log_{10} SL)$$

Diet

Stomach contents were analyzed to characterize dietary composition as a function of body size, sex, and host association. To extract stomach contents, the stomach and gastrointestinal tract from the mid-esophagus to the rectum were removed from freshly thawed fish and weighed to the nearest 0.001 g. The entire digestive tract was weighed full and empty. Aggregate stomach contents were weighed to the nearest 0.001 g. All stomach contents were examined visually and microscopically at various magnifications on a Leica MZ 12.5 dissecting microscope to determine presence/absence frequencies of prey items before being preserved in 10% formalin for a minimum of two weeks. The formalin-preserved gut contents were rinsed with tap water in a 150-micron mesh filter before being visually separated to the lowest identifiable taxa under a dissecting microscope. Separated taxa were placed in aluminum drying pans to be weighed and dried at 80 °C for at least 48 h to obtain a dry weight.

Percent numeric abundance (%N), percent of total volume (%V) and percent frequency of occurrence (%F) were determined from the pooled data of all samples and

was used to construct an index of relative importance (IRI) (Franks et al. 1996) for each taxon using the following formula:

Equation 3: Index of relative importance

$$IRI = (\%N + \%V) \times \%F$$

Taxa with higher IRI values are expected to be of higher dietary importance to the organism. Percent IRI was also calculated by dividing the individual taxon's IRI by the sum total of all IRI values (Franks et al. 1996).

Mean proportion by volume was calculated using the following formula (Chipps and Garvey, 2007):

Equation 4: Mean proportion by volume

$$\boldsymbol{MV_i = \frac{1}{P}\sum_{j=1}^{P}\left(\frac{V_{ij}}{\sum_{i=1}^{Q} V_{ij}}\right)}$$

where i = prey item, j = individual fish, P = number of fish with food in stomach, Q = number of prey categories, V_i = volume of prey. Chipps and Garvey 2007).

Similarly, mean proportion by number was calculated using the formula (Chipps and Garvey 2007):

Equation 5: Mean proportion by number

$$MN_i = \frac{1}{P}\sum_{j=1}^{P}\left(\frac{N_{ij}}{\sum_{i=1}^{Q} N_{ij}}\right)$$

where *i* = prey item, *j* = individual fish, P = number of fish with food in stomach, Q = number of prey categories, N_i = number of prey items in prey category *i*. (Chipps and Garvey 2007).

Stable Isotope Analysis

In order to compare trophic position and source of primary production across ontogenetic age ranges of sharksuckers, stable isotope concentrations of $\delta^{13}C$ and $\delta^{15}N$ were determined using mass spectrometry. Stable isotope analysis (SIA) is a commonly used ecological tool that compares light to heavy isotope ratios of organisms to environmentally available isotope ratios in their ecosystems in order to determine patterns of nutrient uptake and element cycling. SIA provides a broader temporal scope than the direct evaluation of stomach contents. Stomach contents can only supply information on what prey items have been consumed immediately prior to examination. They are limited to a particular moment in time and may give a false impression of the importance of uncommon items. Hard-to-digest prey items or parts, such as exoskeletons or bones, may be overrepresented while rapidly digested prey may be underestimated. Stomach contents give an idea of what prey items have been ingested by a particular fish but do not provide information on dietary items that have already been digested and assimilated by the consumer. SIA complements stomach content analysis because it

provides information on assimilated diet components over a longer time scale (Peterson and Fry 1987, Chipps and Garvey 2007). Carbon and nitrogen are commonly used stable isotopes in ecological studies. Animals incorporate nitrogen and carbon into their own tissues from consumed prey so the ratios of nitrogen and carbon isotopes in their tissues can be used to characterize diet composition. As $\delta^{13}C$ resists trophic magnification, it is possible to trace sources of primary production through a food web by comparing $\delta^{13}C$ to $\delta^{12}C$ isotope ratios (Fry 2006). Similarly, $\delta^{15}N$ to $\delta^{14}N$ ratios indicate trophic positioning as $\delta^{15}N$ concentrations are magnified across increasing trophic levels (Fry 2006). Organisms feeding at the same trophic level on the same food items are expected to accumulate similar $\delta^{13}C$ and $\delta^{15}N$ ratios.

Animals that consume prey items are expected to be enriched in $\delta^{15}N$ relative to their prey. This amplification across trophic levels occurs because $\delta^{15}N$ is preferentially selected for during consumer metabolic processes, while $\delta^{14}N$ is more likely to be excreted (DeNiro and Epstein 1981, Minigawa and Wada 1984, Gannes et al. 1997). This results in the predator incorporating a larger proportion of the heavier nitrogen isotope into their own tissues.

Similarly, metabolic process acting on carbon cause $\delta^{13}C$ values to increase slightly, up to approximately 1.0‰ in consumer muscle compared to prey (DeNiro and Epstein 1978, Tieszen et al. 1983, Peterson and Fry 1987, Vander Zanden and Rasmussen 2001, Michener and Kaufman 2007). Post (2002) calculated mean trophic fractionation of $\delta^{13}C$ at 0.4‰ with a standard deviation of 1.3‰ using data compiled from multiple studies across varying organisms.

Turnover of stable isotope values varies in different tissues, by isotope and across species. Stable isotopes for this study were obtained using white muscle. White muscle is commonly analyzed for stable isotope values in fish because it has a relatively long turnover rate and is less variable than other tissues, such as liver or heart muscle (Pinnegar and Polunin 1999, Sweeting et al. 2005). Isotopic turnover rates are correlated with growth rate and younger, faster growing organisms incorporate new dietary isotopes more rapidly than older animals (Herzka 2005, Sweeting et al. 2005). Isotopic turnover rates of carbon and nitrogen in fish muscle have been shown to occur as rapidly as a few days in larvae to months or even years in older, slower growing fish (Hesslein et al. 1993, Maruyama et al. 2001, Herzka 2005, Sweeting et al. 2005).

The SIA data used in the current study were made available by the FSU Shark Survey in order to compare echeneid isotopic values across size ranges. To determine stable isotope concentrations, the frozen light muscle tissue samples were combusted into gaseous form and the resultant carbon dioxide (CO_2) and nitrogen (N_2) gas samples were analyzed via mass spectrometry (Fry 2006). During this process, positively ionized molecules of carbon and nitrogen were magnetically separated into their separate isotopes in a mass spectrometer and final light-to-heavy isotope ratios of each element were calculated electronically (Fry 2006). Carbon stable isotope values are expressed relative to the PeeDee Belemnite (PDB) international standards while nitrogen values are expressed relative to the concentration of atmospheric nitrogen (Fry 2006). Stable isotope values are described using standard δ notation in parts per thousand (‰), calculated by:

Equation 6: Stable isotope δ notation (Fry 2006)

$$\delta X = [(R_{SAMPLE}/R_{STANDARD}) - 1]\ x\ 1000$$

where *X* is the stable isotope (C or N) and *R* is the ratio of heavy to light isotope, either ^{13}C:^{12}C or ^{15}N:^{14}N (Fry 2006). Samples exhibiting lower ratios of the heavier isotope than the international standard are described as "depleted" and those with higher ratios of the heavier isotope as "enriched." Less negative δ ^{13}C values contain higher ratios of the heavy isotope and so are considered enriched. Analyses were performed at the National High Magnetic Field Laboratory in Tallahassee, Florida. Every 10th sample was duplicated to determine analytical accuracy. Duplicating samples resulted in a mean difference of 0.1‰ in $\delta^{13}C$ (SD = 0.2) and 0.3‰ in $\delta^{15}N$ (SD = 0.3).

In this study, SIA of carbon and nitrogen was used as a complementary measure to stomach content analysis (SCA) in order to evaluate long-term patterns of consumption. Light-to-heavy stable isotope ratios of carbon and of nitrogen were compared across echeneid developmental stages, size classes and sex to evaluate possible ontogenetic niche shifts in dietary composition. As SIA values in the current study were compared across size classes and sex solely to compare intraspecific trophic position and analyze potential ontogenetic dietary shifts, isotope ratios for prey items were not obtained. Stable isotope values were evaluated using analysis of covariance (ANCOVA).

Reproduction

Fecundity Estimation

Gravimetric and auto-diametric methods were used to estimate fecundity of sharksuckers (Klibansky and Juanes 2008). Gonad samples for fecundity estimation were

taken from previously frozen fish. Only ovaries containing visible translucent eggs were included in the estimation. The left and right ovaries were removed from thawed fish and weighed to the nearest 0.001 g before being placed in 10% formalin for a minimum of 2 weeks. Ovaries were removed from formalin, rinsed in water and patted dry before being weighed again to the nearest 0.001g. An approximately 0.1 g central section across both ovaries was excised and then placed in a 150-micron mesh filter and rinsed under running water to separate the oocytes from surrounding tissue. Separated oocytes were placed in 10% formalin and shaken by hand occasionally to further separate oocytes for at least a week before being rinsed again and placed in tap water in a petri dish. A drop of dish soap solution (1 part Dawn® to 19 parts tap water) was added to the dish to reduce surface tension and keep the oocytes from floating at the top of the water. The oocytes were allowed to sit in the water and soap solution for at least 15 minutes before being imaged under a dissecting microscope. The dish of oocytes was then scanned on an HP flatbed scanner in grey scale at a resolution of 1200 dpi. Hydrated oocytes were counted manually for two subsamples per ovary to determine relative batch fecundity. Scans were analyzed in ImageJ to obtain area measurements of 100 hydrated oocytes per ovary. Resulting area measurements were then converted to diameter to calculate mean diameter (Klibansky and Juanes 2008). After imaging, to calculate volume the imaged oocytes and the remainder of the ovary were dried in a drying oven at approximately 80 °C. Oocyte subsamples and ovaries were dried for a minimum of 4 days and were weighed until an asymptote was obtained.

A gonadosomatic index (GSI) was developed for each fish to evaluate seasonality of relative gonad size as a proxy for development. The GSI was calculated as:

Equation 7: Gonadosomatic index (White, Munroe and Austin 2002)

$$GSI = \left((Gonad\ Weight/Somatic\ Weight) \times 100\right)$$

Somatic weights were determined by subtracting gonad weight from total body weight. In order to allow comparison with gonads preserved for histology, weights of frozen specimens were corrected to an equivalent fresh weight.

To evaluate sex-specific variations in reproductive periodicity, GSI values were compared by monthly mean averages across sex.

Reproductive Histology

Gonad samples for histology were initially preserved in Bouin's solution for 24 h and then moved to 70% ethanol. Multiple changes of 70% ethanol failed to rinse all of the yellow staining from the Bouin's solution from the whole gonad samples, so smaller sub-samples were taken from the center of each gonad and rinsed in 70% ethanol to completely remove the excess Bouin's solution. Gonads were photographed and then an approximately 1-cm thick section was removed from the center of each pair of gonads. These 1-cm sub-samples were placed in plastic tissue cassettes (Mega-Cassette®) in fresh 70% ethanol and placed on an agitator. The cassettes were placed into new 70% ethanol whenever the ethanol became discolored. Once the ethanol remained clear the gonad samples were processed. The sub-samples were further sectioned to create 5-mm slices

that were then placed into regular plastic tissue cassettes. The sample cassettes were processed in a Ventana RMC 1530 paraffin tissue processor.

Processed samples were then embedded in Paraplast Plus® paraffin. Samples embedded in paraffin were sectioned on an Olympus Cut 4060 microtome and floated in a distilled water bath at approximately 40 °C. Floating sections were adhered to slides and allowed to dry vertically before being placed horizontally on a warming plate to finish drying. Dry slides were stained with Fisherbrand® Harris's modified hematoxylin and Protocol® alcoholic eosin Y. Stained slides were coverslipped and allowed to dry horizontally before being read.

Histological oocyte stages were identified using the characteristics common to teleost ovaries stained with hematoxylin and eosin as illustrated by Grier et al. (2009) (Table 1).

Note that although Grier et al. (2009) consider cortical alveolar oocytes (CA) to be a step within the primary growth stage, Lowerre-Barbieri et al. (2011) include CA oocytes under secondary growth (Figure 1). Lowerre-Barbieri et al. (2011) base their argument for the inclusion of CA oocytes in secondary growth in that CA oocytes do not occur in immature females and so can be used as a marker for maturation as well as a probable sign of commitment to spawning in the upcoming season. Brown-Peterson et al. (2011) also consider CA oocytes "the definitive marker entry into the developing phase" of oogenesis. Grier et al. (2009) use follicle size as the primary discriminator for three secondary growth (vitellogenic) stages, which they label early secondary growth, late secondary growth and full-grown oocytes.

Table 1: Stages of oogenesis. Adapted from Grier et al. 2009.

Stages of oogenesis		
Primary oogonia	PO	Large, clear cells with spherical nuclei.
Chromatin nucleolus oocyte	CN	Undergo meiosis through early diplotene stage.
Primary growth oocyte	PGO	Large, pale nuclei. Dark basophilic ooplasm. May contain Balbiani bodies.
Perinucleolar step oocyte	PNO	Contain multiple nucleoli circling the germinal vesicle (GV).
Cortical alveoli oocyte	CA	Inclusion of cortical alveoli and/or lipid droplets.
Vitellogenic oocyte	VTG	Increase in size with incorporation of yolk granules.
Stage 1 vitellogenic oocyte	VTG1	Few yolk granules.
Stage 2 vitellogenic oocyte	VTG2	Larger than VTG1, larger yolk granules, CA moved towards zona pellucida (ZP).
Stage 3 vitellogenic oocyte	VTG3	Majority of oocyte is filled with yolk globules.
Oocyte maturation	OM	
Germinal vesicle migration	GVM	Germinal vesicle moves toward animal pole
Germinal vesicle breakdown	GVB	Germinal vesicle breaks down, meiosis resumes.
Hydrated oocyte	HO	Yolk coalesces, hyaline appearance.
Post-ovulatory follicle	POF	Collapsed follicle layers following ovulation
Atretic oocyte	AO	Phagocytosis of oocyte

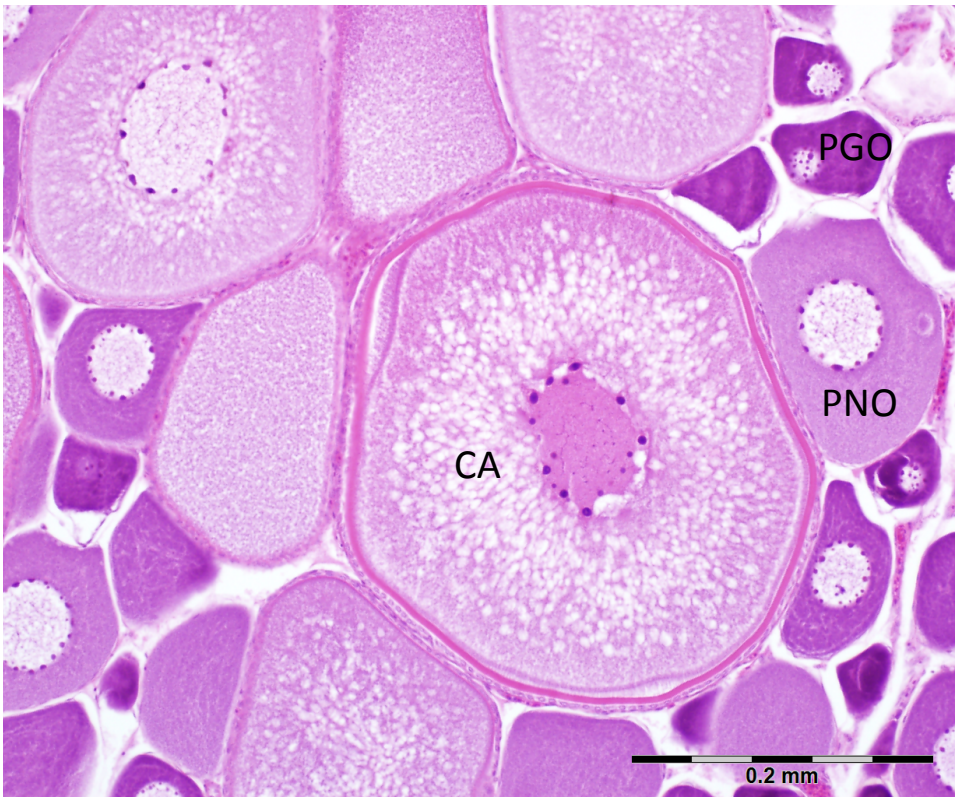

Figure 1: Sharksucker primary growth (PGO), perinucleolar (PNO) and cortical alveolar (CA) oocytes.

Grier et al.'s (2009) early secondary growth oocytes, which are categorized by Brown-Peterson et al. (2011) as primary vitellogenic ooctyes (Vtg1), contain small eosinophilic yolk granules and, in many species, clear lipid droplets begin to collect along the peripheral ooplasm. Grier et al.'s (2009) late secondary growth step oocytes, like Brown-Peterson et al.'s (2011) secondary vitellogenic oocytes (Vtg2) appear similar to primary vitellogenic oocytes but increase in size with the accumulation of increasingly larger yolk granules while the cortical alveoli are pushed towards the zona pellucida. Grier et al. (2009) categorize full-grown vitellogenic step oocytes, also called tertiary

vitellogenic oocytes (Vtg3) by Brown-Peterson et al. (2011), as the final stage of vitellogenesis prior to oocyte maturation (Figure 2).

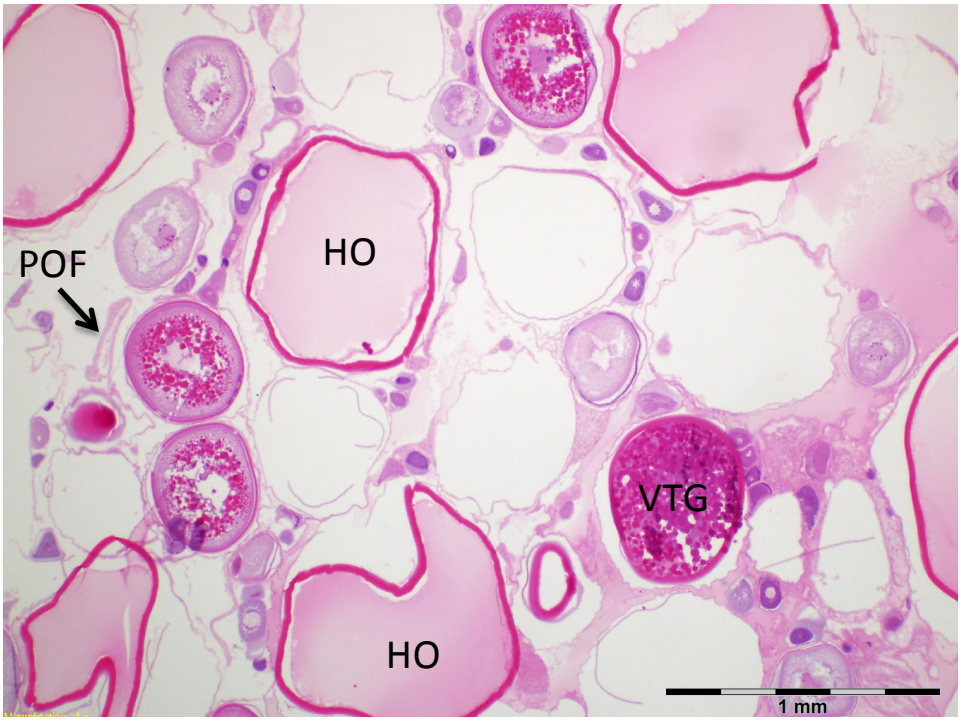

Figure 2: Vitellogenic (VTG), and hydrated (HO) oocytes, and post-ovulatory follicles (POF) in sharksucker ovary.

Hydrated oocytes (HO) are released from the follicle into the ovarian lumen during ovulation, leaving behind histologically apparent post-ovulatory follicle complexes (POF) made up of the collapsed follicle layers (Figure 3). These POFs may degrade as rapidly as within hours or days depending on species and water temperature and can be difficult to distinguish from later stage atretic oocytes particularly in warmwater fishes where resorption can occur rapidly due to increased metabolic rates from exposure to warmer water temperatures (Brown-Peterson et al. 2011, Hunter and

Macewicz 1985, Lowerre-Barbieri et al. 2011). The lumen of the POF remains continuous with the ovarian lumen (Grier et al. 2009).

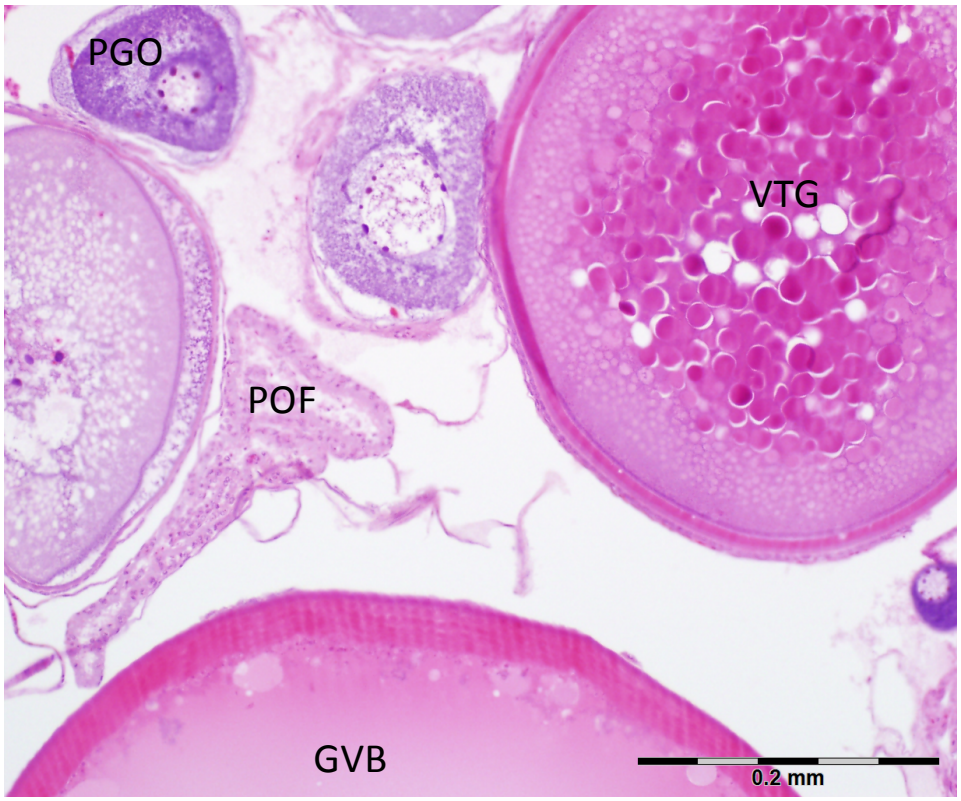

Figure 3: Spawning capable sharksucker ovary with post-ovulatory follicle (POF), vitellogenic oocyte (VTG), and primary growth oocyte (PGO).

Hunter and Macewicz found that oocytes only became completely hydrated within 12 h of ovulation in northern anchovy, which makes them a useful marker of imminent spawning at least in certain species (Hunter and Macewicz 1985). For example, spotted seatrout, a warmwater indeterminate batch spawning species, have been shown to have all three stages of oocyte maturation, hydration and ovulation occur in time periods

of less than 24 h and so the presence of maturing oocytes may mark the active spawning subphase (Brown-Peterson et al. 2011).

Atretic oocytes (AO) are those that do not mature or that are never ovulated and instead undergo atresia (Figure 4, Figure 5). Atresia is characterized by the phagocytosis of the oocyte by follicular cells that are in turn phagocytized by stromal cells (Grier et al. 2009).

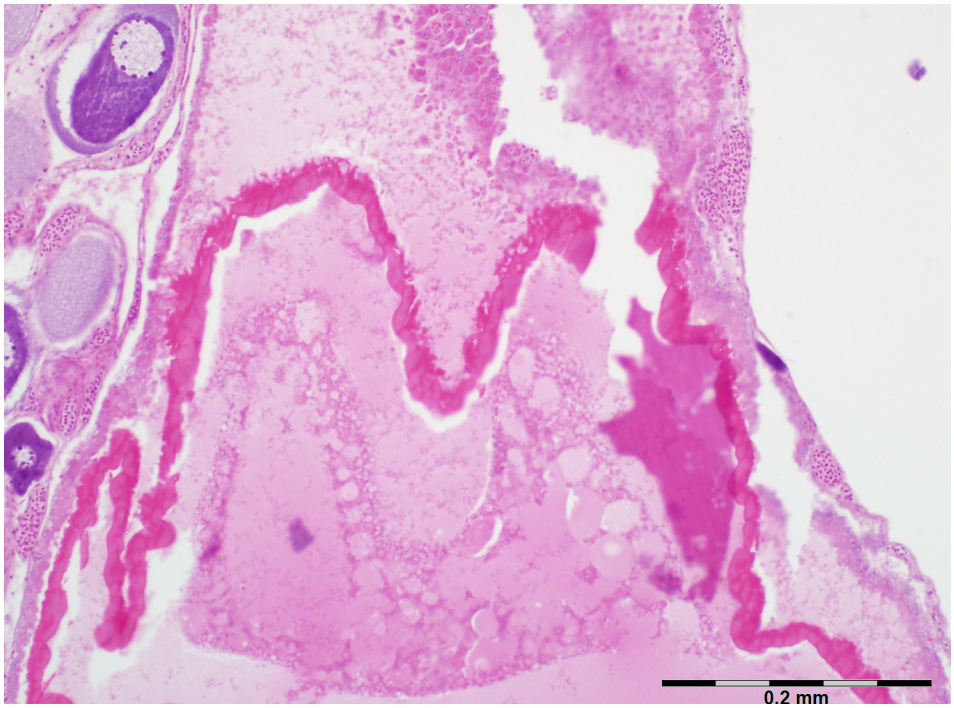

Figure 4: α atresia of a hydrated sharksucker oocyte.

Ovarian atretic states categorize the prevalence of atretic oocytes and/or follicles and can be used to determine the probability of spawning cessation (Hunter and Macewicz 1985).

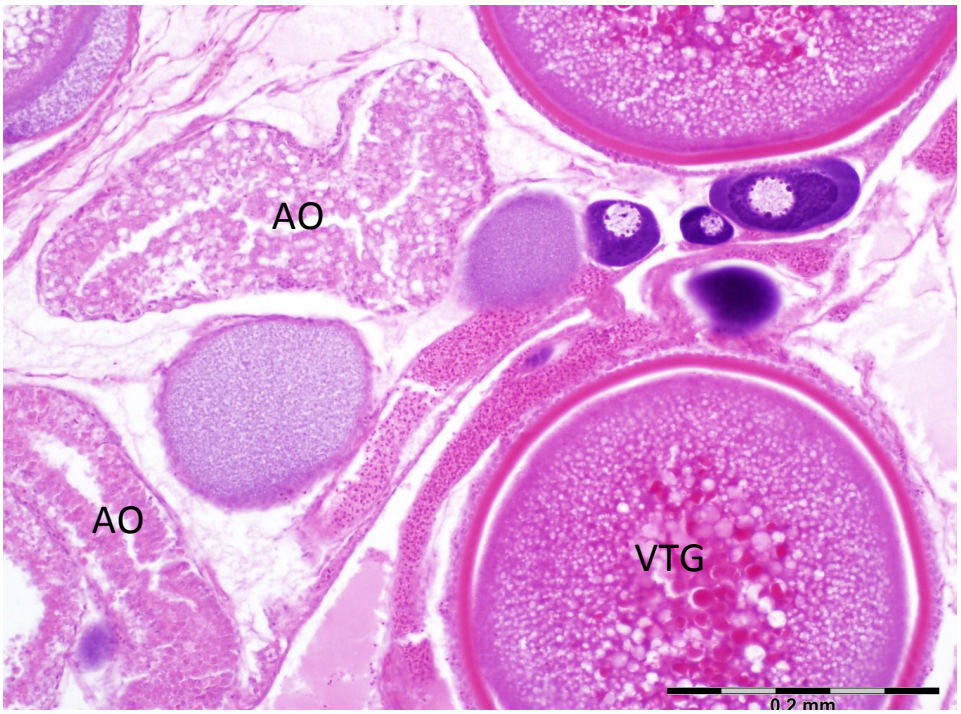

Figure 5: β atretic oocytes (AO) and vitellogenic oocytes (VTG) in sharksucker ovary.

Hunter and Macewicz (1985) categorize four stages of atresia (α, β, γ, δ) (Table 2) and four ovarian atretic states (0, 1, 2 and 3) (Table 3).

Table 2: Stages of atresia. Adapted from Hunter and Macewicz 1985.

Stages of atresia	
α	Disintegration of germinal vesicle, and zona pellucida. Resorption of oocyte by follicular cells.
β	Oocyte has been resorbed. Follicle cells remain and empty cavity may appear similar to older POFs.
γ	Cell nuclei become irregular.
δ	Granular, brownish pigment collects in remaining cells.

Table 3: Atretic ovarian states. Adapted from Hunter and Macewicz 1985.

Atretic state	
0	Vitellogenic oocytes present. No α atresia.
1	<50% of vitellogenic oocytes in α atresia.
2	>50% of vitellogenic oocytes in α atresia.
3	No vitellogenic oocytes present. β atresia present.

Fish in atretic state 1 may still be able to spawn but it is unlikely that fish in atretic state 2 will spawn so atretic state 2 is considered a useful marker for determining spawning cessation (Hunter and Macewicz 1985).

Histologically identified oocyte stages based on the previously summarized criteria were used to categorize each examined sample into one of the five major phases of the female reproductive cycle as described by Brown-Peterson et al. (2011)(Table 4).

The immature phase is found only in fish that have never spawned and is characterized by the presence of oogonia and primary growth (PG) oocytes with no secondary growth oocytes present (Brown-Peterson et al. 2011).

Ovaries in the early developing subphase contain PG oocytes in addition to cortical alveoli (CA) oocytes. Ovaries enter the developing phase once vitellogenesis begins and Vtg1 and Vtg2 oocytes may be present (Brown-Peterson et al. 2011).

Spawning capable fish are those whose oocytes are matured enough to spawn in the current reproductive cycle (Brown-Peterson et al. 2011).

Vtg3 oocytes are present when initially entering this phase, but in batch-spawning species these oocytes mature and are ovulated in multiple batches as the spawning season proceeds so they may not be evident in fish that have recently spawned.

Table 4: Reproductive phases and sub-phases of female sharksuckers. Adapted from Brown-Peterson et al. 2011.

Phase	Histological features
Immature	Oogonia and tightly packed PG oocytes.
Developing	Developing oocytes only through the Vtg2 stage. No POFs. Atresia possible.
Early developing	Presence of developing oocytes only through the CA stage.
Spawning capable	Contain Vtg3 oocytes, OM, and/or POFs. Atresia possible.
Actively spawning	Exhibit signs of spawning readiness: GVM, GVBD, hydration and/or ovulation.
Regressing	Contain POFs and atretic oocytes. CA, Vtg 1 and Vtg2 may be present.
Regenerating	Oogonia and PG oocytes with no more advanced stages present. Late stage atresia or POFs possible.

Spawning capable asynchronous batch spawners that have begun spawning in the current cycle may exhibit POFs along with earlier stage vitellogenic oocytes. Consequently, the presence of Vtg 3 oocytes and/or POFs along with possible oocyte maturation stages and α atresia of late stage oocytes can be used to distinguish the spawning capable phase in batch spawning species (Brown-Peterson et al. 2011). The presence of CA oocytes along with differing stages of vitellogenic oocytes in spawning capable fish provides evidence for indeterminate fecundity in batch spawners (Brown-Peterson et al. 2011).

An actively spawning subphase of the spawning capable phase is characterized by oocyte maturation including germinal vesicle migration and germinal vesicle breakdown,

or the presence of hydrated oocytes, or recent POFs. Brown-Peterson et al. state that recent POFs along with maturing oocytes may be indicative of daily spawning in indeterminate batch spawning warmwater species (2011).

The post-spawning regression phase is characterized by the proliferation of atretic oocytes along with a reduction in vitellogenic oocytes. There may still be POFs present in regression phase ovaries (Brown-Peterson et al. 2011).

Ovaries in the regenerating phase have left the regressing phase and may contain oogonia and PG oocytes but no more advanced classes of oocytes (Brown-Peterson et al. 2011).

Atresia can occur in any reproductive phase but it is most commonly seen in the spawning capable, regressing and regenerating phases (Brown-Peterson et al. 2011).

Ovaries and testes were also examined for the presence of melano-macrophage centers that may contain lymphocytes and can serve a function similar to that of lymph nodes in other vertebrates (Agius and Roberts 2003) (Figure 6).

Identification of the stages of spermatogenesis in histological samples followed the descriptions of Grier and Uribe Aranzábal (2009).

These authors identified 4 main categories of spermatogenesis: primary spermatogonia, secondary spermatogonia; primary and secondary spermatocytes; and spermatids and spermatozoa (Table 5, Figure 7).

Secondary spermatogonia are found within individual spermatocysts (SC) that are surrounded by Sertoli cells. The remaining stages of spermatogenesis occur in these spermatocysts.

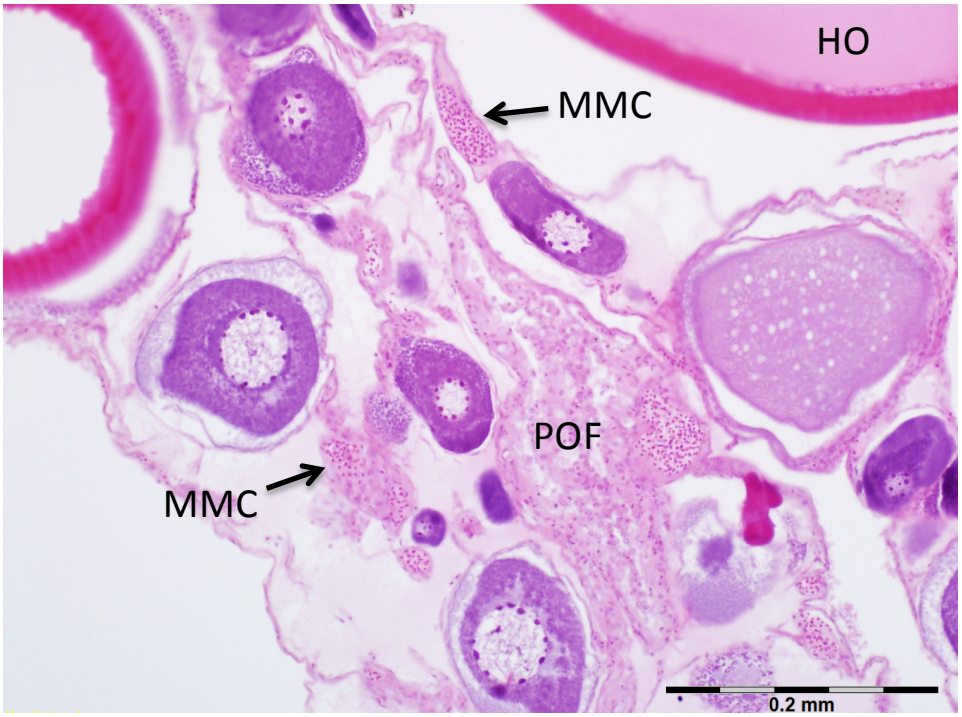

Figure 6: Melano-macrophage centers in sharksucker ovary, along with hydrated oocyte (HO) and post-ovulatory follicle.

Cells in an individual spermatocyst undergo development synchronously. Decreasing size with each division through the spermatid stage is the most obvious histological indicator of the progression of spermatogenesis.

Table 5: Stages of spermatogenesis. Adapted from Grier and Uribe Aranzábal 2009.

Stages of spermatogenesis	
Primary spermatogonia	SG
Secondary spermatogonia	SG2
Primary spermatocytes	SC1
Secondary spermatocytes	SC2
Spermatids	ST
Spermatozoa	SZ

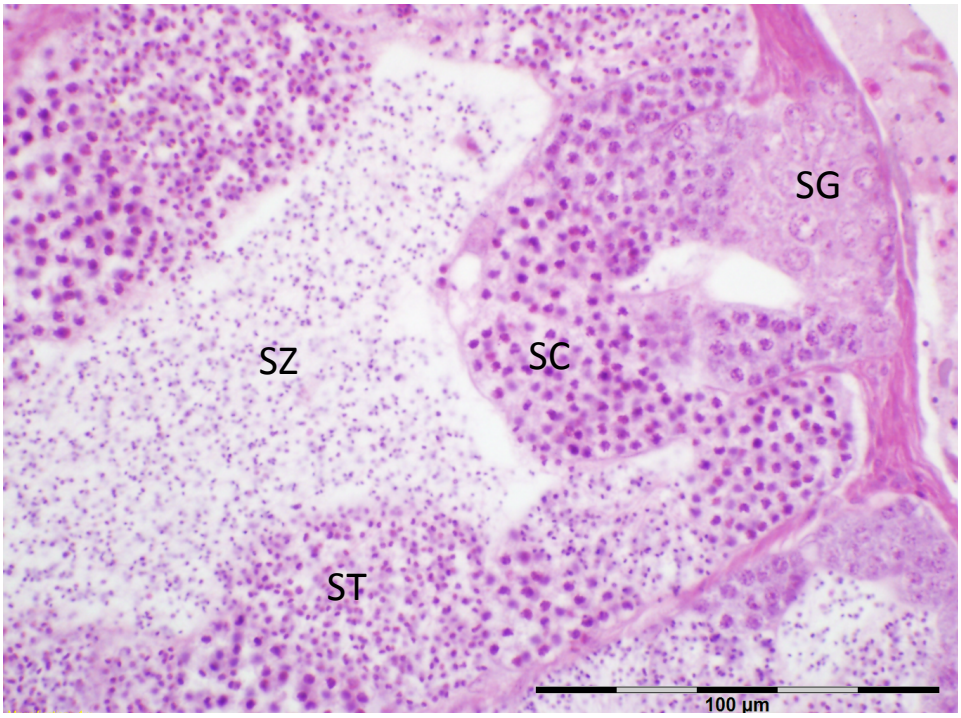

Figure 7: Spermatogenesis in sharksucker testis, showing spermatogonia (SG), spermatocytes (SC), spermatids (ST) and spermatozoa (SZ).

Male histology phases were characterized using the criteria described by Brown-Peterson et al. (2002) for cobia and are in line with the standardized terminologies outlined by Brown-Peterson et al. (2011) (Table 6).

The subphases of the spawning capable class are differentiated by how continuous the germinal epithelium is and by the location of any discontinuous germinal epithelium in the testes (Figure 8, Figure 9, Figure 10). Active spermatogenesis along with the presence of spermatocysts is evident in all of the spawning capable subphases although the presence of spermatogonia in spermatocysts only occurs in the Early-GE subphase and spermatogonia are uncommon in the Mid- and Late-GE subphases (Brown-Peterson et al. 2011).

Table 6: Reproductive phases and sub-phases of male sharksuckers. Adapted from Brown-Peterson et al. 2011.

Phase	Histological features
Immature	Contain only Sg1. Lobules lack lumen.
Developing	Presence of spermatocysts with Sg1, Sc1, Sc2, St and/or Sz. No sz in sperm ducts. Continuous GE.
Early developing	Stages of spermatogenesis through Sc1 present.
Spawning capable	Sz in sperm ducts and/or in lumens.
Early-GE	All GE is continuous.
Mid-GE	GE is discontinuous near sperm ducts but continuous peripherally.
Late-GE	GE is discontinuous throughout.
Regressing	Presence of residual Sz and peripheral spermatogonia. The few spermatocysts present contain only Sc2, St and Sz.
Regenerating	Spermatogonia present but lack spermatocysts. Continuous GE.

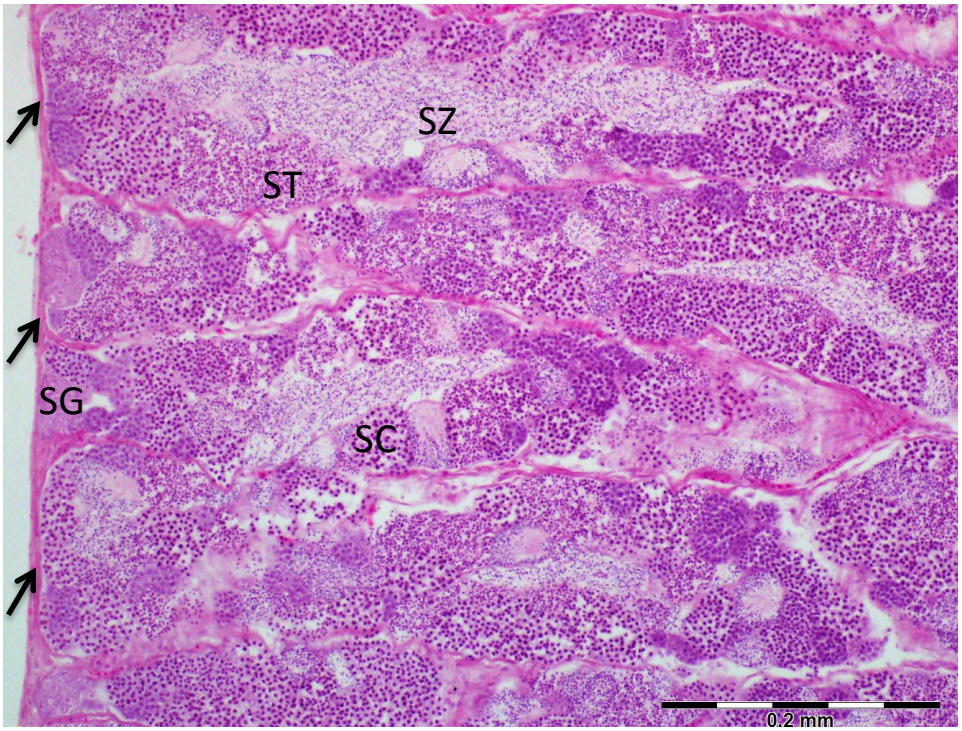

Figure 8: Spermatogenesis in sharksucker testis, showing spermatogonia (SG), spermatocytes (SC), spermatids (ST), spermatozoa (SZ). Arrows indicate continuous germinal epithelium (CGE).

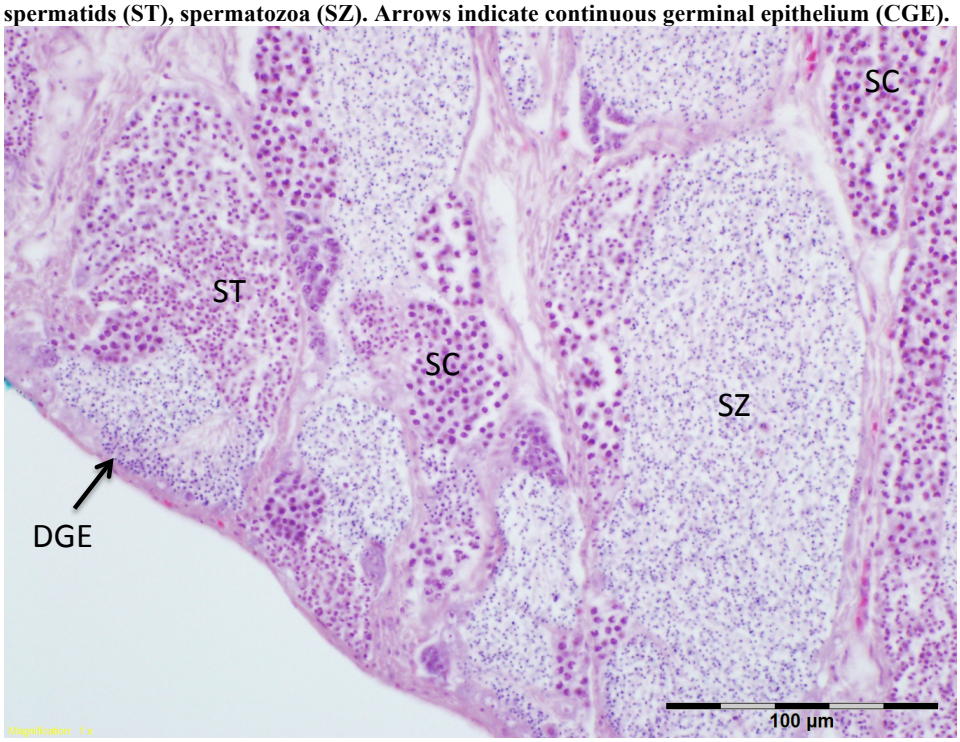

Figure 9: Spermatocytes (SC), spermatids (ST) and spermatozoa (SZ) in a sharksucker testis with discontinuous germinal epithelium (DGE).

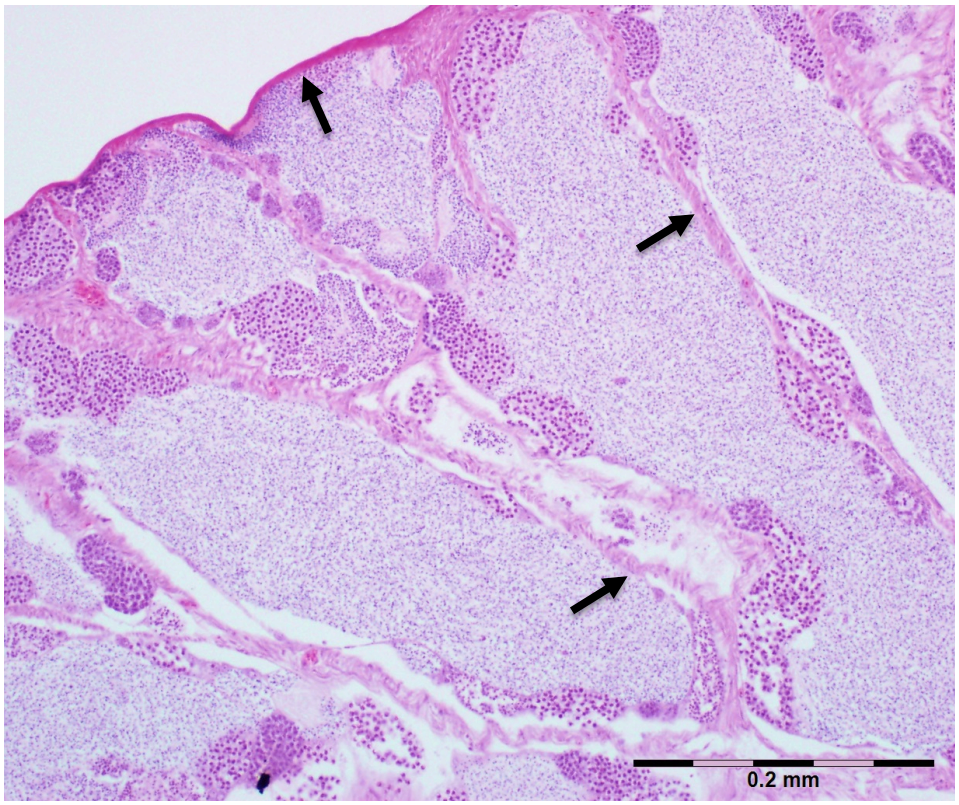

Figure 10: Discontinuous germinal epithelium (DGE) in sharksucker testis (arrows).

RESULTS

Growth

A total of 184 sharksuckers were measured and sexed (Figure 11).

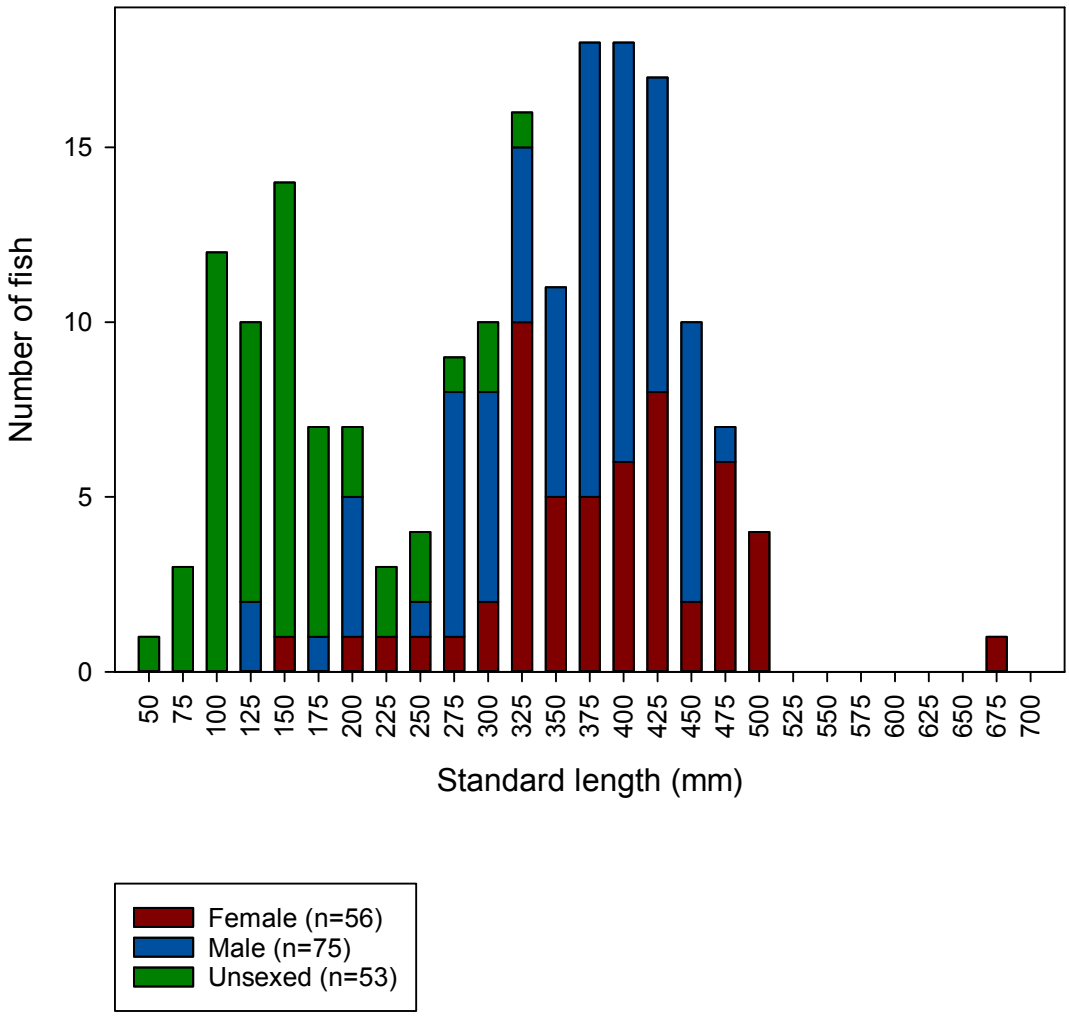

Figure 11: Length-frequency distributions of sharksuckers (n = 184).

Weight could be reliably predicted from length across all sizes and sex categories from an approximately cubic relationship (r^2>0.92 for all equations). Regression formulas are provided for each category in log-log form (Figure 12, Table 7) as well as for untransformed values (Figure 13, Table 8).

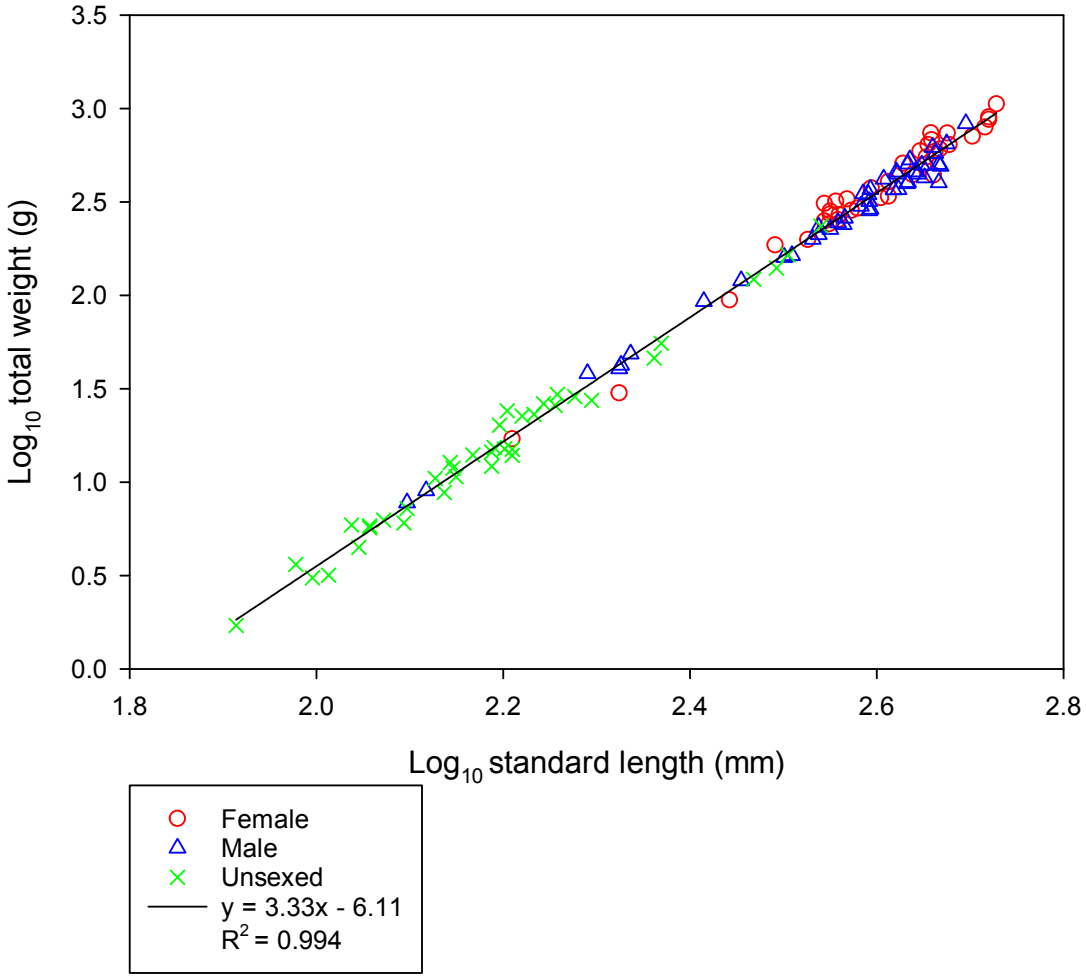

Figure 12: Log_{10} weight-length regression of sharksuckers (n = 121).

Table 7: Log_{10} weight-length regressions of sharksuckers (n = 121).

Weight-length Regressions $Log_{10}WT=a+b(Log_{10}SL)$						
	n	*a*	SE of *a*	*b*	SE of *b*	r^2
Pooled	121	-6.11	0.06	3.33	0.02	0.99
Male	45	-5.90	0.13	3.24	0.05	0.99
Female	38	-6.39	0.24	3.44	0.09	0.97

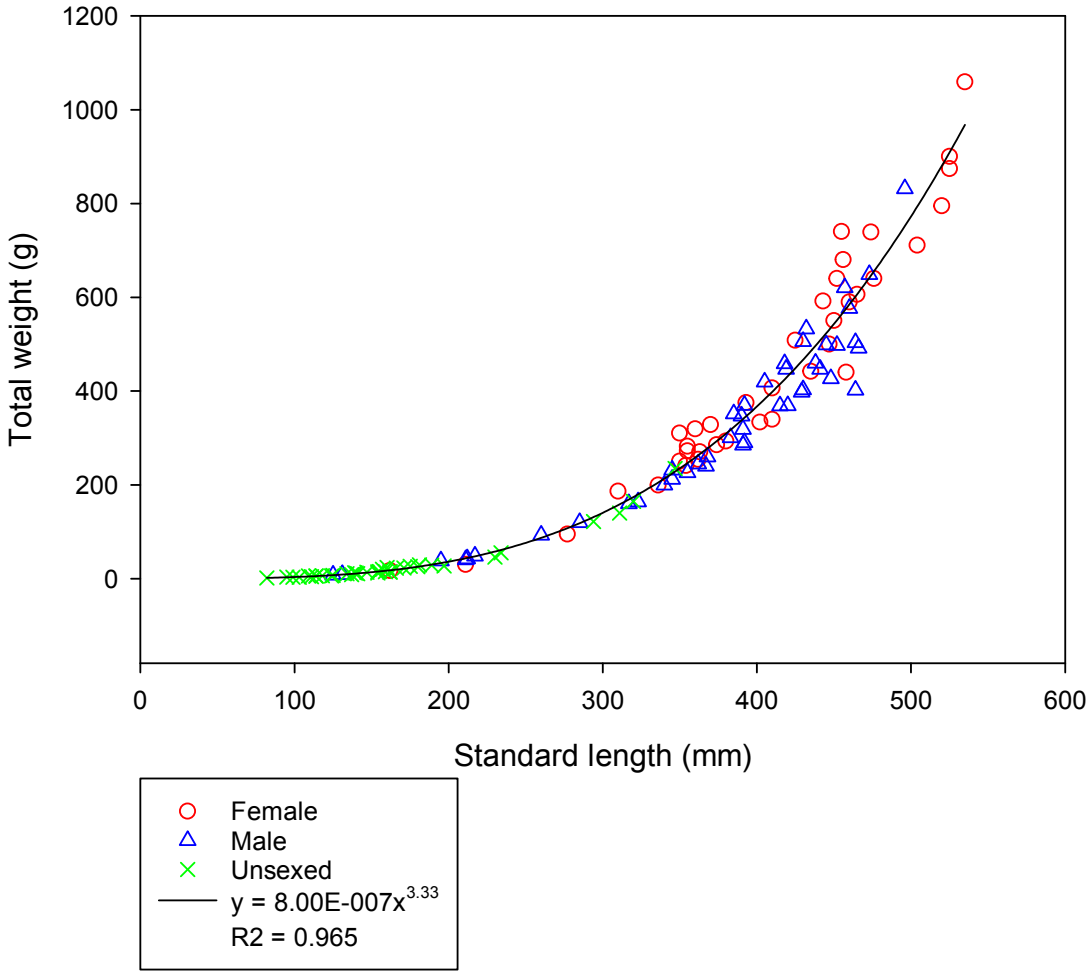

Figure 13: Weight-length relationship of sharksuckers (n = 121).

Table 8: Weight-length regressions of sharksuckers (n = 121).

	Weight-length Regressions $y=ax^b$					
	n	***a***	**SE of *a***	***b***	**SE of *b***	r^2
Pooled	121	8.00E-007	4.67E-007	3.33	0.10	0.97
Male	45	8.74E-007	1.10E-006	3.30	0.21	0.93
Female	38	2.35E-006	2.29E-006	3.16	0.16	0.94

The lowest frequency of opaque increments occurred in spring, and despite the low sample sizes, the pattern conformed to a single annual cycle and the use of a June 1 hatch date (Figure 14).

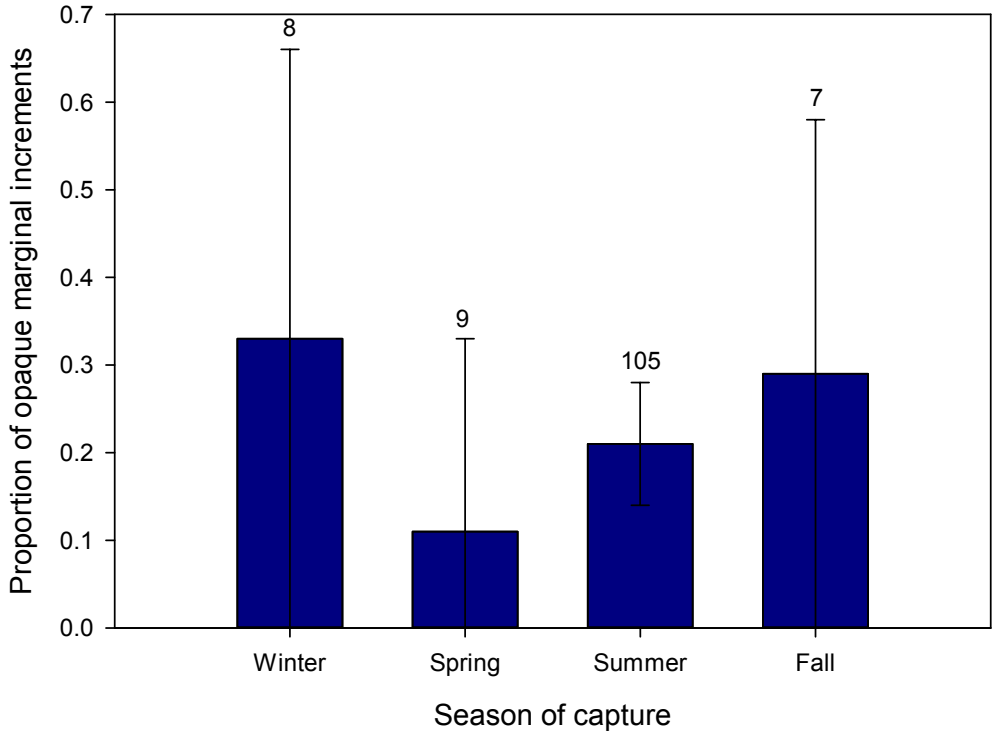

Figure 14: Probability of opaque marginal increment by season (n = 129) Numbers above columns denote sample size. Binomial confidence intervals (95%) are plotted.

Male fish in the growth analysis ranged from 211 mm SL to 473 mm SL and from 40.69 g TW to 649 g TW. Females ranged in length from 210 mm SL to 535 mm SL and in weight from 29.99 g TW to 1059 g TW. Unsexed fish ranged in length from 215 mm SL to 347 mm SL and in weight from 41.92 g TW to 235 g TW

Male sharksuckers aged from otoliths ranged in age from 1 to 8 y and females from 1 to 7 (Table 9,Table 10).

Table 9: Age-length key for male sharksuckers (n = 65).

Age-length key of male sharksuckers in percentages per age group									
SL Length Group (mm)	**Age in Years**								**n of fish in each category**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
200		0.67	0.33						3
250	0.14	0.57	0.29						7
300	0.09	0.73	0.09	0.09					11
350	0.06	0.11	0.39	0.33	0.11				18
400		0.11	0.16	0.42	0.16		0.16		19
450				0.29	0.14	0.43		0.14	7

Table 10: Age-length key for female sharksuckers (n = 46).

Age-length key of female sharksuckers in percentages per age group									
SL Length Group (mm)	**Age in Years**								**n of fish in each category**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
200	0.33	0.33	0.33						3
250		1.00							2
300	0.33	0.67							3
350	0.08	0.54	0.23	0.15					13
400			0.71	0.14		0.14			7
450			0.44	0.22	0.22	0.11			9
500			0.22	0.33	0.22	0.11	0.11		9

Von Bertalanffy growth parameters for pooled male and female sharksuckers are $L\infty = 466.66$, $K = 0.56$ and $t0 = -0.28$ (Figure 15, Table 11).

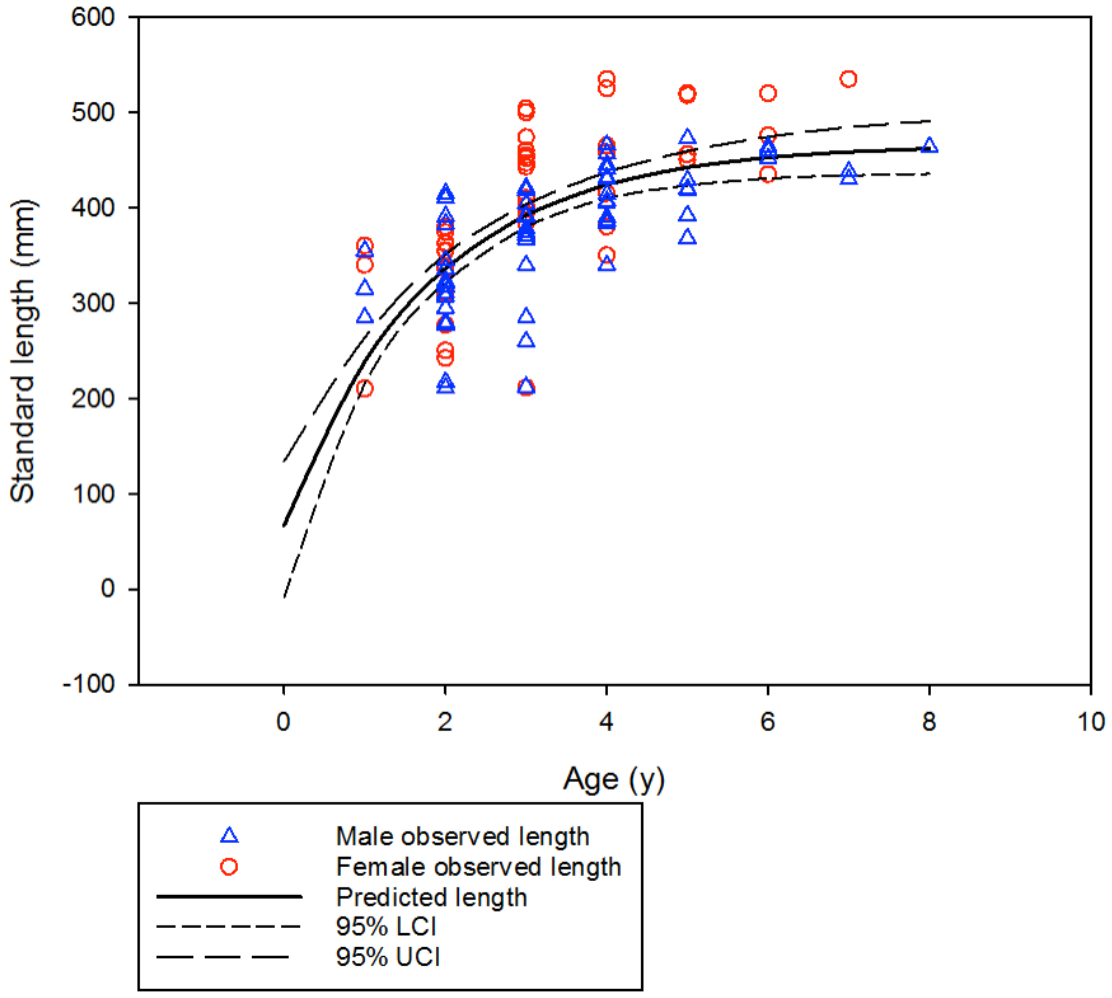

Figure 15: Observed and predicted length-at-age data with 95% confidence intervals for sharksuckers from the von Bertalanffy growth model (n = 113).

Table 11: Pooled von Bertalanffy parameters for sharksuckers (n = 113).

L_∞	K	t_0	n
466.66	0.56	-0.28	113

The von Bertalanffy growth model parameter L∞ was significantly different for male and female sharksuckers (p = 0.05) so growth was also modeled separately by sex.

By age-2 males achieved 73% of their asymptotic length (324 mm SL), whereas females reached 68% (349 mm SL) (Figure 16, Table 12, Figure 17, and Table 13).

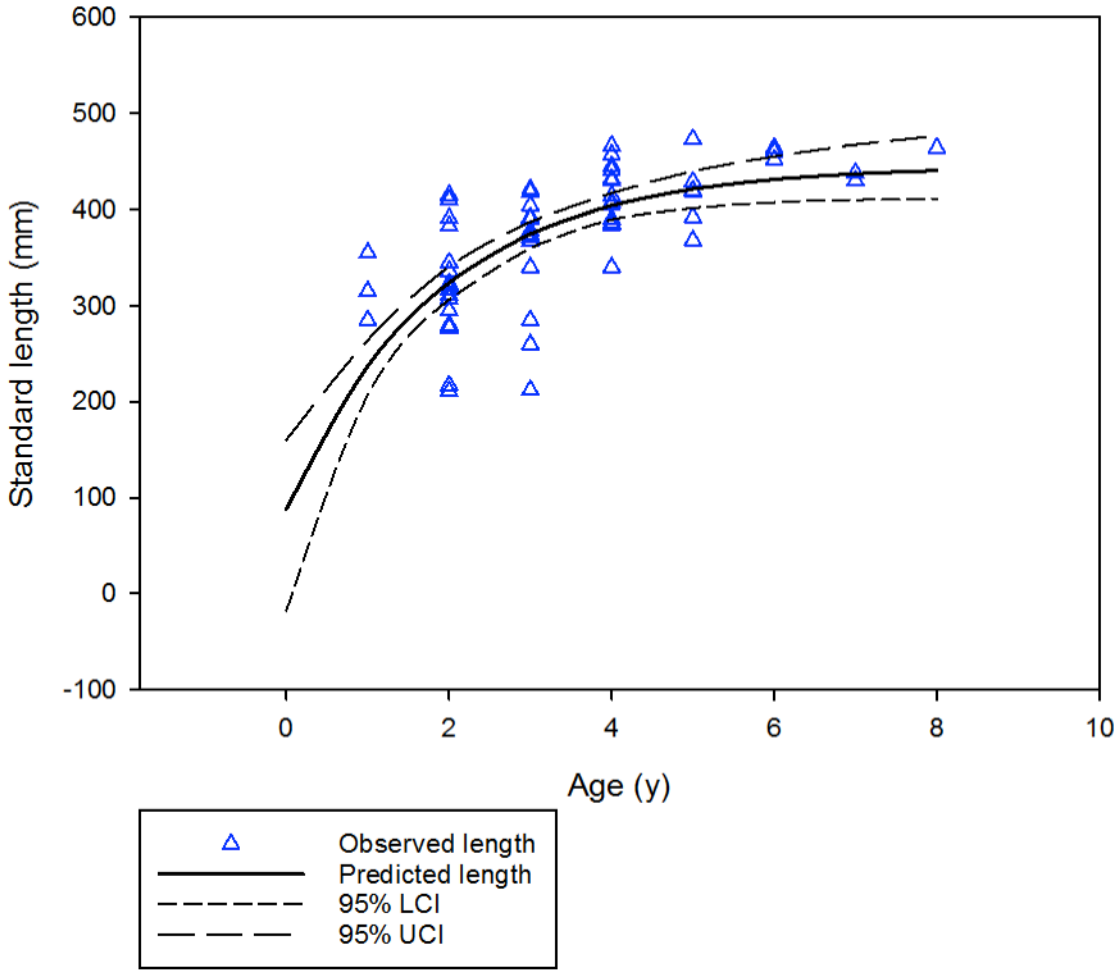

Figure 16: Observed and predicted length-at-age data with 95% confidence intervals for male sharksuckers from the von Bertalanffy growth model (n = 66).

Table 12: von Bertalanffy growth model parameter estimates for male sharksuckers.

L_∞	K	t_0	n
445.41	0.54	-0.41	66

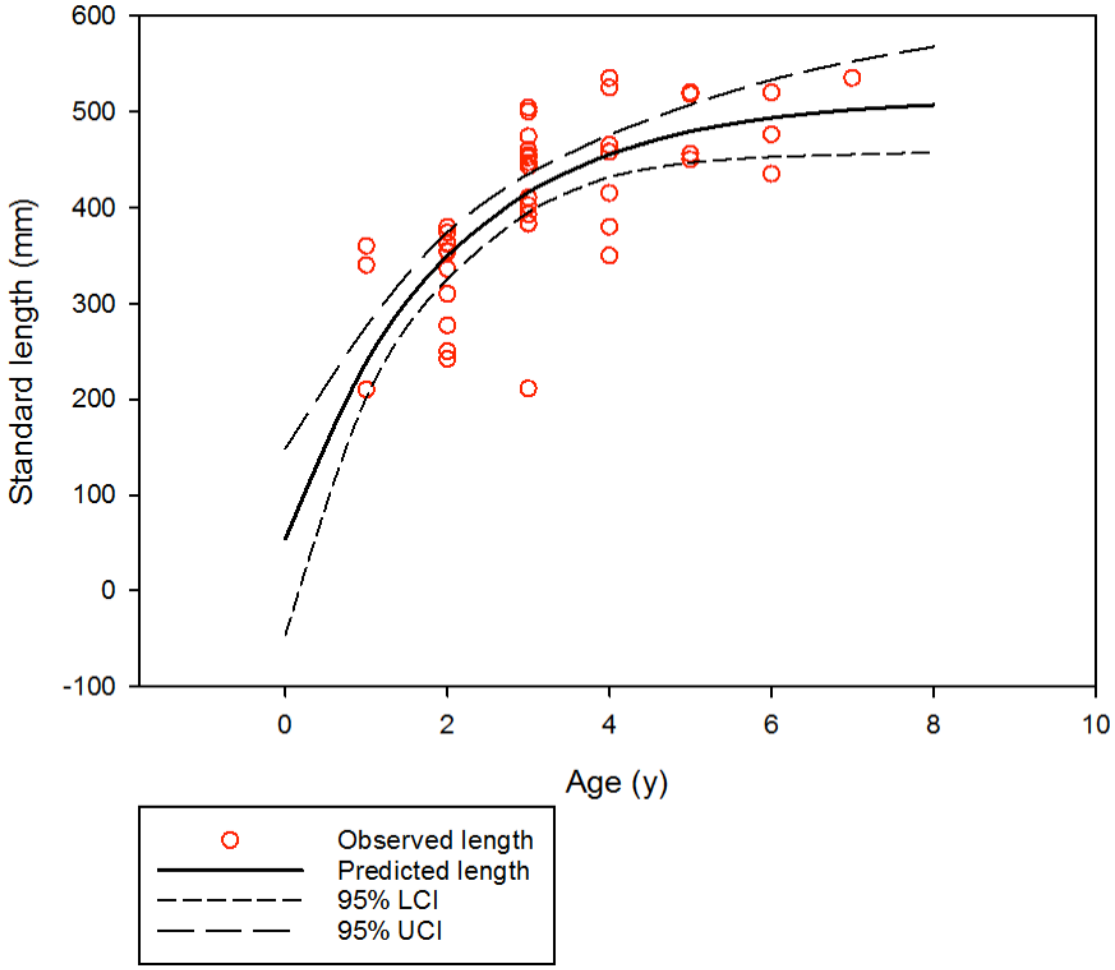

Figure 17: Observed and predicted length-at-age data with 95% confidence intervals for female sharksuckers from the von Bertalanffy growth model (n = 47).

Table 13: von Bertalanffy growth model parameter estimates for female sharksuckers.

L_∞	K	t_0	n
514.69	0.51	-0.22	47

Diet

Frequency of Occurrence of Prey Items

Of the 179 sharksucker stomachs examined, 105 (59%) contained identifiable diet items (Table 14).

Table 14: Frequency of occurrence of empty stomachs. Numbers in parentheses denote percentage out of all stomachs (n = 179).

Category	All n=179	<249 mm SL n=55	>250 mm SL n=124
All stomachs	179	55	124
Stomachs with identifiable contents	105 (59%)	37 (67%)	68 (55%)
Empty stomachs	32 (18%)	2 (4%)	30 (24%)

Parasitic digenetic trematodes occurred in 32 stomachs (30%), and along with bait and detritus were excluded from consideration as diet items (Table 15).

Table 15: Frequency of occurrence of parasitic trematodes. Numbers in parentheses denote percentage out of stomachs with identifiable contents (n = 105).

Category	All n = 105	<249 mm SL n = 37	>250 mm SL n = 68
Digenetic trematode	32 (30%)	3 (8%)	29 (43%)

Detritus, including all unidentified granular or sand-like substances, as well as items that appear anthropological in origin such as plastic beads and woven fibers, occurred in 19 stomachs (18%). Vegetative items such as blades of grass were found in 27 separate stomachs (26%). A total of 39 stomachs (37%) contained contents that were

too digested to be positively identified and 24 stomachs (23%) had only pink or yellow mucus. An additional 11 stomachs (10%) contained only digenetic trematodes with no prey items. For analysis, stomachs with identifiable contents totaled 105).

Fish (in part or whole) had the highest frequency of occurrence and were found in 58 sharksucker stomachs (55% out of 105). Whole fish were rarely observed but fish flesh, non-sharksucker fish scales, teeth, bones, spines, and fin rays were common. Crustaceans were the second most frequently occurring prey item (48%).

Within the crustacean prey item category, euphausiid, stomatopod and larval decapod crustaceans occurred in 25 stomachs (24%) while adult decapods (crabs) occurred in 10 stomachs (10%). Ectoparasitic copepods occurred in 22 stomachs (21%). Ectoparasitic copepods are analyzed separately below. Amphipods were found in 10 stomachs (10%), and non-parasitic copepods were found in 8 (8%). Crustacean eggs of various types were found in 16 stomachs (15%) and usually occurred in connection with ectoparasitic copepods. Ostracods were found in two stomachs (2%) and one stomach had a parasitic isopod (1%).

Other taxa found in diet included non-crustacean eggs (1%), gastropods and bivalves (17%), echinoderms (4%), annelid worms (2%) and sponges (3%).

Frequencies of occurrence of prey items broken down by size class of sharksucker (<249mm SL vs. >250mm SL) are listed in Table 16.

Table 16: Dietary categories by size class. Numbers in parentheses denote percentage out of stomachs with identifiable contents (n = 105).

Category	All n = 105	<249 mm SL n = 37	>250 mm SL n = 68
Crustacean	50	29 (78%)	21 (31%)
Planktonic decapod and euphausiid	25	11 (30%)	14 (21%)
Adult crab	10	1 (3%)	9 (13%)
Ectoparasitic copepod	22	17 (46%)	5 (7%)
Other copepod	8	7 (19%)	1 (1%)
Amphipod	10	8 (22%)	2 (3%)
Cladoceran	4	3 (8%)	1 (1%)
Ostracod	2	1 (3%)	1 (1%)
Isopod	1	1 (3%)	0
Fish	58	17 (46%)	41 (60%)
Eggs	17	11 (30%)	6 (9%)
Mollusk	17	6 (16%)	13 (19%)
Planktonic gastropod and bivalve	6	4 (11%)	2 (3%)
Benthic gastropod and bivalve	12	2 (5%)	10 (15%)
Echinoderm	4	2 (5%)	2 (3%)
Sponge	3	0	1 (4%)
Annelid	2	0	2 (3%)
Vegetation	27	5 (14%)	22 (32%)
Detritus	19	3 (8%)	16 (24%)
Other	7	1 (3%)	6 (9%)
Unidentified	64	30 (81%)	32 (47%)

Ectoparasitic Copepods as Prey Items

The majority of ectoparasitic copepods occurred in fish <249 mm SL (17 out of 37 stomachs, 46%). Ectoparasitic copepods were found in only 5 stomachs of 5 fish >250 mm SL (7% of 68 total).

Of the 22 sharksucker stomachs containing ectoparasitic copepods, 20 (91%) were associated with an elasmobranch host (Table 17).

Table 17: Host associations for sharksuckers that consumed ectoparasitic copepods (n = 22).

Month of Capture	Location	Host Species	Host TL (mm)	Sharksucker SL (mm)	Parasitic Copepods (n)
June	Bend	Marine turtle sp.	NA	82	5
May	Keys	*Carcharhinus limbatus*	NA	95	4
October	Bend	*Carcharhinus limbatus*	2690	103	7
January	Keys	*Carcharhinus limbatus*	920	109	3
January	Keys	*Carcharhinus limbatus**	1590	111	1
May	Keys	*Carcharhinus limbatus*	1510	124	2
January	Keys	*Carcharhinus limbatus*	1530	125	2
February	Keys	*Carcharhinus leucas*	1990	125	3
February	Keys	*Galeocerdo cuvier*	2690	134	1
April	Bend	*Carcharhinus limbatus*	1030	137	7
January	Keys	*Carcharhinus limbatus**	1590	139	1
October	Bend	*Carcharhinus limbatus*	1590	141	6
February	Keys	*Carcharhinus limbatus*	1260	155	3
NA	NA	*Carcharhinus leucas*	NA	171	1
February	Keys	*Carcharhinus leucas*	2030	189	2
February	Keys	*Carcharhinus leucas*	2230	211	6
September	Bend	*Negaprion brevirostris***	2800	217	1^
September	Bend	*Negaprion brevirostris***	2800	250	2
June	Bend	*Negaprion brevirostris*	2010	279	3
July	Bend	None	NA	280	6
June	Bend	*Carcharhinus leucas*	1900	430	1
May	Keys	*Ginglymostoma cirratum*	2300	458	1
Total:		**n = 18**			**67**
Mean:			**1910**	**185**	**3**
Min:			**920**	**82**	**1**
Max:			**2800**	**458**	**7**

* = Same host *C. limbatus*

** = Same host *N. brevirostris*

^ = Also n = 1 Parasitic isopod

Associated elasmobranch total lengths ranged from 920 mm to 2800 mm with a mean total length of 1910 mm. Associated elasmobranchs were represented by 18

individual hosts across 5 species. The majority of the associated elasmobranchs were carcharhinids (n = 18, 94%) and the remaining shark was a member of the Orectolobiformes (Ginglymostoma cirratum). Ectoparasitic crustaceans were found in sharksuckers captured in both the Keys and Big Bend locations and across multiple capture months.

Index of Relative Importance of Prey Items

Pooled IRI for the major prey categories "fish," "crustacean," "mollusk," "other," and "unidentified" were constructed for small (<249 mm SL, n = 21) and for large (>250 mm SL, n = 24) sharksuckers (Table 18 and Table 19).

Table 18: Pooled index of relative importance by percentage of diet items in sharksuckers <249mm SL (n = 21).

Prey	Individual Prey Items (N)	%N	Dried Volume (g)	%V	%F	IRI	%IRI
Crustacean	775	0.61	0.2539	0.41	0.95	0.9702	0.73
Fish	12	0.01	0.1491	0.24	0.48	0.1191	0.09
Mollusk	470	0.37	0.0666	0.11	0.19	0.0908	0.07
Other	4	0.00	0.0025	0.00	0.19	0.0014	0.00
Unidentified	12	0.01	0.1473	0.24	0.57	0.1413	0.11
Total	1273		0.6194			1.3228	
Sample N = 21							

Table 19: Pooled index of relative importance by percentage of diet items in sharksuckers >250mm SL (n = 24).

Prey	Individual Prey Items (N)	%N	Dried Volume (g)	%V	%F	IRI	%IRI
Crustacean	120	0.64	4.4496	0.26	0.46	0.4124	0.40
Fish	23	0.12	6.3878	0.38	0.63	0.3110	0.30
Mollusk	11	0.06	0.2048	0.01	0.08	0.0059	0.01
Other	12	0.06	3.6347	0.21	0.46	0.1271	0.12
Unidentified	22	0.12	2.3445	0.14	0.67	0.1698	0.17
Total	188		17.0214			1.0262	
Sample N = 24							

Crustaceans comprised the largest component of the pooled index of relative importance of sharksuckers <249 mm SL (n = 21, Figure 18). Crustaceans made up 60.9% of the numerical abundance, 41.0% of the volume, and 73.3% of the pooled IRI. Fish were only 0.9% of the numerical abundance but totaled 24.1% of the volume and 9.0% of the pooled IRI. Mollusks (predominantly planktonic gastropods) made up 37% of the numerical abundance, 10.8% of the volume and 6.9% of the pooled IRI while other prey items made up 0.0% of the numerical abundance, 0.4% of the volume and 0.1% of the pooled IRI. Unidentified prey items composed 0.9% of the numerical abundance, 23.8% of the volume and 10.7% of the pooled IRI.

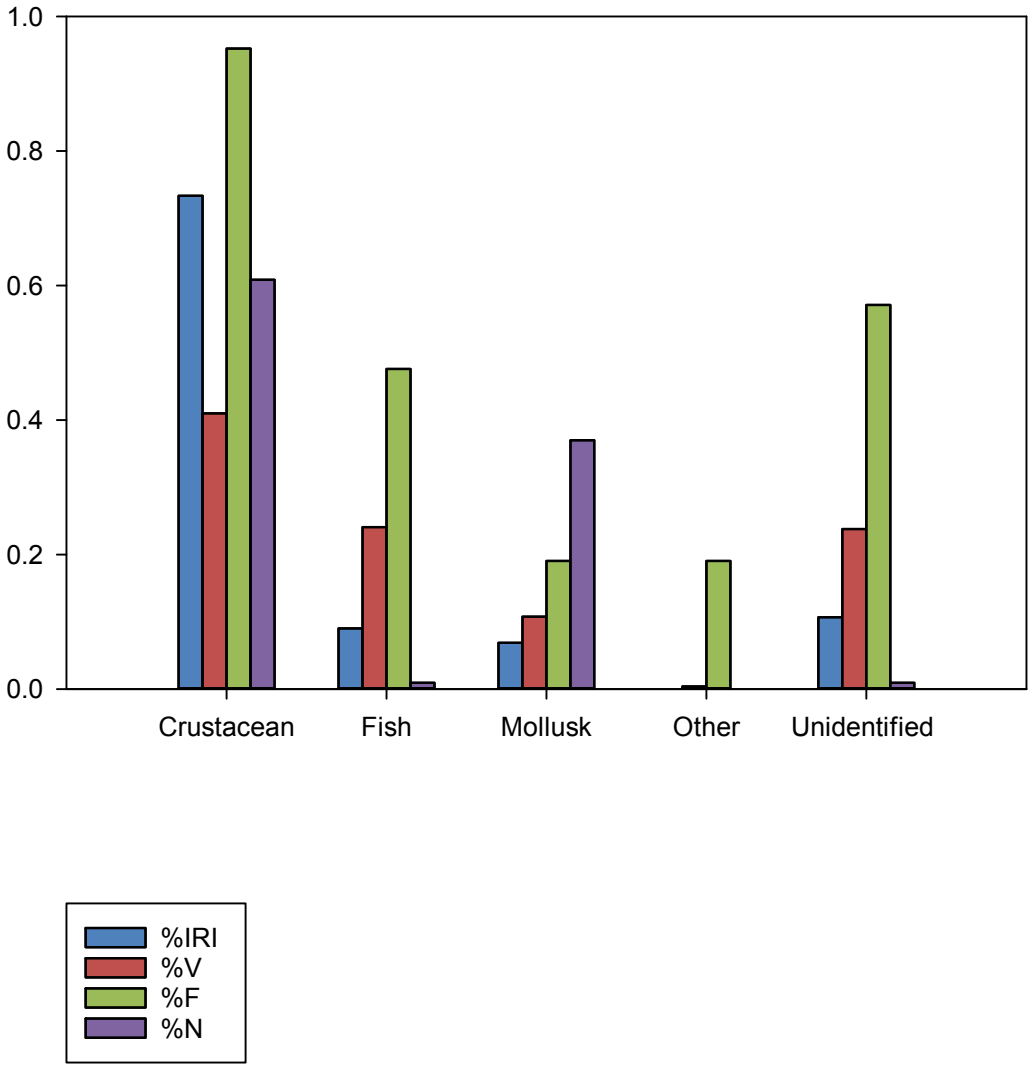

Figure 18: Pooled index of relative importance of sharksuckers <249 mm SL (n = 21).

Fish and non-crustacean prey were more important in the diet of sharksuckers >250 mm SL (Figure 19). Crustaceans still comprised 63.8% of the numerical abundance, 26.1% of the volume and 42.2% of the pooled IRI of the larger sharksuckers. However, fish were also important, composing 12.2% of the numerical abundance, 37.5% of the volume, and 30.3% of the IRI. Mollusks were 6.0% of the numerical abundance, 1.2% of the volume and 0.6% of the pooled IRI.

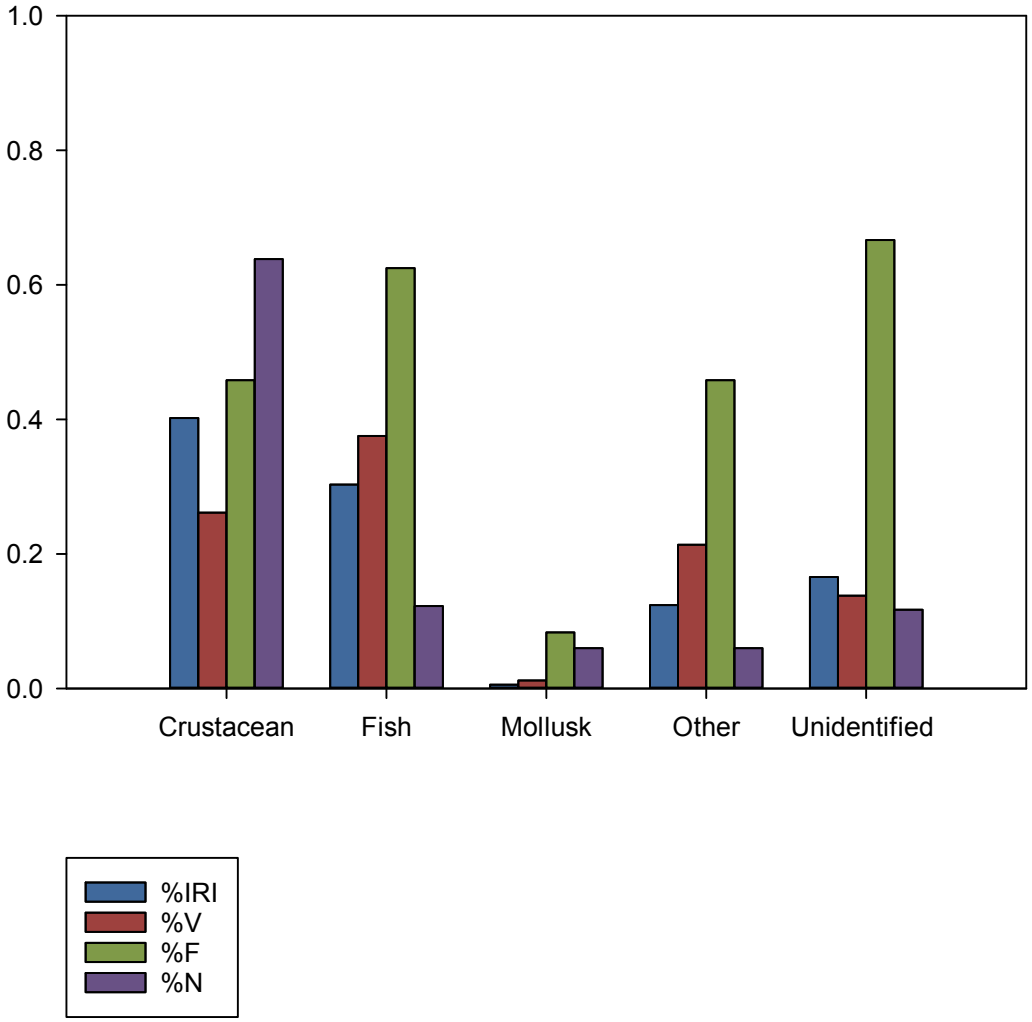

Figure 19: Pooled index of relative importance of sharksuckers >250 mm SL (n = 24).

Other prey made up 6.0% of the numerical abundance, 21.4% of the volume, and 12.4% of the IRI. Unidentified prey items were 11.7% of the numerical abundance, 13.8% of the volume, and 16.5% of the IRI.

Mean Proportion of Prey Items

Crustacean prey made up the largest dietary component of small sharksuckers (<249mm SL) in mean proportion by number (MN) (Table 20, Figure 20).

Table 20: Proportion of sharksucker diet by MN.

Prey	MN	
	<249mm SL	>250mm SL
Ectoparasitic copepod	0.36	0.05
Other crustacean	0.39	0.20
Fish	0.11	0.33
Echinoderm	0.00	0.03
Mollusk	0.09	0.02
Sponge	0.00	0.01
Unidentified	0.06	0.36

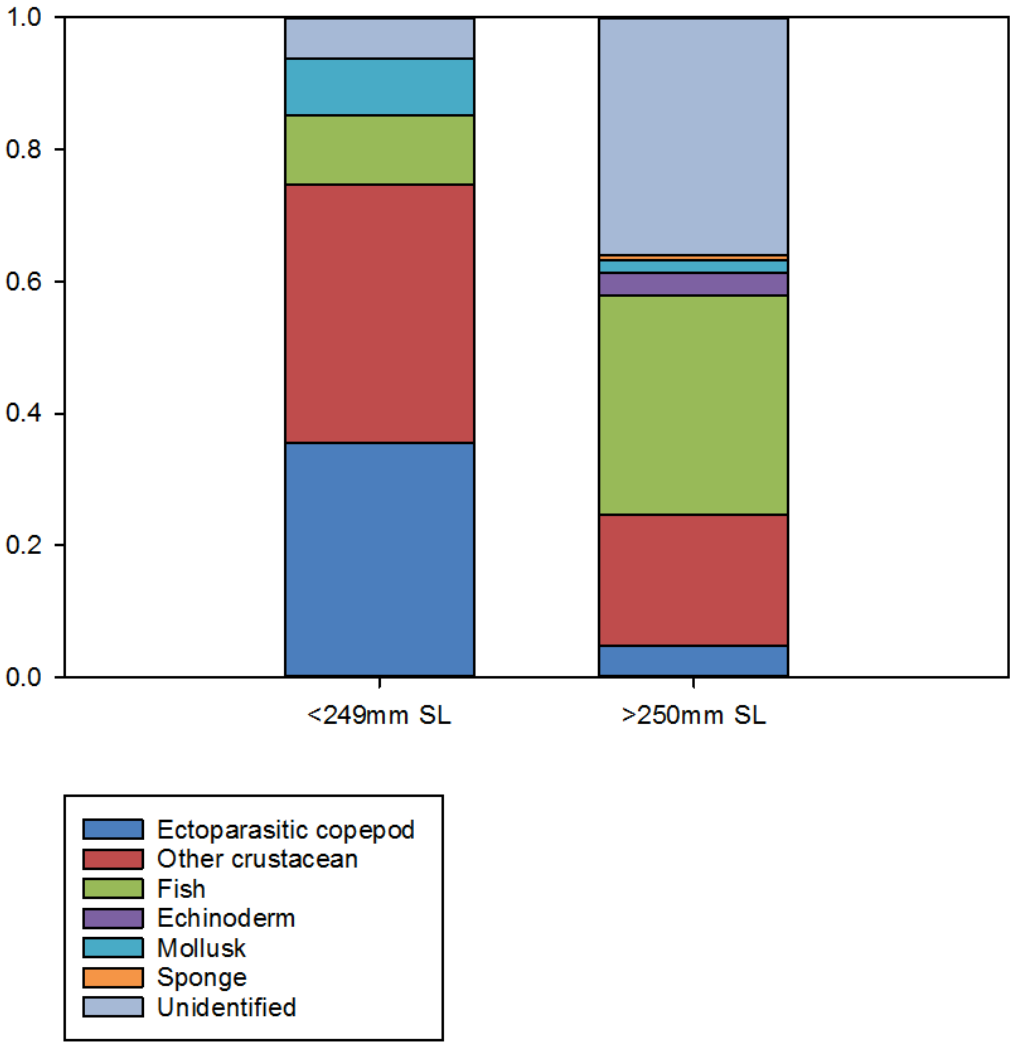

Figure 20: Proportion of sharksucker diet by MN.

Ectoparasitic copepods comprised 36% of the total MN while other crustaceans were 39%. Crustacean prey were less common in sharksuckers >250mm SL. Ectoparasitic copepods were only 5% MN in large sharksuckers while other crustacean prey was 20%. Fish made up a larger proportion in the diet of large sharksuckers (33%) but was only 11% of the MN of sharksuckers <249mm SL. Gastropod and bivalve prey constituted 9% MN of the small sharksuckers but only 2% of the large. Unidentifiable items were the largest proportion of large sharksucker MN at 36% but were only 6% in the small size category. There were no echinoderm or sponge prey in small sharksuckers but they composed 3% and 1% of the large sharksuckers MN, respectively.

Crustacean prey also made up the bulk of mean proportion by volume (MV) in small sharksuckers. Ectoparasitic copepods were 20% of the MV of sharksuckers <249mm SL while other crustacean prey contributed 34% (Table 21, Figure 21). Ectoparasitic copepods did not contribute to the MV of sharksuckers >250mm SL but other crustaceans made up 13%. Fish were 15% of the MV of small sharksuckers but 32% MV of large sharksuckers. Gastropods and bivalves comprised 8% of the MV of sharksuckers <250 mm SL and 4% MV in large fish. Sponges did not contribute to the MV of either size category, and echinoderms did not contribute to the MV of small sharksuckers. Holothurian echinoderms made up 8% of the MV of sharksuckers >250mm SL, however. Unidentified items were 23% of the MV of small sharksuckers and 43% MV of larger.

Table 21: Proportion of sharksucker diet by MV.

Prey	MV	
	<249mm SL	>250mm SL
Ectoparasitic copepod	0.20	0.00
Other crustacean	0.34	0.13
Fish	0.15	0.32
Echinoderm	0.00	0.08
Mollusk	0.08	0.04
Sponge	0.00	0.00
Unidentified	0.23	0.43

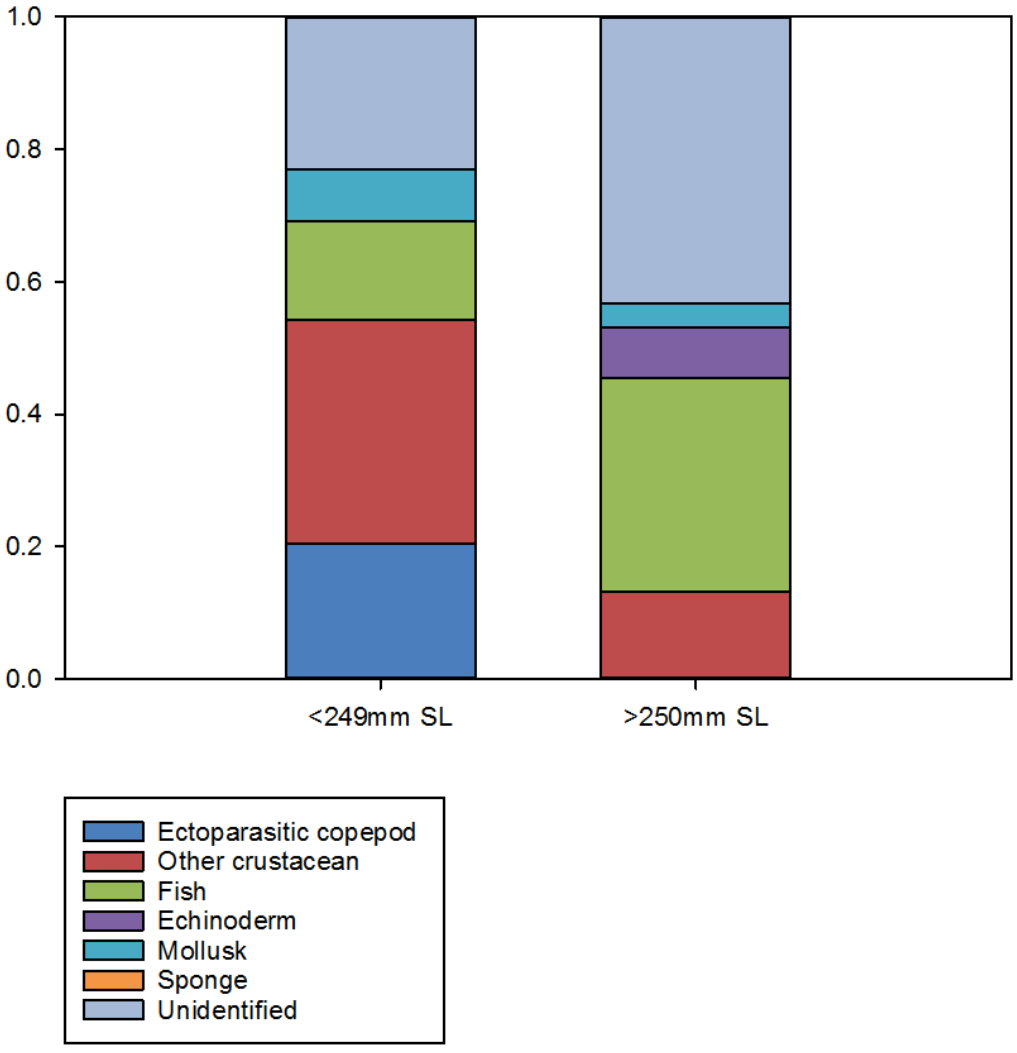

Figure 21: Proportion of sharksucker diet by MV.

Stable Isotope Analysis

Carbon and nitrogen values were derived from the white muscle of sharksuckers (n 240) from the Big Bend (n = 95) and Keys (n = 145) sampling areas (Figure 22, Figure 23). Sharksucker $\delta^{15}N$ ranged from 6.2 to 17.2 (n = 240, mean = 12.0, SD = 1.88) and $\delta^{13}C$ ranged from -11.1 to -24.3 (n = 240, mean = -16.2, SD = 1.91).

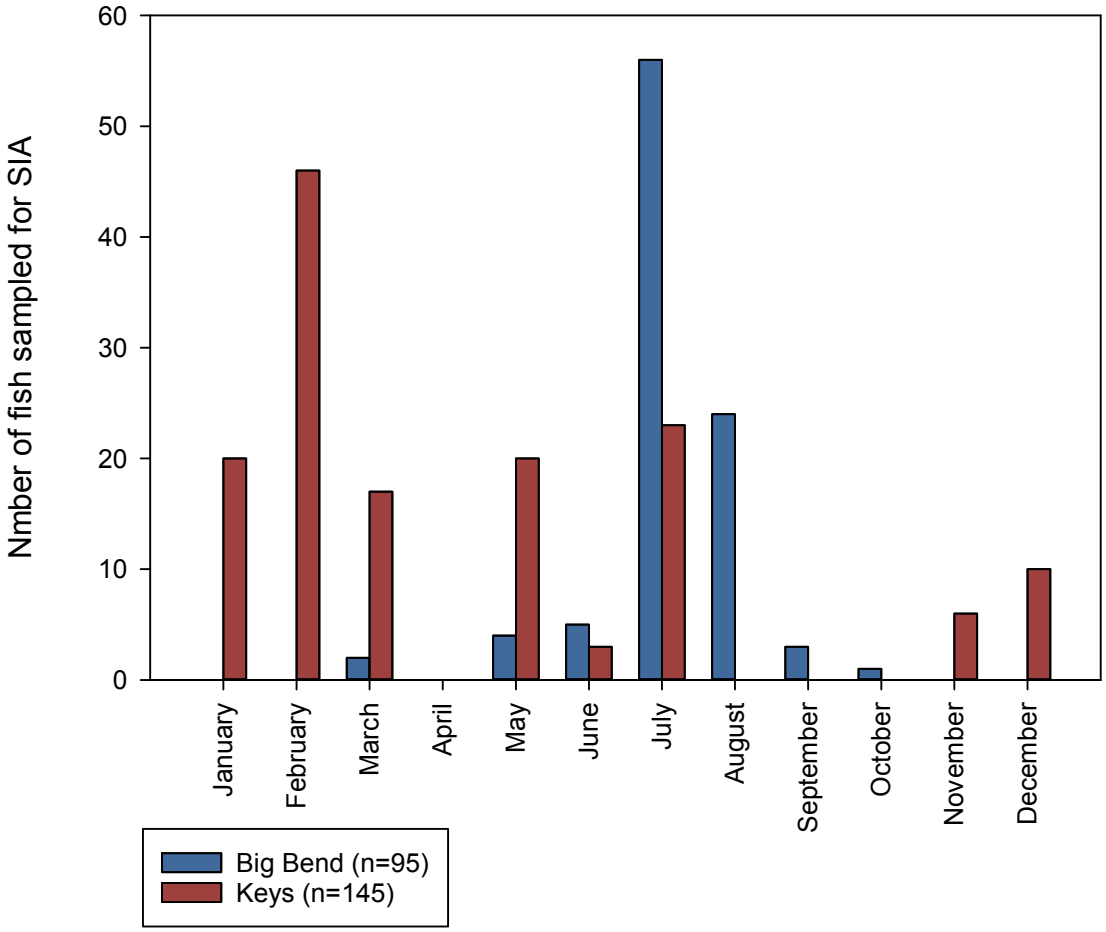

Figure 22: Number of fish sampled for stable isotope analysis by month and location (n = 240).

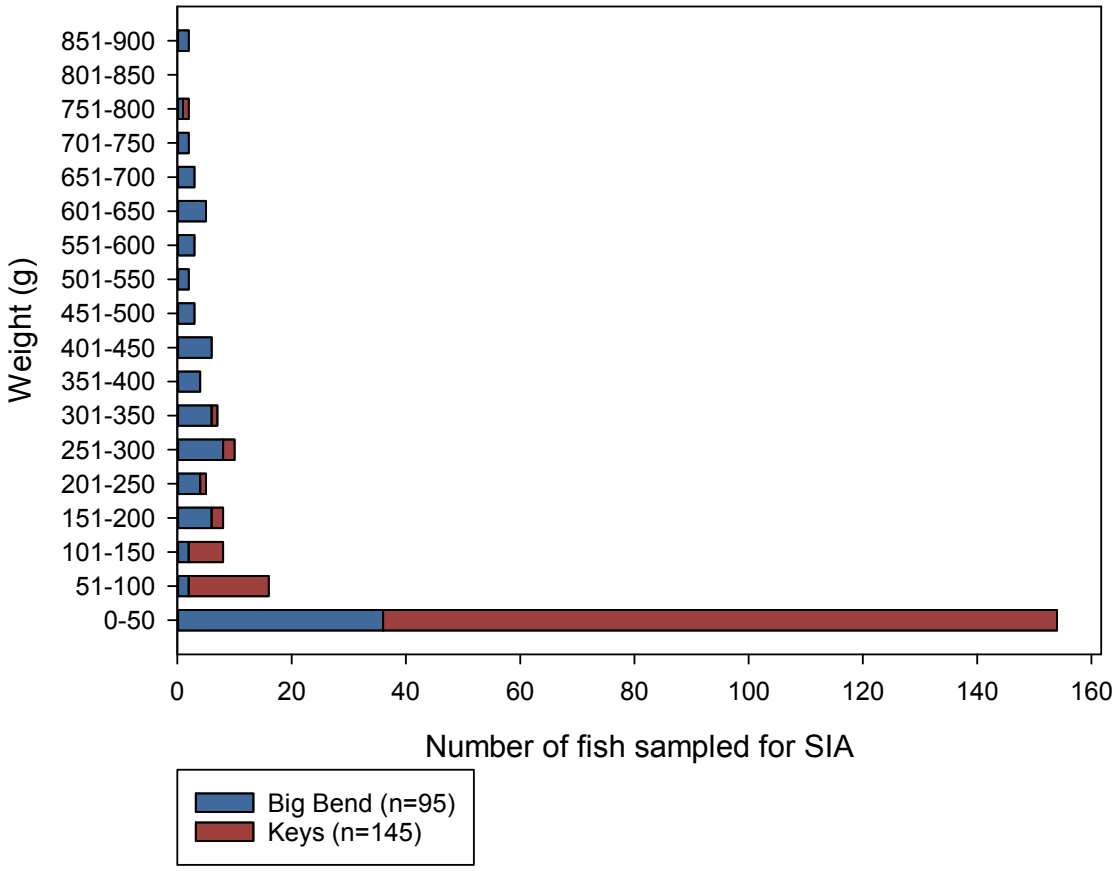

Figure 23: Number of fish sampled for stable isotope analysis by weight and location (n = 240).

Analysis of covariance revealed an interaction effect between sharksucker body size and study area (Big Bend vs. Keys) in both C and N analyses. Small sharksuckers had similar $\delta^{15}N$ values in Big Bend and Keys samples: however, sharksuckers from the Big Bend area showed a significant decrease in $\delta^{15}N$ values with increasing weight in g (ANCOVA, R-square = 0.3, $p<0.01$, Table 22 and Figure 24).

The least squares means of $\delta^{15}N$ for sharksucker weights of 5, 250 and 650g are shown in Table 23.

Table 22: Analysis of covariance of $\delta^{15}N$. Sample area (Big Bend vs. Keys) as a single factor and sharksucker weight in g. as covariate. Asterisks denote non-significant values.

$\delta^{15}N$	R-square = 0.3
Keys	y = (-0.0006*)X +12.6
Big Bend	y = (-0.0051)X + 12.6

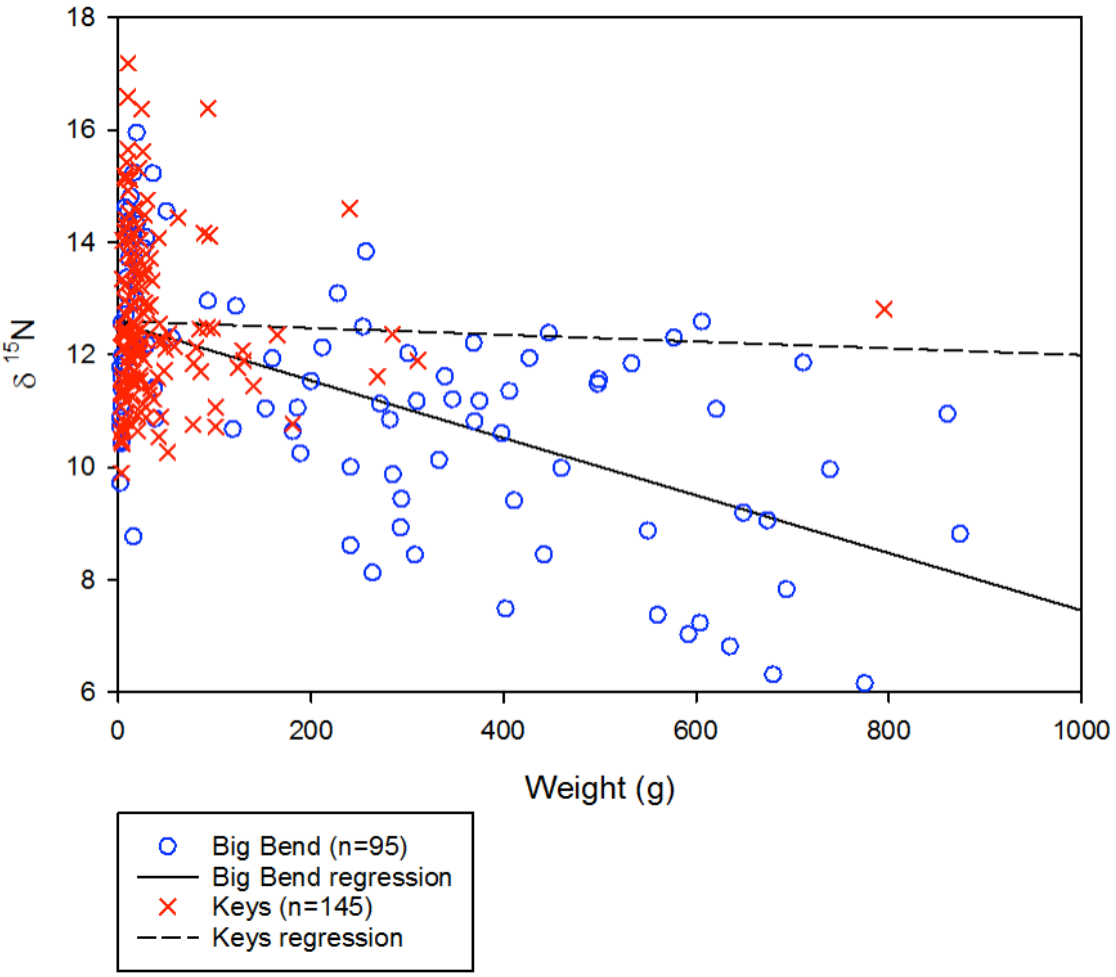

Figure 24: Analysis of covariance of $\delta^{15}N$. Sample area (Big Bend vs. Keys) as a single factor and sharksucker weight in g. as covariate.

Table 23: Least squares means with confidence intervals of $\delta^{15}N$ at various sharksucker weights. Asterisks denote non-significant values.

Weight (g)	Location	LSM	LCI	UCI
5*	Bend	12.5	12.1	13.0
	Keys	12.6	12.3	12.9
250	Bend	11.3	11.0	11.6
	Keys	12.5	11.7	13.2
650	Bend	9.2	8.6	9.8
	Keys	12.2	10.3	14.1

Small sharksuckers in the Keys were enriched in $\delta^{13}C$ values compared to sharksuckers in the Big Bend area. In the Big Bend area, sharksucker $\delta^{13}C$ values significantly increased with body size (ANCOVA, R-square = 0.11, $p<0.01$, (Table 24 Figure 25).

The least squares means of $\delta^{13}C$ for sharksucker weights of 5, 250 and 650g are shown in Table 25.

Table 24: Analysis of covariance of $\delta^{13}C$. Sample area (Big Bend vs. Keys) as a single factor and sharksucker weight in g. as covariate. Asterisks denote non-significant values.

$\delta^{13}C$	**R-square = 0.11**
Keys	y = (-0.0019*)X – 15.9
Big Bend	Y = (0.0522)X – 17.5

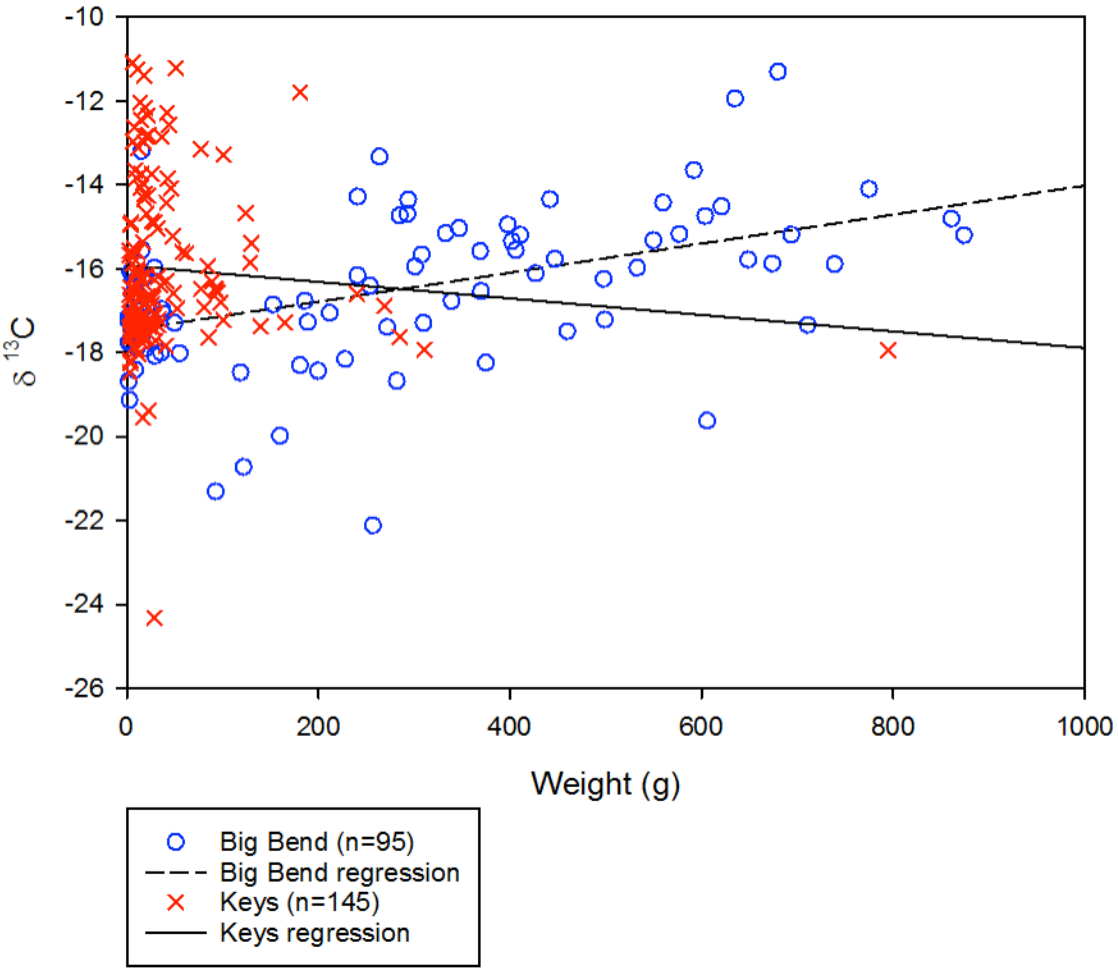

Figure 25: Analysis of covariance for $\delta^{13}C$. Sample area (Big Bend vs. Keys) as a single factor and sharksucker weight in g. as covariate.

Table 25: Least squares means with confidence intervals of $\delta^{13}C$ at various sharksucker weights. Asterisks denote non-significant values.

Weight (g)	Location	LSM	LCI	UCI
5	Bend	-17.5	-18.0	-16.9
	Keys	-15.9	-16.3	-15.6
250*	Bend	-16.6	-17.0	-16.2
	Keys	-16.4	-17.2	-15.6
650*	Bend	-15.2	-15.9	-14.5
	Keys	-17.2	-19.4	-15.0

Elasmobranch Associations

A total of 338 sharksuckers were sampled between October 2009 and September 2012. Out of the 338 fish collected, 220 (65%) were captured in association with a host species (Table 26).

Table 26: Species composition of associated hosts (n = 162).

Host Species	n
Elasmobranchs	
Carcharhinus acronotus	3
Carcharhinus isodon	1
Carcharhinus leucas	18
Carcharhinus limbatus	66
Galeocerdo cuvier	5
Ginglymostoma cirratum	19
Negaprion brevirostris	17
Sphyrna lewini	2
Sphyrna mokarran	18
Pristis pectinata	9
Teleosts	
Bagre marinus	1
Epinephelus itajara	1
Other	
Marine turtle sp.	2
Total	**162**

An additional 14 sharksuckers were probably associated with an elasmobranch host species but due to inconsistent labeling procedures these host associations could not be confirmed and these samples are not included in the calculations of host/shark associations. Data on associated host species for an additional 7 fish were lost so the actual sample of sharksuckers associated with a host may have been slightly higher.

Additionally, as sharksuckers were often observed detaching from their host to attack a baited hook, those fish that were captured on the longline may have been attached to a host immediately preceding the time of capture. Sharksuckers were considered to be associated with a host species if they were physically attached to the host or if they were observed swimming in close proximity to the host. 216 sharksuckers were associated with elasmobranchs, 2 with teleosts and 2 with marine turtles. Host elasmobranch species included 9 species of sharks and 1 species of sawtooth. Interestingly, one sharksucker was captured along with a *Remora remora* that was associated with the same *G. cuvier* host. A single host often supported multiple sharksuckers so the actual number of hosts is lower than the number of associated sharksuckers. The values shown here only quantify the association of sampled sharksuckers to their known hosts and somewhat reflect the relative capture rates of host elasmobranchs. They do not represent the total number of elasmobranchs captured by the coastal shark survey so they do not quantify the incidence or species of elasmobranchs with associated symbionts compared to the total number and types of elasmobranchs captured.

Reproduction

Gonadosomatic indices (GSI) for sharksuckers peaked in August for both males and females (Table 27,Figure 26). GSI were highly variable, particularly in female fish. The highest GSI values occurred in female fish. All GSI >3.00 occurred in fish captured in July or August except for one large female fish captured in April that also had a high GSI. GSI values in sexed fish ranged from 0.04 to 11.34 with a mean of 2.32 (n = 120, SD = 2.21).

Table 27: Gonadosomatic indices for sharksuckers by sex and month (n = 120). Numbers in parenthesis denote standard deviation.

Month	Male n	Male GSI	Female n	Female GSI
February	2	0.16 (0.06)	1	0.16 (0.00)
April	0	NA	3	3.42 (2.43)
May	3	0.51 (0.17)	3	0.88 (0.81)
June	12	0.82 (0.74)	3	0.93 (1.28)
July	19	2.13 (1.20)	15	3.38 (2.98)
August	31	2.38 (1.09)	21	3.91 (3.45)
September	3	0.89 (0.64)	3	0.55 (0.36)
October	0	NA	1	0.59 (0.00)

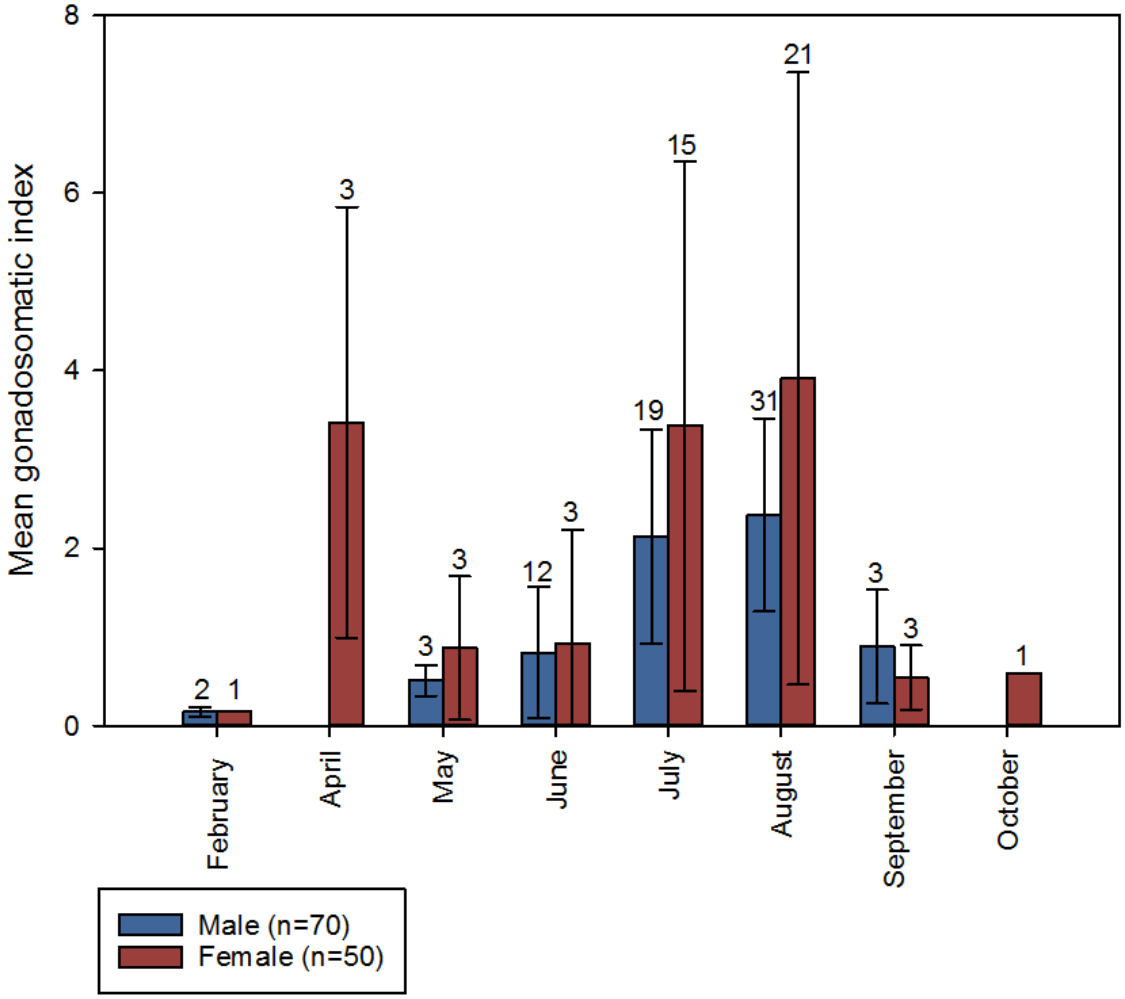

Figure 26: Mean gonadosomatic indices of sharksuckers by sex and month (n = 120).

Relative batch fecundity was positively correlated with GSI ($r^2 = 0.82$, $p<0.05$) (Figure 27).

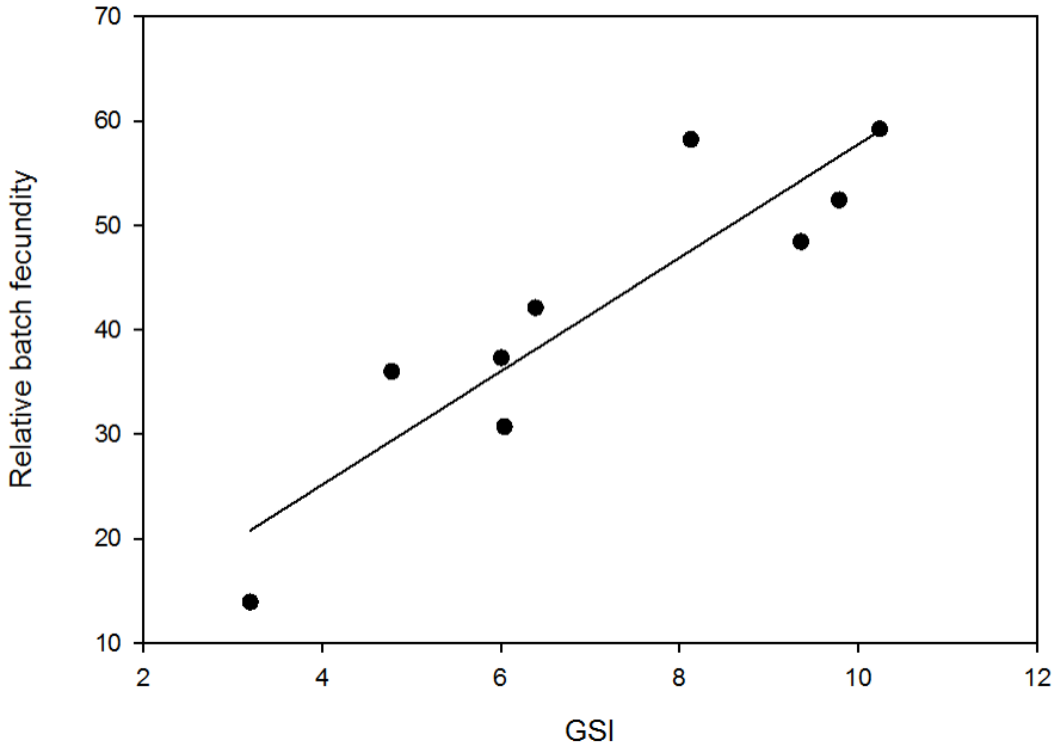

Figure 27: Relationship between relative batch fecundity and GSI in sharksuckers (n = 9, $r^2 = 0.82$).

Hydrated oocytes ranged in number from 10,192 to 42,151 (n = 9, mean = 21,968; SD = 10,280). Relative batch fecundities ranged from 13.9 to 59.2 hydrated oocytes per gram ovary-free body weight (OFBW) (n = 9, mean = 42.0; SD = 14.5, Table 28). Mean diameters of formalin preserved hydrated oocytes were between 1040 and 1623 µm (mean = 1327, SD = 171).

Table 28: Relative batch fecundity of sharksuckers using ovary-free body weight (OFBW) (n = 9).

ID	SL (mm)	OFBW (g)	Hydrated Oocytes	Mean Oocyte Diameter (µm)	Relative Batch Fecundity
1	535	735.68	10192	1623	13.9
2	518	870.11	42151	1253	48.4
3	362	194.98	11538	1127	59.2
4	415	403.23	21110	1388	52.4
5	410	359.72	20930	1320	58.2
6	455	655.52	24422	1040	37.3
7	520	786.29	33109	1416	42.1
8	435	408.34	14682	1380	36.0
9	504	638.68	19579	1393	30.7

Reproductive Histology

Female Reproductive Histology

For histological study, 14 female fish (162 to 476 mm SL) were examined (Table 29). Each ovary contained oocytes in the perinucleolar primary growth stage and oocytes in the cortical alveolar secondary growth stage.

Table 29: Categorization of female sharksucker histological samples (n = 14).

Female Histological Samples		
Phase	**Subphase**	**n**
Immature		0
Developing		1
	Early developing	0
Spawning capable		0
	Actively spawning	4
Regressing		8
Regenerating		0
Not categorized		1

One ovary was not categorized due to the infiltration of multiple red blood cells throughout the ovarian lumen, implying the breakdown and resorption of oocytes. This ovary contained oocytes through the CA stage but no vitellogenic oocytes and so would have been classified as early developing phase; however, the presence of the red blood cells indicated that the ovary may instead be in the regressing phase. A second ovary classified as developing had multiple CA oocytes, a few early vitellogenic oocytes and some atretic oocytes, but no post-ovulatory follicles (Figure 28 A). The remaining 12 ovaries contained varying stages of vitellogenic oocytes, often in states of atresia. Four ovaries contained VTG2 and VTG3 oocytes along with evidence of POFs and atretic oocytes. These four fish were classified as actively spawning sub-phase of the spawning capable phase (Figure 28 B). Three of the samples categorized as spawning capable contained some alpha atretic oocytes but these made up fewer than fifty percent of the vitellogenic oocytes and so these fish were classified as atretic state 1. One fish in the actively spawning sub-phase did exhibit multiple oocytes in the early stages of atresia indicating atretic state 2 but these atretic oocytes included hydrated oocytes. The proliferation of atretic oocytes indicates that this fish was entering the regressing phase but the presence of hydrated oocytes along with POFs implies recent spawning ability, likely within the past 24 h (Hunter and Macewicz 2003, Brown-Peterson et al. 2011).

The remaining 8 ovaries contained varying stages of vitellogenic oocytes and multiple atretic oocytes with no apparent POFs and were classified as regressing phase (Figure 29).

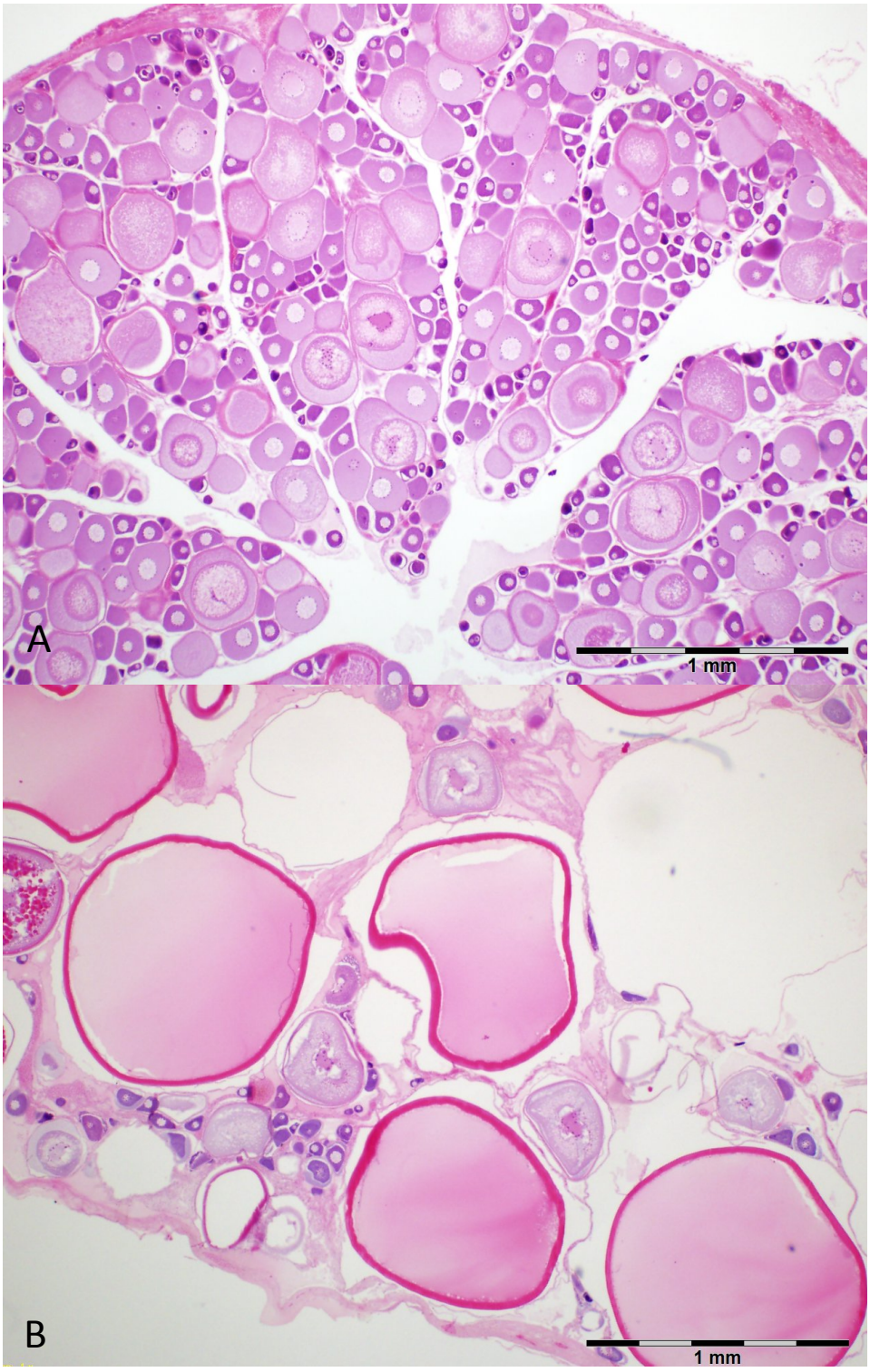

Figure 28: Developing (A) and spawning capable (B) sharksucker ovaries.

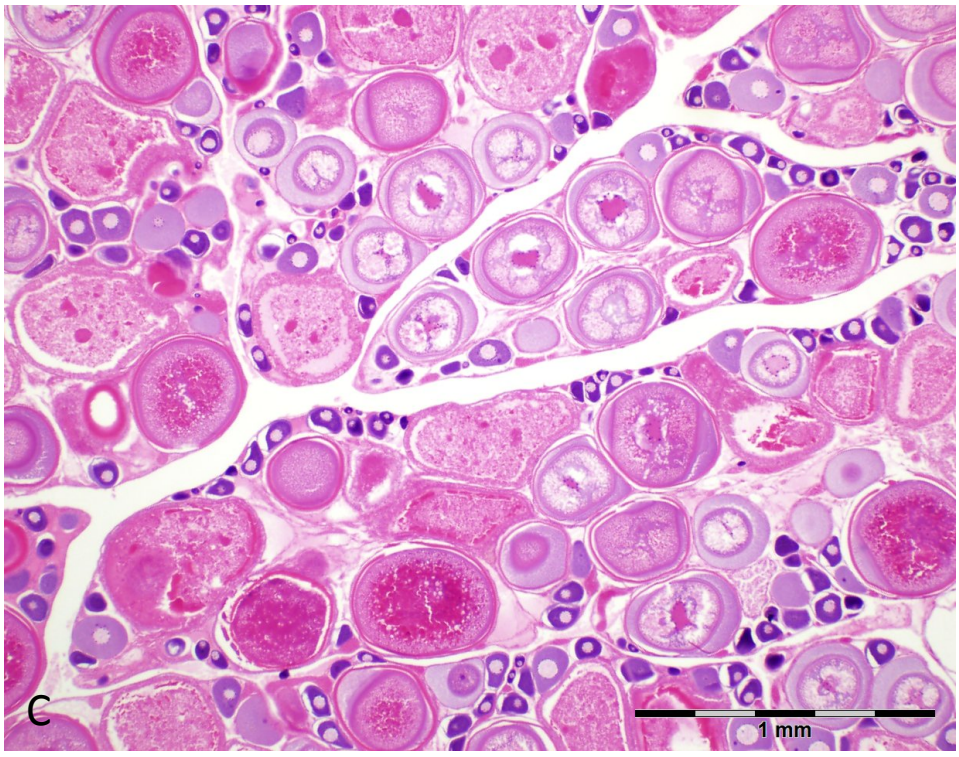

Figure 29: Regressing phase sharksucker ovary.

All regressing ovaries contained some atretic vitellogenic oocytes, 7 exhibited greater than 50% atresia consistent with atretic state 2 and the remaining fish exhibited rates of vitellogenic atresia at below 50% consistent with atretic state 1. No immature or regenerating phase ovaries were observed. Melano-macrophage centers were observed in 71% (n = 14) of ovaries examined.

Male Reproductive Histology

Similarly to cobia and many other teleosts, male sharksuckers exhibited unrestricted spermatogonial testes (n = 19, 131 to 466 mm SL). Unrestricted testes are those in which spermatogonia occur throughout the germinal epithelium including along

the lining of the lobules as opposed to solely along the periphery as in those found in restricted testes (Grier and Uribe-Aranzábal 2009).

The smallest male fish examined histologically for this study measured 131 mm SL with a total weight of 9 g. The gonads of this fish were not macroscopically identifiable as to sex and were fixed and sectioned along with a section of intestine. The small size of the gonads made determination of the reproductive phase difficult even histologically so they were not categorized but they did appear to contain residual spermatozoa and some spermatocysts with little to no lumen.

All of the remaining male histological samples were from fish measuring >285 mm SL and all were reproductively mature individuals classified as spawning capable phase with spermatozoa in the lumen of the lobules (Brown-Peterson et al. 2011) (Table 30, Figure 30).

Table 30: Categorization of male sharksucker histological samples (n = 19).

Male Histological Samples		
Phase	**Subphase**	**n**
Immature		0
Developing		0
	Early developing	0
Spawning capable		0
	Early GE	0
	Mid-GE	10
	Late-GE	8
Regressing		0
Regenerating		0
Not categorized		1

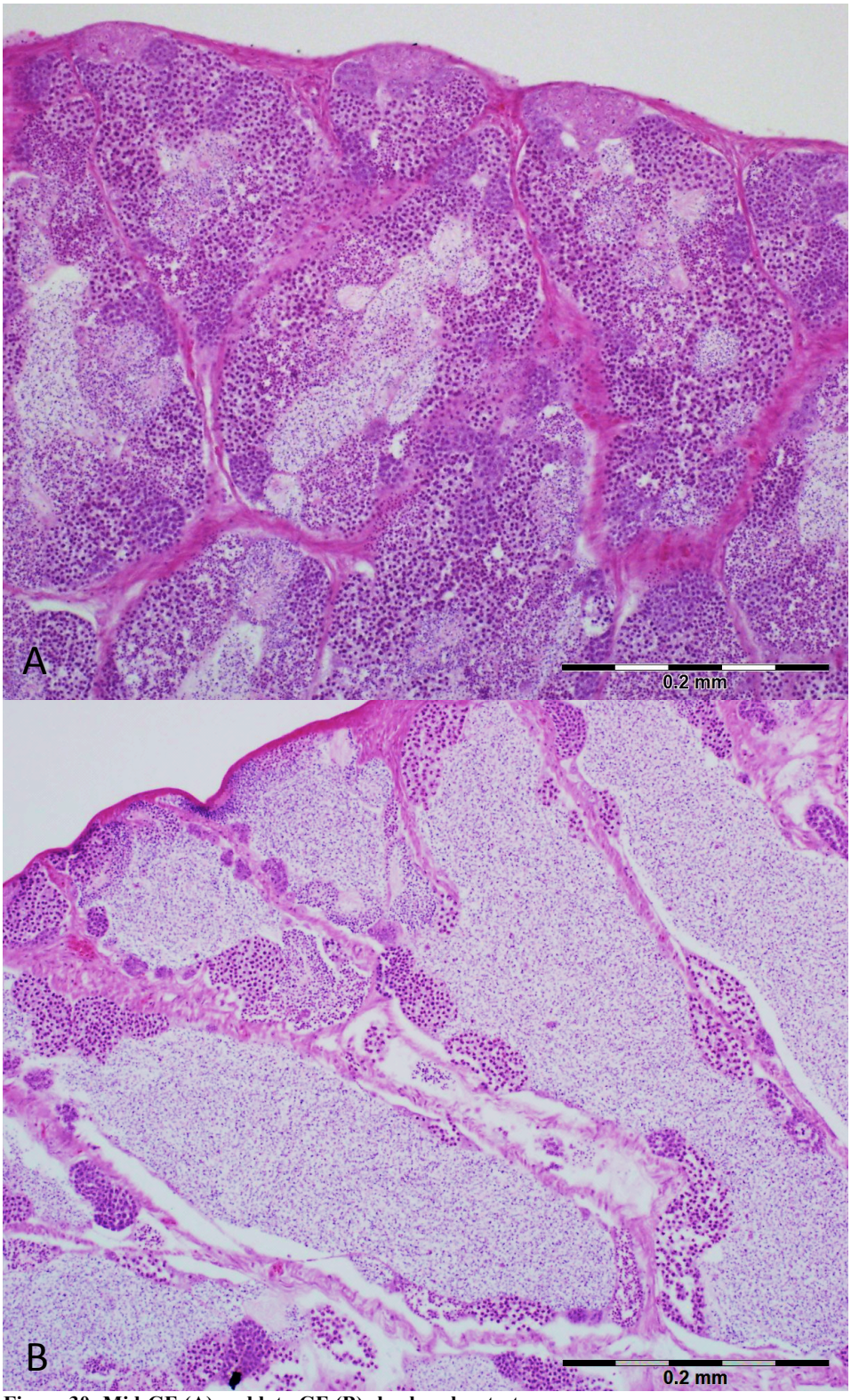

Figure 30: Mid-GE (A) and late-GE (B) sharksucker testes.

It was not possible to determine whether any of these males were actively spawning as the defining criteria for this sub-phase is "the release of milt when gentle pressure is placed on the abdomen (Brown-Peterson et al. 2011)." This procedure was not carried out during the field surveys.

Of the 18 spawning capable male fish, ten fish were mid-GE subphase and eight were late-GE subphase based on distribution of germinal epithelium. Four fish in the late-GE subphase had only one or two small areas of discontinuous germinal epithelium along the terminal edges of peripheral lobules and appeared to be entering the late-GE subphase. A different fish in the late-GE subphase had relatively few spermatocysts compared to the other males indicating that its spawning period was almost finished.

Melano-macrophage centers (MMC), also known as brown bodies or macrophage aggregates, were evident in all of the testes sampled histologically.

DISCUSSION

Growth

Sharksucker growth is adapted to a symbiotic lifestyle, balancing a trade-off between the need to grow large enough to successfully live freely without a host when necessary without getting so large that they are unable to find hosts large enough to support them. Female sharksuckers appear to reach longer body lengths than males (Lester et al. 2004, this study). As greater somatic size in female fish allows for the production of larger numbers of eggs, female sharksuckers likely attain ultimately larger sizes because larger females receive a fitness benefit in the form of higher batch fecundities (Arendt 2010). Conversely, sexually mature male sharksuckers are likely to receive a fitness benefit from diverting resources away from somatic growth and into gamete production. Smaller sizes for males allows for earlier sexual maturation and can benefit lifetime reproductive fitness (Cole 1954, Arendt 2010). Body size has important implications for fitness and resource partitioning. Larger body sizes incur a metabolic cost and organisms must balance trade-offs in higher energy requirements against improved reproductive outcomes (Werner and Gilliam 1984, Peters 1986, Arendt 2010). Many organisms exhibit discrete shifts in habitat and other resource usage with ontogeny as larger body sizes require disparate trade-offs in foraging and predator avoidance. Ontogenetic dietary and habitat shifts are common in fishes, presumably reducing interspecific competition while allowing for exploitation of diverse niches over time

(Werner and Gilliam 1984). Sharksuckers are able to exploit an unusual niche as a symbiont but even this specific niche appears to be partitioned by size, with juvenile sharksuckers known to attach to smaller hosts, including teleosts and chelonians, and adults associating with elasmobranchs (Sazima et al. 1999). These small sharksuckers may be more reliant on their hosts for food and protection than adult sharksuckers. The absence of ectoparasitic copepod prey in the diet of large sharksuckers supports the idea of an increasingly facultative symbiont with size. Juvenile sharksuckers may be more reliant on consumption of host parasites while adult sharksuckers are less host dependent and feed on larger and more diverse prey items. Larger body size decreases risk of predation so the protection offered by the relatively larger size of a host organism may offer a critical refuge for younger sharksuckers (Werner and Gilliam 1984).

Sharksuckers reach a maximum length of approximately 1 m SL (Collette 1999a). The largest sharksucker sampled for this study measured 715 mm SL. Growth rates are not stable over time and the amount of resources allocated to somatic growth are often shifted to allow for greater reproductive output (Blanckenhorn 2000, Lester et al. 2004). Growth in reproductively immature fish is predominantly somatic and linear while growth in sexually mature fish slows due to the diversion of resources away from somatic growth (Lester et al. 2004). Therefore, these von Bertalanffy growth parameters are useful for sharksuckers within the age ranges examined (1 to 8 y). As only a single specimen longer than 535 mm and none older than 9 y was evaluated, the incorporation of older fish in the model might help refine the asymptote. Growth in younger fish may not follow a von Bertalanffy curve.

More precise size-at-age estimations of smaller sharksuckers could not be resolved as evaluation of very young juveniles to validate periodicity of "daily" otolith increments was not possible.

Aging was performed using the assumption that singular otolith increments form annually in sharksuckers as they do in many teleost species, including closely related cobia (Franks et al. 1999) and dolphinfish (Furukawa et al. 2012). The annual deposition of otolith increments could not be validated by marginal increment analysis in this study due to the limitations of small sample sizes outside of the months of July and August. Confidence levels based on cumulative binomial probabilities do support the likelihood of the deposition of a single opaque increment in the summer months but this assumption will need to be validated in the future with a more temporally varied sample set. The violation of the assumption of annual increment deposition (i.e. whether increments form more or less frequently) would require reevaluation of the assigned ages either up or down respectively.

Diet

Analysis of sharksucker stomach contents supports diet specialization across size ranges in sharksuckers from the Gulf of Mexico. Sharksuckers across both size ranges fed on crustaceans, fish and mollusks. Ectoparasitic and planktonic crustaceans made up the bulk of the diet of sharksuckers <249 mm SL but were less important to sharksuckers >250 mm SL. Conversely, fish made up a greater part of the diet of larger sharksuckers, both by volume and frequency.

Previous stomach content analyses for echeneid species have reported multiple food items including planktonic organisms, such as amphipods, copepods, and decapods; parasitic copepods; fish, mollusks and crustaceans; and fecal matter from host organisms (Strasburg 1962; Cressey and Lachner 1970; E. H. Williams et al. 2003). Echeneids display varying amounts of dietary specialization, with the Echeneiinae exhibiting generalist feeding preferences, whereas many of the remora species are specialized feeders. Because sharksuckers display facultative symbiosis as opposed to the more specialized obligate symbionts of the remora family, their dietary preferences are likely to be more generalized and as expected a wide variety of food items were found to occur in sharksucker stomachs. Independent, free-living sharksuckers presumably forage for multiple prey items and those living with hosts may feed on the remains of the hosts' prey and/or potentially on the hosts' feces, in addition to foraging for their own prey. Scavenging behavior is consistent with the presence of partial fish and decapod remains from relatively large prey items in the stomachs of some of the examined sharksuckers. Shark teeth were found in the stomachs of 2 sharksuckers, presumably having been ingested as a byproduct of being embedded in prey released by an associated elasmobranch. Echeneids have also been seen to venture short distances away from their hosts to forage independently and in this way might be exposed to a wider range of potential prey items, although their consumption patterns are expected to be limited by gape-size limitations on physically symbiotic species. Fragments of host prey are also an important part of sharksucker diets. Consumption of fish prey may be underestimated relative to organisms with hard parts that take longer to digest in this study.

Sharksuckers in the Gulf of Mexico ingest a wide variety of prey taxa including ectoparasitic copepods which is consistent with the findings of Cressey and Lachner who found parasitic "copepods or isopods" in 16% of their specimens (Cressey and Lachner 1970). As expected, ectoparasitic copepods made up a small but integral part of the sharksucker diet, particularly at the juvenile stage, while the rest of their food items are probably obtained opportunistically (Cressey and Lachner 1970). Cressey and Lachner divided their specimens into four size groups and found the highest proportions of ectoparasitic copepods in their second smallest size group, 86–125mm SL (40% of stomachs in sample contained parasitic copepods) and in their next larger size group, 126–165 mm SL where 33% of stomachs contained parasitic copepods. The stomachs of the smallest fish (57–85 mm SL) exhibited an 11% frequency of occurrence of ectoparasitic copepods while in their largest SL category, 166–630mm SL only 9% of stomachs contained parasitic copepods. Additionally, no sharksuckers over 311mm SL examined in the Cressey and Lachner study contained ectoparasitic copepods (Cressey and Lachner 1970).

A broader diet with reduced consumption of crustacean ectoparasites in adult sharksuckers is to be expected due to the increased caloric needs of larger, older fish. Juvenile sharksuckers stationed in sheltered coral reef locations may be exposed to multiple host organisms with novel infestations of ectoparasitic crustaceans that can provide an important source of food for this life stage (Sazima et al. 1999).

The presence of ectoparasitic copepods in the stomachs of sharksuckers in the current study implies that adults will continue to consume ectoparasitic copepods

depending on availability, but the presence of larger crustacean and fish prey items indicates that ectoparasites are a relatively unimportant source of energy for adult fish.

Hunger is a known source of bias in diet studies of fish captured with baited hooks. Bait was easily identified and removed from the analysis but the high percentage of empty stomachs and stomachs with heavily digested contents in this study may point to a hunger bias (Garvey and Chipps 2007). In addition, digestion of stomach contents would continue until fish were completely frozen; therefore, variable periods between capture and freezing also contributed to empty stomach and degraded stomach contents. Actual consumption rates are likely higher.

Stable Isotope Analysis

Significant shifts in stable isotope values for sharksuckers supports the concept of an increasingly facultative symbiont with size.

Small sharksuckers exhibited similar $\delta^{15}N$ values in both sampling areas, which is expected of fish feeding on similar prey items at the same trophic level. Larger sharksuckers in the Big Bend area were depleted in $\delta^{15}N$ compared to smaller sharksuckers from both areas and relative to similarly-sized fish from the Keys. Larger fish were expected to be enriched in $\delta^{15}N$ as large fish are often able to feed at higher trophic levels than smaller fish due to increased predator efficiency with growth (i.e., larger gape sizes and ability to attack larger prey items) (Romanuk et al. 2011). A possible explanation for the seemingly incongruous result of nitrogen depletion relative to increased size is the relative contributions of ectoparasitic copepods in the diet of sharksuckers of different size ranges.

Sharksuckers < 249 mm SL consumed ectoparasitic copepods much more frequently than larger sharksuckers. Assuming the ectoparasitic copepods consumed by small sharksuckers have similar $\delta^{15}N$ values to their elasmobranch hosts this could explain the higher relative $\delta^{15}N$ levels seen in smaller sharksuckers. Smaller sharksuckers consuming ectoparasitic copepods that have been feeding on elasmobranchs would then be expected to show higher $\delta^{15}N$ values than the larger sharksuckers that are consuming a broader variety of prey items across trophic levels. Deudero et al. (2002) looked at carbon and nitrogen stable isotope ratios in ectoparasites and compared them to isotopic rations in host tissues. These authors found that copepod parasites displayed a variety of nitrogen isotopic ratios in relation to their host species; some were enriched whereas others were depleted. This is consistent with other studies of ectoparasites. Iken et al. (2001) reported that gill-feeding ectoparasitic copepods were enriched ~3‰ compared to their hosts. Pinnegar et al. (2001) examined two species of ectoparasites on fish (an isopod and a copepod) and found that their isotopic signatures were comparable to that of their hosts and were neither significantly depleted nor enriched (2001). Although they came to the conclusion that stable isotope analysis of fish parasites does not provide useful information on food webs, comparable isotope values between hosts feeding at high trophic levels (such as elasmobranchs) and their ectoparasites is consistent with enrichment in $\delta^{15}N$ in organisms that then consume those ectoparasites (Pinnegar et al. 2001). Sharksuckers feeding on ectoparasites that previously fed on elasmobranchs would then be expected to be nitrogen enriched relative to sharksuckers feeding on organisms from lower trophic levels, even if the ectoparasites were slightly

nitrogen depleted relative to the elasmobranch host. Daly et al. (2013) reported a white muscle $\delta^{15}N$ mean isotopic value of 13.5‰ for bull sharks (*Carcharhinus leucas*) in the Southwest Indian Ocean. A single captive lemon shark (*Negaprion brevirostris*) had a lipid extracted $\delta^{15}N$ white muscle value of 13.65‰ (Hussey et al. 2010).

Stomach content analysis is subject to multiple biases including the difficulties inherent in distinguishing partially digested food items, the risk of overestimating the value of rare items along with potentially magnifying the relative importance of other prey by relying on the presence of indigestible hard parts that may be retained for longer time periods. While the analysis of stomach contents provides a temporally limited view of the actual prey consumed by an individual fish at a particular moment in time, the analysis of stable isotope values can increase temporal resolution by providing an idea of what prey items are actually being assimilated. A change of ~3.4 ‰ in $\delta^{15}N$ is generally considered indicative of a shift between trophic levels from prey to predator and relatively large changes of isotope values have been used as support for ontogenetic niche shifts in vertebrate species (DeNiro and Epstein 1981, Minagawa and Wada 1984, Post 2002, Fry 2006, Sweeting et al. 2007, Lee Cruz et al. 2012). While the size-related differences in $\delta^{15}N$ values of sharksuckers from the Gulf of Mexico in the current study are statistically significant, these differences are not large enough to unequivocally indicate a complete change in trophic level such as might be seen during a shift from a crustacean diet to a more facultative diet. However there are multiple possible explanations for the smaller shift seen here, including ontogenetic change in prey

consumption patterns, differential fractionation of isotopes due to physiological characteristics and temporal or spatial alterations in foraging locations.

Sharksuckers are likely to continue to consume prey opportunistically across their lifetime and it seems possible that the change seen in $\delta^{15}N$ values here could result from a shift away from the consumption of ectoparasitic parasites that have been feeding on elasmobranchs, and other small crustacean and zooplankton prey by juveniles, to a reliance on larger, free-living crustacean and teleost prey by mature adults, including prey items already stunned or killed by their host.

The ectopasitic copepods evaluated by Deudero et al. (2002) had lower $\delta^{13}C$ carbon values relative to associated host species. They suggest that this depletion is due to high concentrations of lipids in blood as the metabolic production of lipids preferentially retains ^{12}C over ^{13}C (Vander Zanden and Rasmussen 2001, Deudero et al. 2002). Consumption of ectoparasitic copepods depleted in ^{13}C in could contribute to the variation in carbon isotopic values seen between small and large sharksuckers in the Big Bend. Smaller sharksuckers feeding on ectoparasitic copepods might be expected to have lower $\delta^{13}C$ than larger sharksuckers. Small sharksuckers in the Keys were more enriched in $\delta^{13}C$ than fish from the Big Bend area and they did not differ significantly from larger sharksuckers.

Primary carbon sources in the Gulf of Mexico include phytoplankton, macroalgae, seagrasses, epiphytes or terrestrial sources.

Spatially, $\delta^{13}C$ values can vary by latitude, depth and in proximity to landmasses. Higher latitudes are $\delta^{13}C$ depleted compared to lower latitudes, inshore ecosystems are

enriched relative to offshore environments and benthic food webs are enriched versus pelagic (Fry and Parker 1978, Hobson et al. 1994, Cherel and Hobson 2007, Radabaugh and Peebles 2014). Moncrieff and Sullivan (2001) compared the carbon isotopic values of producers and consumers off the coast of Mississippi and calculated a mean $\delta^{13}C$ of -12.2‰ for *Halodule wrightii*, -17.5‰ for epiphytic algae, -15.8‰ for sand microflora, -21.8‰ for phytoplankton, and -16.8‰ for macroalgae such as *Sargassum* sp. Seagrass species common in both the Big Bend and Keys include turtle grass (*Thalassia testudinum*), manatee grass (*Syringodium filiforme*) and shoal grass (*H. wrightii*) with mean $\delta^{13}C$ values of -10.9‰, -5.9‰, and -11.5‰ respectively (GMP 2004, Hemminga and Mateo 1996). Particulate organic carbon from terrestrial sources in the northern Gulf of Mexico is more depleted than marine sources at -23.8‰ to -26.8‰ (Wang et al. 2004). The mean carbon values for sharksuckers in this study (-16.2‰ in the Keys and -17‰ in the Big Bend) indicate a marine source of carbon and most closely approximate the values of epiphytic algae, macroalgae and sand microflora. Sharksucker carbon values correlate most closely with inshore algae and benthic carbon sources, not the comparatively enriched seagrass or depleted phytoplankton and terrestrial carbon signatures. Fry (1984) found that planktonic and benthic algae were the most important primary producers in an eastern Florida lagoon while Moncrieff and Sullivan (2001) showed that epiphytic algae were 46% of the total and 60% of the benthic primary production in an inshore community in the northern Gulf of Mexico.

Variation in isotope values for small sharksuckers captured in the Big Bend sampling area versus the Keys could indicate a difference in predation patterns between

the locations, but they could also be influenced by temporally different sampling patterns and/or spatial variation in atmospheric isotope inputs. Although fish were sampled from the Keys throughout much of the year, with 52% captured over the months of December, January and February, most of the fish caught in the big bend area were captured during the summer (89% during June, July and August) and no fish were sampled from the Big Bend area during the months of December, January or February (Figure 22). This introduces the possibility that temporal (i.e. seasonal) changes in prey availabilities could also have contributed to the observed spatial differences in isotope values. Geochemical isotope concentrations and ecosystem inputs of $\delta^{13}C$ and $\delta^{15}N$ vary spatially and temporally in aquatic ecosystems and it is possible that the differences seen in sharksucker isotope values between the Big Bend and Keys sampling areas are due to this spatial variation (Post 2002, Fry 2006). In addition to the disparate temporal sampling between the study locations, the mean weight of fish captured in the Keys was significantly less than that of fish sampled from the Big Bend area and only one large fish was sampled from the Keys (Figure 23).

Reproduction

Reproductive Histology

Sexually mature sharksucker ovaries examined histologically in this study exhibited multiple stages of oocyte development including both pre-vitellogenic and vitellogenic stages. This supports the assertion that sharksuckers are a batch spawning species that possess indeterminate fecundity, which allows for the continued recruitment

and maturation of oocytes across an extended spawning season (Murua and Saborido-Rey 2003). All fish sampled histologically were captured during the month of August and the presence of multiple regressing female fish at this time suggests that the sharksucker spawning season in the eastern Gulf of Mexico begins earlier in the year and is winding down in the late summer although many fish may still be capable of spawning. All male fish sampled histologically were undergoing active spermatogenesis, producing spermatozoa and capable of spawning. The capture of sexually mature male sharksuckers in predominantly the mid-to-late-GE classes of spermatogenesis in proximity with female sharksuckers leaving the spawning capable phase of oogenesis and entering into the regressive phase indicated extended spawning periodicity in sharksuckers. All 18 mature male sharksuckers sampled were capable of spawning during the month of August. As the female fish sampled for histological analysis over the same time period exhibited a broader range of reproductive readiness, the spawning season of sharksuckers likely extends over multiple months of the year. An extended spawning season with female fish becoming spawning capable at multiple times would encourage an extended spawning capable phase in male fish as it is advantageous to be producing readily accessible mature spermatozoa throughout the entire range of time that female fish are likely to be producing eggs. Males that continue to produce spermatozoa late into the spawning season would be consistent with a female reproductive cycle where females enter the regressing phase asynchronously. These reproductively ready males would retain the ability to fertilize any available spawning capable females even late in the spawning season.

Due to the limited temporal nature of this study it is not possible to determine exactly how long the spawning period might last: however, it begins some time prior to the month of August and likely corresponds with the presence of warm waters and ample food supply, which are available in the mid-summer to early autumn months in the temperate environment of the eastern Gulf of Mexico.

All sharksucker testes and 71% of ovaries examined histologically exhibited melano-macrophage centers. Teleost melano-macrophage centers can contain lymphocytes and may serve a function similar to that of lymph nodes in other vertebrates (Agius and Roberts 2003). The presence and size of melano-macrophage centers in teleost reproductive tissues may increase with age due to their possible role in the phagocytosis of unnecessary or damaged cells (Agius and Roberts 2003). Melano-macrophage centers in the testes of common snook are believed to phagocytize spermatozoa and, because they have been shown to increase in size and number late in the spawning season they may be an indicator for the regression phase (Grier and Taylor 1998). The presence of melano-macrophage centers in sharksucker testes may imply proximity to the end of a spawning season with testes beginning to enter into a post-spawning regression state; however, all testes sampled still exhibited spermatocysts in multiple stages of spermatogenesis indicating a continuation of spawning readiness. Melano-macrophage centers in ovaries have been associated with atretic oocytes and may possibly indicate a post-spawning regression state; however, this is complicated by the fact that these centers have also been show to occur as a response to environmental stresses including pollution (Agius and Roberts 2003). Brown-Peterson, et al. (2002)

noted that they did not observe any melano-macrophage aggregates in their study of cobia testes, although Lotz et al. (1996) did identify macrophages in conjunction with atretic follicles in cobia ovaries.

Incomplete removal of the Bouin's solution in some samples appeared to result in brittle ovarian samples that sectioned poorly in some cases. Larger vitellogenic, maturing and hydrated oocytes seemed particularly susceptible to tearing during sectioning which may have led to an underestimate of the occurrence of these oocyte stages. Testes samples did not appear to be as negatively affected by the means of fixation. Other histological artifacts include localized chattering either due to dulling microtome blades or fixation-related brittleness in the fixed tissues; however, these issues did not affect the overall conclusions of the histology results.

Fecundity

Batch fecundity refers to the number of oocytes matured and spawned at a single time, and relative batch fecundity is the number of oocytes spawned at a single time per g of ovary-free body weight. Hydrated oocytes were used to evaluate batch fecundity because the use of smaller oocytes to determine potential fecundity may overestimate actual fecundity when rates of atresia are unknown (Hunter and Macewicz 2003).

The mean relative batch fecundity for sharksuckers of 42.0 hydrated oocytes per g OFBW was comparable to similar values reported for cobia. Brown-Peterson et al. (2001) reported mean relative batch fecundity of 53.1 eggs/g OFBW in cobia in the southern United States based on counts of the largest oocytes.

The mean number of hydrated oocytes for sharksuckers of 21,968 was lower than that reported for dolphinfish. Alejo-Plata et al. (2011) reported batch fecundity of hydrated oocytes in dolphinfish in the Mexican Pacific is between 45,022 and 1,930, 245 per individual with an overall mean of 466,410. Smaller fish in the Alejo-Plata et al. study had a lower mean fecundity of 52,700 hydrated oocytes per fish (2011).

Determination of realized fecundity (the number of viable eggs actually spawned over an entire spawning season) was beyond the scope of the current study and will require further investigation to determine spawning frequency of sharksuckers. The use of hydrated oocytes to estimate fecundity may underestimate actual fecundity as females may have already spawned. However, none of the fish evaluated exhibited oocytes in the lumen of the ovary so it is unlikely any were actively spawning. Oocyte counts were calculated using dried weight of the entire ovary and are likely to be somewhat overestimated due to the presence of ovarian tissue.

Reproductive Periodicity and Water Temperature

Sharksuckers were recorded spawning in captivity in an aquarium in Japan in 1974 (Nakajima et al. 1987). These captive fish had been imported from Singapore and spawned repeatedly over a period of 140 days across the months of June through December, with a gap from early August to mid-September (Nakajima et al. 1987). This report of captive spawning spanning over multiple months supports the hypothesis of an extended spawning period in wild sharksuckers. According to Nakajima, et al. (1987), the captive sharksuckers they observed only exhibited their breeding behaviors when the

water temperature was maintained between 27.5 °C and 30.5 °C, and they stated that no spawning activity occurred at temperatures below 25 °C.

Water temperatures at the time of capture of the fish in the Gulf of Mexico for the current histological study were uniformly high (31.1 °C to 31.9 °C.) This is higher than the temperature range that the captive spawning fish were exposed to in the Nakajima article and well above the lower spawning temperature threshold of 25 °C they described (Nakajima et al. 1987). These authors also did not discuss any water temperature above 30.5 °C so it is uncertain at what upper temperature threshold spawning behavior may be inhibited in sharksuckers. It appears likely, however, from the presence of apparently reproductively active fish of both sexes in the current study, that the upper thermal limit is greater than 31.9 °C.

Comparisons with Cobia and Dolphinfish

The smaller sizes and reduced swimming abilities necessary to a symbiotic lifestyle restrict the predatory ability of echeneids relative to the closely related cobia (*Rachycentron canadum*) and dolphinfish (*Coryphaena hippurus*). Sharksuckers reach a maximum size of about 1 m, roughly half the maximum sizes of cobia (~2 m) and dolphinfish (~2.1 m) (Collette 1999a-c). Small cobia are similar in appearance and coloration to sharksuckers (Smith & Merriner 1982).

Larger cobia are predominantly female (Shaffer & Nakamura 1989) and in the Gulf of Mexico, the von Bertalanffy growth parameters L_∞ and K are significantly different for female (L_∞ = 1,555.0 mm; K = 0.272; t_0 = -1.254 mm) and male cobia (L_∞ = 1,170.7 mm; K = 0.432; t_0 = -1.150 y) (Franks et al. 1999).

Unlike sharksucker and cobia, dolphinfish are sexually dimorphic (Massutí & Morales-Nin 1997, Alejo-Plata et al. 2011). Dolphinfish growth is very rapid and reported von Bertalanffy growth parameters for dolphinfish include L_∞ = 1457 mm FL with K = 2.19 and t_0 = -0.046 y off Puerto Rico (Rivera & Appeldoorn 2000), and L_∞ = 1299 mm FL with K = 1.08 off North Carolina (Schwenke & Buckel 2008). Von Bertalanffy growth curves were not significantly different between male and female dolphinfish in either of these studies.

Dolphinfish are often found in association with floating objects where they may be preying on fish seeking shelter but they are not known to associate with other animals (i.e. sharks) (Alejo-Plata et al. 2011). Similarly, cobia associate with inanimate objects and artificial structures but they are also frequently found in association with elasmobranchs (sharks and rays) (Smith & Merriner 1982) and sea turtles (Shaffer & Nakamura 1989).

Like sharksuckers, cobia are migratory batch spawners with an extended spring and summer spawning season (Lefebvre & Denson 2012). Cobia in the Gulf of Mexico generally migrate northward in spring from the Florida Keys where they overwinter (Shaffer & Nakamura 1989, Franks et al.1999).

Dolphinfish are also asynchronous batch spawners that spawn over extended periods in summer and again in winter (Massutí & Morales-Nin 1997, Alejo-Plata et al. 2011). Dolphinfish in the tropical Pacific migrate to productive upwelling zones in the prior to spawning (Alejo-Plata et al. 2011).

Life History Adaptations of the Sharksucker

Sharksuckers occupy an unusual niche and their life-history adaptations enable an uncommon marine vertebrate symbiosis.

Sharksucker ultimate size is limited by the size constraints imposed by host size availability. Greater physical sizes at maturity can allow for improved foraging, decreased risk of predation and increased fecundity but at the cost of being unable to locate a host large enough to support a phoretic relationship. Sharksuckers appear to gain many of the expected benefits of increased size by proxy from their hosts (i.e. lowered predation risk and access to prey disturbed or dismembered by the host). This allows for a trade-off wherein sharksuckers grow large enough to forage independently from their hosts if necessary while attaining mature sizes that are substantially smaller than host species. Sharksuckers grow more slowly than their sister groups but develop the capacity for symbiosis very rapidly. Juvenile sharksuckers are capable of attachment by 35 days after hatching (Nakajima et al. 1987) and it has been suggested that symbiotic attachment is obligatory by 40 to 80 mm in length (Strasburg 1964). The rapid development of the cephalic disk implies attachment is a priority for juvenile development and is further evidence of the importance of symbiosis for young sharksuckers.

Sharksucker diet is also adapted to their symbiotic life style. Sharksuckers exhibit a decreasing dietary dependence on ectoparasitic copepods with growth and a concurrent facultative increase in consumption of larger, more diverse prey items. The pronounced presence of ectoparasitic copepods in the diet of smaller sharksuckers in this study

suggests the intriguing possibility of obligate symbiosis in young sharksuckers, shifting to facultative symbiosis with age.

Parasitic trophic interactions affect energy flow and production through food-webs and may serve to limit predator populations (Minchella & Scott 1991, Kuris 2008, Lafferty et al. 2008, Minchella & Scott). This suggests that elasmobranchs hosting sharksuckers may be gaining a fitness benefit from opportunistic cleaning. Unfortunately, it is difficult to determine how detrimental ectoparasitic copepods might be to an individual host in the absence of sharksucker associations and how valuable the removal of the parasites would be in relation to the potential metabolic costs of hosting a sharksucker (e.g., increased hydrodynamic drag, loss of potential prey items to consumption of the sharksucker, physical irritation caused by the remoras sucking disc) (Brunnschweiler 2006).

Successful recruitment depends on the survival of fish at early life stages. This could leave sharksucker populations particularly vulnerable to the availability of suitable hosts (i.e. high enough densities of host animals such as elasmobranchs that are carrying ectoparasitic copepods) at critical juvenile life stages. Sharksuckers are known to move between hosts and in this way may increase the likelihood of encountering a parasitized animal. Sharksucker recruitment may in this way be dependent upon elasmobranch population density.

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