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Title of Thesis: SEXUALITY AND SEXUAL MATURITY OF THE ARC-EYE
HAWKFISH, *PARACIRRHITES ARCATUS* (CIRRHITIDAE)

Number of Pages: 53 Pages (including abstract and approval pages)

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AN ABSTRACT OF THE THESIS of Mildred A. Kelokelo for the Master of Science in
Biology presented, May 09, 2019

Approved:  _____

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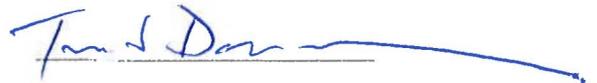
Title: Sexuality and Sexual Maturity of the Arc-eye Hawkfish, *Paracirrhites arcatus*
(Cirrhitidae)

Hermaphroditism, or sex change in animals and plants, is very common and has been reported for many marine reef fishes. Since the discovery of this phenomenon, hypotheses and techniques have been developed to try to understand the proximate and ultimate causes of sex change in fishes. Knowledge of the sexuality and reproductive systems of marine reef fishes is important for informing conservation and resource managers. Reproductive analysis is a basic need for theoretical and practical applications. Hawkfishes (Cirrhitidae) are a family of mostly small-sized reef fishes valued in the ornamental fish trade, dive tourism and occasionally in subsistence fishing. Sexual patterns in hawkfishes have been studied both in natural conditions and captivity, and a few species have been confirmed as being protogynous hermaphrodites. Gonads of *Paracirrhites arcatus*, a relatively common species on Guam's shallow coral reefs, were examined to evaluate sexual patterns and reproductive cycles. Histological analysis of gonads shows the presence of transitional individuals with both developing

spermatogenic tissue and degenerating ovarian tissues suggesting that protogynous hermaphroditism is expressed in this species.

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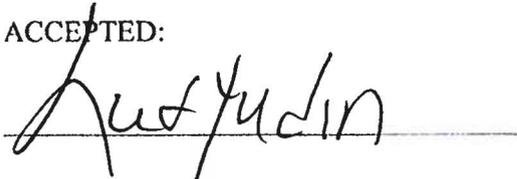


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SEXUALITY AND SEXUAL MATURITY OF THE ARC-EYE HAWKFISH,

***PARACIRRHITES ARCATUS* (CIRRHITIDAE)**

BY

Mildred A. Kelokelo

**A thesis submitted in partial fulfillment of the
requirements for the degree of**

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Introduction

Sex change resulting in simultaneous or sequential hermaphroditism is common among plants and animals, and especially fishes (Policansky 1982; Thresher 1984).

Simultaneous hermaphroditism, in which an individual performs both female and male functions during mating, has been found in some species such as the hamlets of the genus *Hypoplectrus* (Serranidae) (Fischer 1980; 1981). Sequential hermaphroditism is more common and occurs in an individual that changes sex during part of its life, and usually remains the latter sex throughout its life. Three primary types of sequential hermaphroditism are known to occur among marine and freshwater fishes: protogyny, bidirectional hermaphroditism, and protandry.

In protogynous hermaphroditism, individuals change sex from female to male. This reproductive strategy may convey an advantage to individuals that change sex at a certain size or age by maximizing fitness, in terms of multiple mating opportunities per unit time to gain higher lifetime reproductive ability. Protogyny is common among mating systems monopolized by larger males and a female-biased sex ratio (Robertson and Choat 1973; Robertson and Warner 1978; Warner and Robertson 1978; Warner 1984a). This sexual pattern may be advantageous for females to reproduce while small and only change into a male to increase reproductive success and remain within the mating group (Warner 1988). Thus, being a small female in a harem may be more advantageous than being larger because smaller individuals will focus mainly upon mating opportunities with males while very large females may have to devote resources towards sex change instead of egg production with corresponding costs against reproductive success (Warner 1975; 1984).

Protogyny is the most common sex change strategy (Warner 1984), and is known to occur in various reef-fish families. Most notable are wrasses (Labridae), parrotfishes (Labridae: Scarinae), marine angelfishes (Pomacanthidae), seabasses and groupers (Serranidae, with the groupers now classified as Epinephelidae by some), and hawkfishes (Cirrhitidae) (Cole 2010; Robertson and Warner 1978; Craig and Hastings 2007; Kobayashi et al. 1985; Kobayashi and Suzuki 1992; Sadovy and Donaldson 1995). Multiple mating strategies are employed in protogynous fishes and these include harem mating systems, where dominant males control two or more females within a mating group; this strategy is common among reef-dwelling protogynous fishes (Thresher 1984; Warner 1984; Nakazono et al. 1985). Alternatively, some protogynous fishes may utilize a lek-like system, in which sexually active males establish and defend temporary mating territories while attempting to attract females for courtship (Loiselle and Barlow 1978). In this system, females select whom they mate with although males may engage in aggressive courtship while attempting to mate. Unlike harem mating systems, where a dominant male guards and mates with up to several females within a temporary mating territory, once mating is accomplished in a lek-like system the females leave the lek site and the males abandon the mating territory until the next mating period (Thresher 1984).

Serial bidirectional hermaphroditism is considered quite uncommon and appears to be limited mainly to species with specialized microhabitat requirements, such as obligate-coral dwellers; for example, this strategy has been found in coral-dwelling gobies (i.e., *Gobiodon* spp) (Nakashima et al. 1996; Munday et al. 1998). These serial bidirectional hermaphrodites begin as females and change sex into males depending on body size, age, and the social organization within isolated branching corals where they live. A single male

and one or more females comprise a typical mating group. Under certain circumstances, such as the immigration of a larger and more dominant male into the coral head, the resident male can change sex back into a female to retain residence in the coral but also to realize some mating success with the new male, usually as the largest female in the group. In the 'risk of movement' model proposed by Nakashima et al. (1996) and Munday et al. (1998), predation pressure on these reef fishes makes movement between neighboring coral heads to seek other mates highly risky and dangerous, especially for small and sparsely distributed species such as gobies. This reproductive strategy may be advantageous for sedentary and stay-at-home individuals. They can switch genders as the need arises, such as when a mate dies or when the sex ratio is strongly skewed towards a specific gender, rather than risk predation while attempting to seek mates elsewhere.

Protandry is the opposite of protogyny and occurs when a male changes sex into a female. Social groups are composed of a larger female and up to several males. Sex change occurs when the female moves to another mating group or dies, thus allowing the largest male in the group to change sex. Although studies are limited, protandrous hermaphroditism has been reported for 21 genera of marine fishes within the families Centropomidae, Gonostomatidae, Muraenidae, Platycephalidae, Pomacentridae, Sparidae, and Creediidae (Sadovy de Mitcheson and Liu 2008; Langston 2004). Well-studied protandrous fishes include the anemonefish, *Amphiprion clarkii* (Hattori and Yamagisawa 1991) and the barramundi, *Lates calcarifer* (Moore 1979).

The discovery of this hermaphroditism among plants and animals including fish, has led to the development of hypotheses and investigatory techniques to try to understand how, when and why fish change sex, and what cues cause them to do so.

Suggested factors or conditions that trigger sex change include environmental conditions such as temperature, life history parameters such as size of an individual relative to others in the social group, the sex ratio of the social group, and local density of the social organization and behavior; all influence the timing and initiation of sex change (Mrosovsky and Pieau 1991; Buston 2003; Warner et al. 1996; Warner and Swearer 1991; Jones 1980; Collin et al. 2005; Munday 2002; Shapiro 1984; Wright 1989; Lutnesky 1994; Warner 1984; Shapiro 1987; Ross 1990; Kuwamura and Nakashima 1998). Other than natural causes of sex change, the drastically changing environment induced by human activities plays a role in sex change among marine organisms because of the pollutants released into the waterways that change the chemical composition of the surrounding environment; some of these pollutants alter an organism's endocrine system, and hence, reproductive system. Studies on long-term impacts of pollutants on the reproduction of fish indicate that pollution has a harmful effect upon sex determination, the development of gonads, and on the number of eggs produced (Kime 1995).

Different approaches are employed to examine sex change among fishes. One approach is the use of biological markers, including morphological structures and coloration, that have been used as indicators of sex change in many marine fish species (Cole and Robertson 1988). Research suggests that sex change is related more to body size (Jones 1980), but other factors, such as age and social behavior, are involved. For example, changes in the shape of dorsal fin spines and rays have been linked to sex change in gobies (Gobiidae) (Cole and Robertson 1988). The use of structural markers, however, has its drawbacks given that sex differentiation is very difficult to detect for several taxa because of the presence of shared markers between sexes in species that lack

sexual dimorphism. The visual or macroscopic examination of gonads to evaluate sex and maturity, while used commonly, is often misleading and can result in an underestimation or overestimation of mature individuals within a sampled population. One study on the reproductive cycle of snappers (Lutjanidae) in the genus *Lutjanus* found that macroscopic analysis misdiagnosed 47% of specimens examined (Longenecker et al. 2013). Other similar studies reported that an average of 50% of specimens are misdiagnosed (Grandcourt et al. 2011; 2006; Vitale et al. 2006). Inaccurate macroscopic analysis can lead to the design and implementation of fisheries management strategies or policies that inadvertently promote the depletion of fisheries resources because of inaccurate sex determination. Thus, the use of histological methods to analyze fish gonadal structure to determine patterns of sexuality and maturity is essential.

Histological analysis to evaluate and determine sexuality and maturity among hermaphrodites is becoming increasingly common and is required to diagnose hermaphroditism (Sadovy and Shapiro 1987a; 1987b; Sadovy and Domeier 2005; Sadovy de Mitcheson and Liu 2008; West 1990; Tyler and Sumpter 1996; Blazer 2002). This technique is now used routinely for sex verification, assessment of reproductive phase, or quantification of atresia (Blazer 2002). Histological examination has shown that during protogynous sex change ovarian tissues degenerate leaving only the ovarian wall, while testicular tissues may develop from the former ovarian lamellae or proliferate from the wall bordering the lumen radially or linearly (Cole and Shapiro 1992).

Several criteria have been commonly used to diagnose functional hermaphroditism including: a) detailed gonadal histological slides that show several phases of sex change; b) simultaneous occurrence of mature testicular and ovarian tissues

in gonads; and c) observations of functional sex change in identified individuals (Sadovy and Shapiro 1987b; Sadovy and Domeier 2005; Sadovy de Mitcheson and Liu 2008). In addition, certain criteria, while not reliable for independent validation of hermaphroditism, can support a determination of hermaphroditism, including the presence of a bisexual gonadal phase, sex ratio that is biased by sex and age, and sexual dimorphism (Sadovy de Mitcheson and Liu 2008). Although time-consuming and expensive when compared to the visual or macroscopic analysis used for reproductive studies, histology is an essential tool because it allows development of definite criteria to determine and confirm sexual patterns and describe other aspects of fish reproductive biology (Alonso-Fernández et al. 2011). Results from studies that utilize histology as an investigative tool can be informative in the future selection of fisheries conservation strategies and can allow improvements in the efficacy of management options. Through histological analysis many protogynous hermaphrodites have been confirmed including *Chrysoblephus puniceus* (Sparidae: Garratt 1986), *Thalassoma cupido* (Labridae: Meyer 1977), *Lethrinus rubrioperculatus* (Lethrinidae: Ebisawa 1997), *Neocirrhites armatus* (Cirrhitidae: Sadovy and Donaldson 1995) and *Sufflamen chrysopterus* (Balistidae: Takamoto et al. 2003).

Many fish species are important for coastal and island nations that rely heavily on marine resources as a source of food and income. The increased human population sizes in these nations and improved fishing technology have led to unsustainable fishing practices that have put pressure on many important fish stocks. An understanding of the important aspects of reproductive biology, such as sexuality, reproductive cycle, follicle development process, and time of spawning helps to develop better management and

conservation practices to sustain fish populations. For hermaphroditic fish species, the problem is especially acute. For example, parrotfishes may be overfished because all large individuals are usually terminal-phase males; if their numbers are depleted without replacement via sex change the population size and sex structure will be skewed towards smaller initial-phase males and females (Hawkins and Roberts 2003). To alleviate potential problems caused by this phenomenon, fisheries management policies and regulations have been implemented to sustain fisheries using tools that include setting species-specific body size limits, catch quotas, and fishing seasons. However, for these measures to be successfully applied, a sound knowledge of the life history and biology of the fish species is required. Important parameters such as size-at-maturity estimates of males and females, length-weight relationships, fecundity, spawning periodicity, and above all, sexuality should be well defined.

The hawkfishes (Cirrhitidae) consist of 33 species in 12 genera that are distributed in coastal coral and rocky reefs in the tropical and subtropical Indo-Pacific and Atlantic oceans (Randall 1963; 2001; 2005). Twelve species have been reported from Micronesia, and ten species are known to occur in the waters of Guam (Donaldson and Myers 1988; Myers 1999; Myers and Donaldson 2003). Most species in the family are relatively small, usually less than 250 mm total length (TL), but one species, *Cirrhitus rivulatus* (Eastern Pacific), reaches over 500 mm TL (Thompson et al. 1979). Their vibrant colors, acclimatization to captivity and easy upkeep have made many hawkfishes popular in the aquarium trade (Burgess et al. 1991). Other species are valued in dive tourism, and larger-sized species are taken as food, usually as bycatch, in subsistence or artisanal reef fisheries. All species are carnivorous with a diet consisting of small fish, crustaceans, or

small invertebrates. In coral and rocky reef systems, their habitats range from outer reef flats and spur and groove zones to reef slopes at depths of over 100 m (Chave and Mundy 1994; Myers 1999; Randall 2005; Donaldson unpublished manuscript). Species of at least five genera are often associated with corals of the genus *Pocillopora*, as well as sea fans and gorgonians (Donaldson 1989; Myers 1999; Kane et al. 2009) and are thus vulnerable to the effects of coral bleaching because of elevated sea temperatures.

Hawkfishes studied thus far have an harem mating system in which a single male is dominant over two or more females (Thresher 1984; Donaldson 1987; 1990; 1999). The male defends these females from rival males and courts and spawns with each female sequentially. Females within these social groups may exhibit territoriality towards one another. This system and behavior have been observed in several species including, *Amblycirrhitus bimacula*, *Cirrhitichthys aprinus*, *C. aureus*, *C. falco*, *C. oxycephalus*, *Cirrhitops fasciatus*, *Ci. hubbardi*, *Cirrhites pinnulatus*, *Cr. rivulatus*, *Cyprinocirrhites polyactis*, *Neocirrhites armatus*, *Oxycirrhites typus*, *Paracirrhites arcatus*, *P. forsteri*, *P. hemisticus*, *P. nisus*, and *P. xanthus* (Donaldson 1987; 1990; 1999; unpublished data; Donaldson and Colin 1989). Facultative monogamy exists also in at least two obligate coral-dwelling species, *N. armatus* and *O. typus*. Here, group size and composition are limited by microhabitat (i.e., *Pocillopora* coral, sea fans or gorgonians) size with smaller microhabitats restricting the group structure to just a single monogamous pair (Donaldson 1989; 1999). However, in a recent study by Kadota and Sakai (2016) a stable mating relationship in *Paracirrhites forsteri* was confirmed which was previously described by Donaldson (1990) as harem polygyny. Unlike in a harem polygyny where a large male guard several females and mates with them, the mating system observed in the population

of *P. forsteri* studied showed that during mating each female appeared on a prominent coral head located within a male territory, and the largest males would monopolize mating opportunities with the females. With mating complete, most females left the male home territories during the morning and early afternoon. This mating system has not been found in other hawkfishes and may be due to the availability of resources such as shelter, feeding and predation (Kadota and Sakai 2016). Many protogynous hermaphrodites, such as hawkfishes and angelfishes, and small protandrous species, have relatively small-sized mating groups with patchy distributions that are often limited by the availability of suitable microhabitats such as corals or host anemones (Donaldson 1987). With the destruction of these microhabitats, population structures are greatly affected. Hawkfishes studied thus far have four principal microhabitat associations (Donaldson 1987; 1990). These include obligate coral-dwelling (*Neocirrhites armatus* and *Oxycirrhites typus*), facultative coral-dwelling (e.g., *Paracirrhites arcatus*, and *P. forsteri*), non-coral substratum-dwelling (e.g., *Cirrhitichthys falco*), or water column-dwelling (*Cyprinocirrhites polyactis*).

Previous studies on hawkfishes were based on extensive field observations to understand its reproductive behavior, spawning, mating systems, and other reproductive aspects (Donaldson 1986; 1987; 1988; 1989; 1990). Until recently, gonad histology has been used to understand the pattern of sexuality in two genera, *Cirrhitichthys* and *Neocirrhites* with only some species being confirmed as protogynous hermaphrodites (Kobayashi et al. 1985; Kobayashi and Suzuki 1992; Sadovy and Donaldson 1995; Sadovy de Mitcheson and Liu 2008). Many other species remain to be investigated, and

this study's main objective is to describe the sexual pattern of *P. arcatus*, a species common on shallow reefs of Guam in the Mariana Islands.

Paracirrhites arcatus, is a facultative coral-dwelling species that forms harems (Donaldson 1990). This species ranges from East Africa to the Hawaiian Islands and Tahiti (Randall 2005). *Paracirrhites arcatus* is a popular aquarium fish species because of its relatively small size (to 140 mm TL) and ease of care in captivity. This species lives in relatively shallow (< 30m) reef areas containing large, living coral heads. *Paracirrhites arcatus* habitually lies motionless on the upper surface of living coral heads waiting for prey that it ambushes with a swift attack from its perch. Its diet is composed of crustaceans and small fishes that swim near it and often does not need to venture away from its home in search of food (Hiatt and Strasburg 1960).

This study aims to describe sexual pattern in *P. arcatus*. Because some members of the Cirrhitidae have been confirmed protogynous hermaphrodites, this suggests that other species, including *P. arcatus*, are protogynous (Sadovy and Donaldson 1995). To evaluate protogyny in *P. arcatus*, histological examination of gonads from individuals sampled from a given population should reveal that larger individuals are male and have transitioned from female to male, while smaller individuals should be female and lack evidence of transition. Using the characteristics of transition in protogyny described by Sadovy and Shapiro (1987) will help to evaluate protogyny in *P. arcatus*.

Fecundity and spawning periodicity were also considered initially for this study to better understand how fecund an individual female may be during a spawning period and estimate the average size at which a mature female individual is most fecund.

Fecundity and spawning cycles are important for understanding the reproductive biology

of many marine reef fishes. When understood clearly, these parameters provide information to assist in developing effective management and conservation strategies to sustain marine resources. However, because of logistical difficulties and the loss of microhabitat supporting *P. arcatus* at Orote Point because of crown-of-thorns starfish (*Acanthaster planci*, Acanthasteridae) predation upon corals favored by this hawkfish, sample sizes were too small to quantitatively investigate this aspect of reproductive biology of *P. arcatus*. Thus, fecundity and spawning cycles over time cannot be addressed here.

Methods

Site Description

The principal sampling site was the shallow reef on either side of Orote Point, Guam. Orote Point (13.44°N, 144.63°E) is located on the Orote Peninsula and lies on the western coast of Guam. The entire peninsula is defined by dramatic cliffs that drop steeply to the sea and inshore depths greater than 20 m may be reached quickly. Shallow reef flats and reef terraces occur adjacent to low-lying land features such as Orote Point Beach on the Apra Harbor side of the point and Orote Point Cove on the opposite side of the point (Figure 1). At the onset of the study, both supported abundant populations of *P. arcatus* that utilize *Pocillopora eydouxi* (Pocilloporidae) and other corals for shelter and perching points. Unfortunately, many of these corals were killed by foraging crown-of-thorns starfish, especially in Orote Point Cove, during this study. The decline

in the numbers of *P. arcatus* seen in the cove afterwards appeared to correspond to this loss of microhabitat (T.J. Donaldson, unpublished data).

Sample collection and processing

A total of 139 *P. arcatus* individuals was collected using SCUBA at depths of 4-9 m collected with hand nets and a small (diameter = 100 cm) surround net. Specimens were collected in May and June 2017 and February, May, June, October, and November 2018.

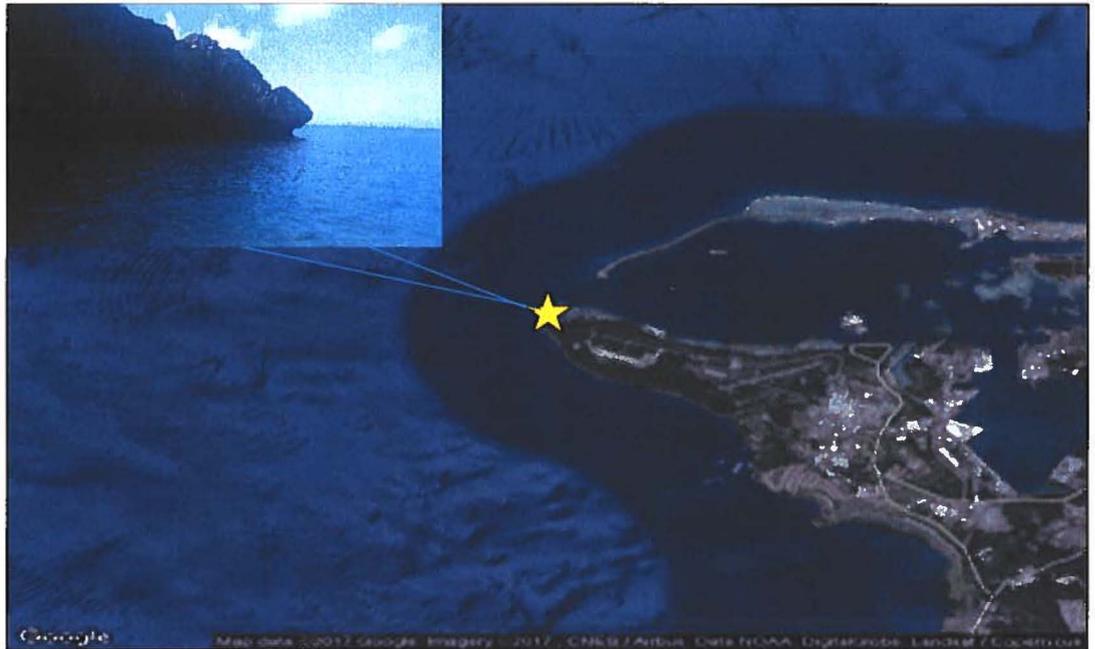


Figure 1: Orote Point, Guam showing the location of the principal sampling site. Google Earth Image © University Libraries GIS Research and Map Collection.

Once caught, fish were placed in plastic bags, carried to the surface, labeled, and kept on ice until taken to the laboratory, where they were frozen (-20°C) until processing. The specific histological techniques used were based upon methods described in Longenecker et al. (2013b). Laboratory work was conducted at the University of Guam Marine Laboratory, the UOG Biological Sciences Histology Laboratory, and the UOG Biological Sciences Microscope Laboratory.

All specimens were processed and examined using the following procedures. Total length (TL) and standard length (SL) were measured to the nearest 0.1 cm using a metric rule. Total length (TL) of a fish was measured from the tip of the snout with the mouth closed to the tip of the longest fin ray of the caudal fin. Standard length (SL), was measured from the tip of the snout with the mouth closed to posterior end of the caudal peduncle (i.e. excluding the caudal fin). Specimens were weighed using a digital balance to the nearest 0.1 g. For fish larger than 50 mm TL, gonads were extracted by first removing the caudal fin and caudal peduncle behind the anal opening with a scalpel or scissors, and then using either of the same tools to remove the head behind the gill arch. Then, a small incision was made through the abdomen to remove the gonads. Based on experience, removing gonads from individuals less than 50 mm TL was difficult, therefore for fishes smaller than this size the entire abdominal area was preserved in Dietrich's fixative (30 mL of 95% ethanol, 10 mL formaldehyde solution, 2 mL glacial acetic acid, and 58 mL water). No decalcification process was used for specimens placed in this fixative. All gonad samples from fish >50 mm TL were placed in wells that contained Dietrich's fixative and a specimen tag was placed with the gonad sample to identify each. Samples remained in the fixative for at least

24 hours. Gonad tissues were dehydrated in a graded ethanol series that was changed every hour with a stepwise increase in concentration (25%, 50%, 75%, and 95%). This was followed by a second immersion in 95% ethanol for an additional hour to remove water from the tissues. Then, gonad tissues were processed with two changes of JB4 infiltration solution (a water-soluble monomer compound) following kit instructions where the first infiltration was for ~24 hours followed by a second change for about an hour. The tissues were then embedded in a plastic resin used in the preparation of samples for high-resolution light microscopy. After embedding, tissue samples were sectioned using a microtome fitted with a glass knife and binocular dissecting microscope. For each tissue sample, ten sections approximately 5-7 μm thick were made and each section mounted on glass slides, stained with Toluidine Blue solution, and dried on a hot plate set to low temperature for at least a few minutes or until the tissue mounts dried completely. Gonad tissues were staged using a compound microscope (10X and 40X) fitted with a digital camera, with photographs taken and then examined to determine sex and maturity status.

The staging of oocyte development and sperm maturity was based on methods found in (Grier 1981) and Wallace and Selman (1981). Females were categorized based on four stages according to egg size and maturation. A Stage I oocyte shows primary growth where the cytoplasm is observed to stain darkly and is uniform, and the oocyte shape is ellipsoid or circular. Females with Stage II oocytes have cytoplasm with the presence of non-staining lipid vesicles, whereas Stage III (vitellogenic) oocytes have a cytoplasm mixture of non-staining lipid vesicles and have stained, round and uniform vitellin (yolk protein) globules present. Stage IV was divided into

two stages; IVa maturation and IVb hydration oocyte. In Stage IVa, the yolk protein forms into larger vitellin globules or forms a large cytoplasm-like structure, whereas in Stage IVb the cytoplasm shrinks and stains more lightly than Stage IVa. Evidence of maturity in female *P. arcatus* was considered for individuals in Stage IV. Staging of males was based upon the presence of visible spermatozoa (sperm cells with tails). The presence of sperm tails was used as an indicator of male maturity. Gonad sections were examined to detect individuals in the transitional stage to test the hypothesis that *P. arcatus* is a protogynous hermaphrodite using methods described by Sadovy and Shapiro (1987). Following the examination of gonad sections to evaluate protogynous hermaphroditism, data were grouped into 1 cm TL size classes to evaluate the size at which sexual transition occurs. Size class was also used to help determine the size structure at which most individuals are females, males, or transitional.

Statistical analysis

For evidence of protogyny, a two-sample t-test was used to test for a sex-based bimodal size distribution. To determine size at sex change, the range of sizes at which sex change occurs was investigated. Here a curvilinear regression analysis was performed on body size against the proportion of females and males, then tested with a two-sample t-test of mean lengths to test for significance. The sex ratio was also calculated and given as males: females (M: F) using the formula: nM/nF where nM equals the total number of males and nF equals the total number of females. To test for significance, the chi-square (χ^2) test was used to determine differences between sex from a 1: 1 sex ratio.

Results

Between 31 May 2017 and 20 November 2018, 15 trips were taken to collect samples of *P. arcatus* for reproductive analysis. Although sampling was initially planned to follow monthly lunar cycles, this was not followed due to logistical difficulties, mainly bad weather conditions making access to the site difficult. Overall, 139 specimens were collected, of which 111 were processed successfully. These were composed of females (64%), males (22%), and transitional individuals present (14%). Mean lengths of female, male, and transitional fish were 6.7 cm (TL), 8.3 cm (TL), and 6.0 cm (TL), respectively (Table 1).

Indications of size classes for males, females and transitional fish are shown in Figure 2 and demonstrate a sex-bias within the size frequency distribution of the sampled population. Males were generally larger than females, with females ranging from 3.5 – 8.5 cm TL and males from 4.4 – 11.0 cm TL. The smallest sexually mature female was 5.2 cm TL, and most females were observed to be mature with the presence of large vitellin globules (Figure 5). With the exception of a single immature male (4.4 cm TL), all male individuals that were sampled were determined to be mature based on the presence of spermatogenic tissue (sperm tails) (Figure 6). Size classes of 5, 6, 7, and 8 cm TL had an overlap of mature female and male individuals with females being dominant in the smaller size classes (i.e., 5, 6, and 7 cm TL). In larger size classes, male individuals predominated, and female individuals were no longer observed. Comparisons of male and female *P. arcatus* body sizes indicated differences between the sexes (Figure 3). A two-sample t-test showed that mean lengths of male and female individuals were significantly different ($p = <0.001$).

All gonads processed were bilobed and symmetrical, as described for another hawkfish, *Neocirrhites armatus*, by Sadovy and Donaldson (1995). Gonads were clearly female or male in function because mature tissue of both sexes did not co-occur.

Immature females (Stage I and II) had previtellogenic oocytes. As maturity progressed lipids accumulate within the cytoplasm (Stage III), with the development of vitellogenic oocytes (Stage IV). Gonads of both sexes had a distinct lumen, and gonadal lamellae contained spermatogenic and ovarian tissue. During development of testes, transitional individuals contained varying numbers of previtellogenic oocytes or early stage degenerating vitellogenic oocytes. Development of spermatogenic tissue was observed to begin on the exterior surface of the gonad and progress slowly throughout the tissue. Spermatogenic tissue was found either in isolated cysts scattered throughout ovigerous lamellae during transition as ovarian tissues degenerated.

The estimated size at maturity for half of the females *P. arcatus* within the population was 7.4 cm TL (Figure 8). The sex ratio for *P. arcatus* where M: F was 0.33: 1 ($\chi^2= 23.253$, $df = 1$, $p = <0.001$), thus indicating a predominance of females within the population. A total length of 7 cm was estimated to be the size at which half the female individuals in the population would have changed fully into males.

Table 1: Summary of the length (cm TL) distribution for each sex of *Paracirrhites arcatus*.

	Female	Male	Transitional
<i>n</i>	71	24	16
Mean	6.7	8.3	6.2
SD	0.981	1.477	1.354
SE	0.116	0.301	0.339
C.I of Mean	0.232	0.623	0.722
Median	6.8	8.65	6.3
Min	3.5	4.4	4.4
Max	8.5	11	8.9
Range	5	6.6	4.5

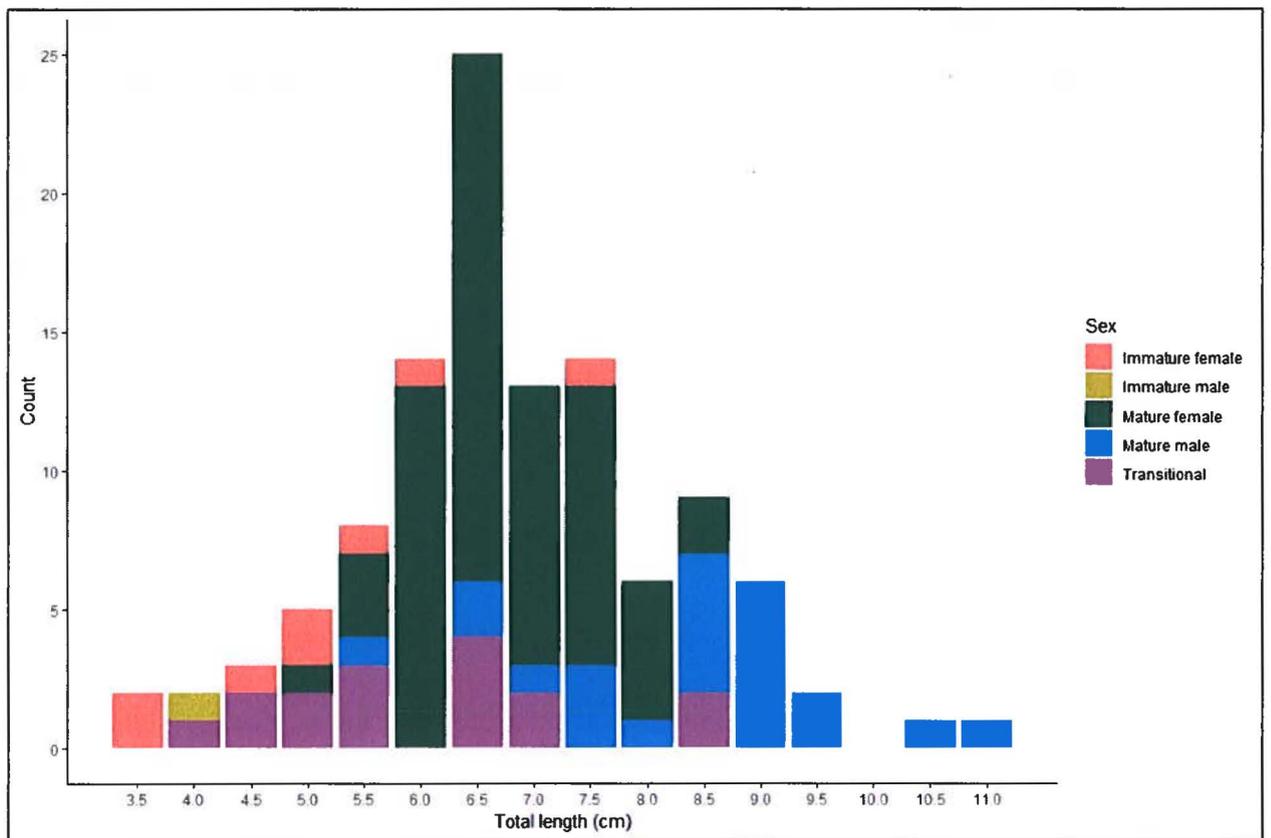


Figure 2: Size frequency distribution of *Paracirrhites arcatus*, $n = 111$.

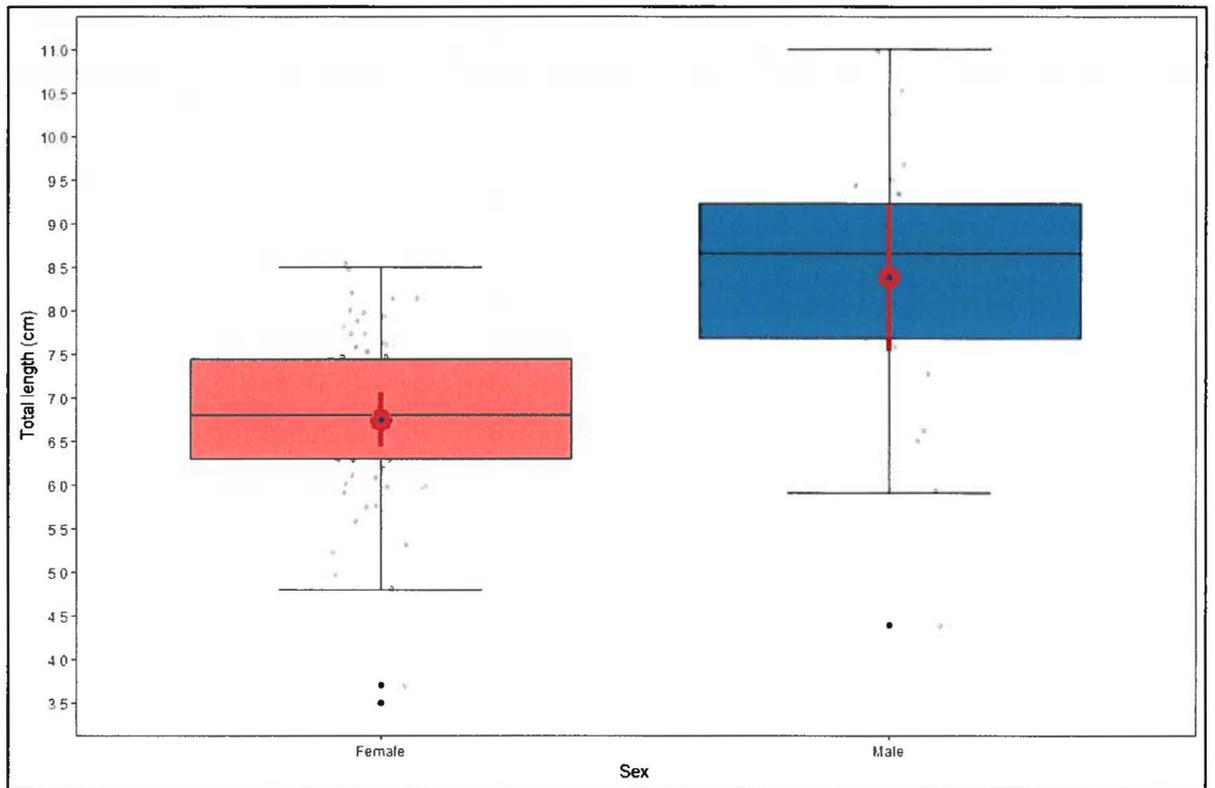


Figure 3: Boxplot, dot plot and standard error of the mean (SEM) plot for total lengths of each sex of *Paracirrhites arcatus*. Gray dots = sample data points, Black dots = outlier, Blue dot = mean, Red = 99% confidence interval.

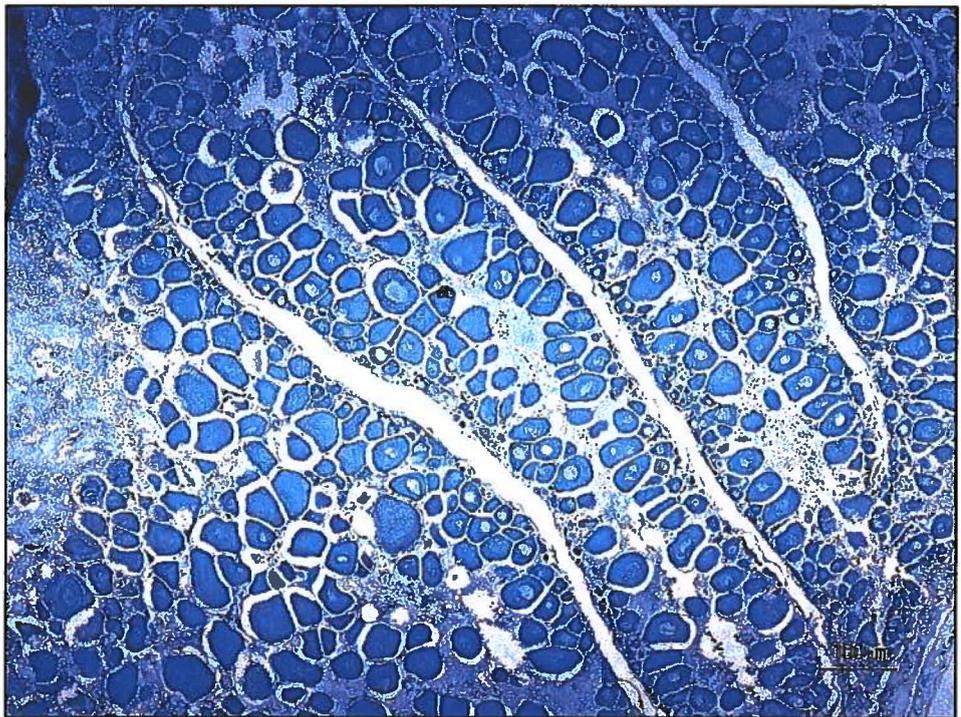


Figure 4: Stage I (top image) and Stage II (bottom image) oocyte of *Paracirrhites arcatus*, with total body length of 5 cm and 7.5 cm, respectively. Magnification: 50X.

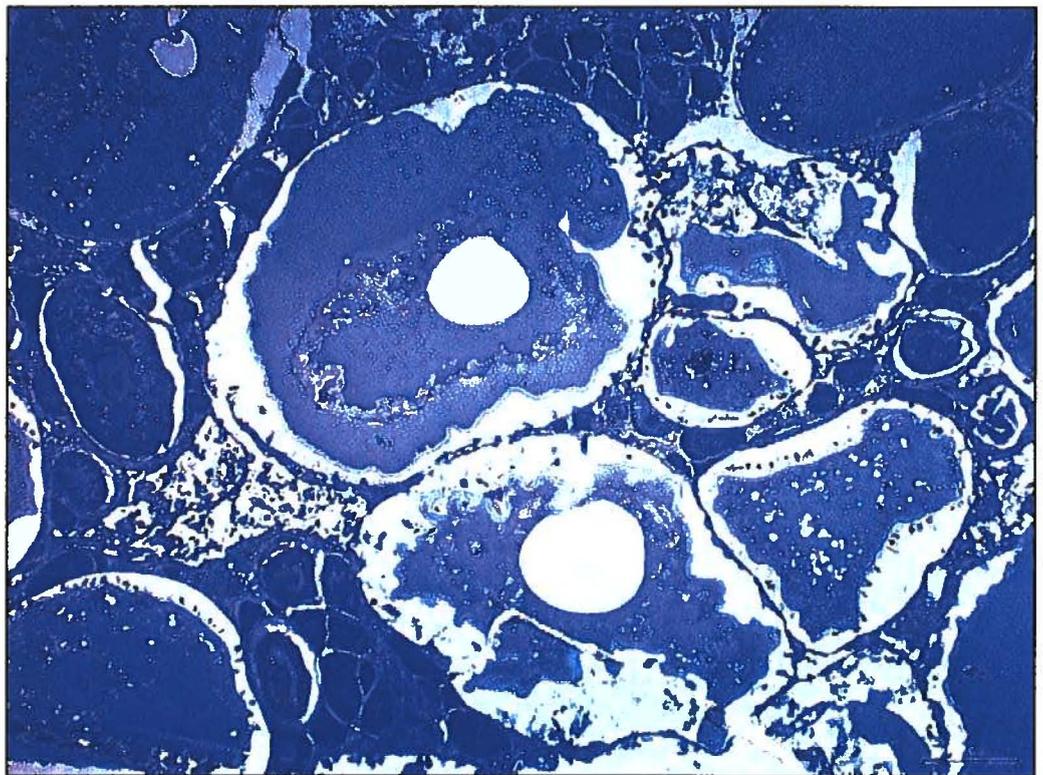
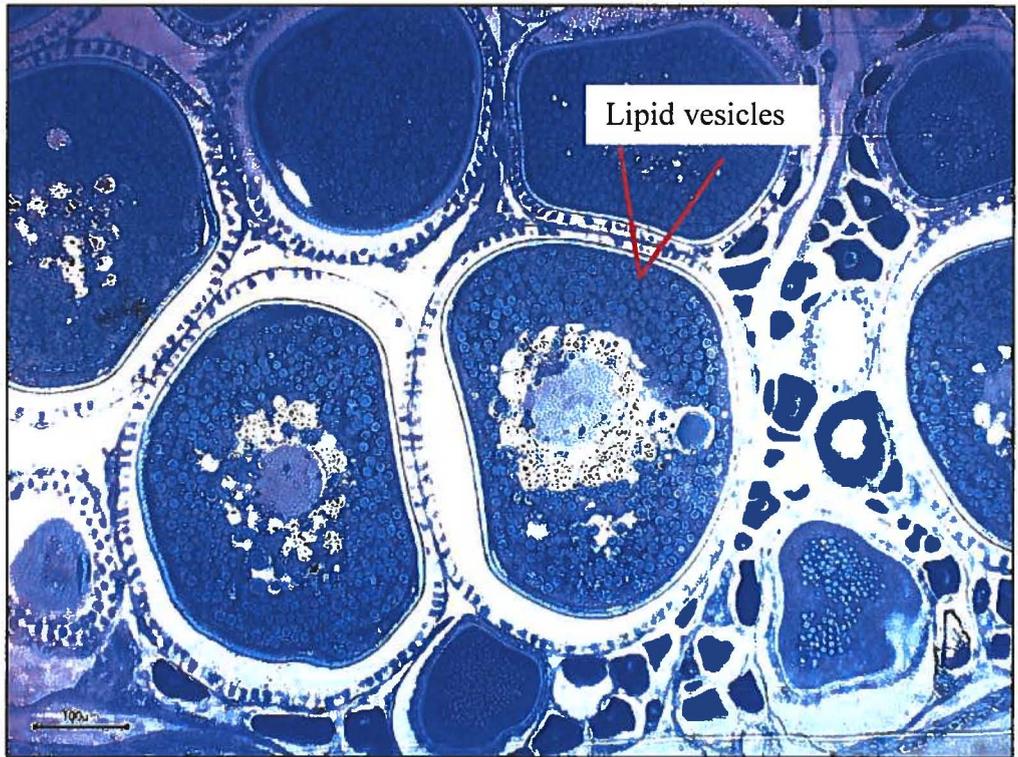


Figure 5: Stage III (top) and IV (bottom) oocyte of *Paracirrhites arcatus* with total body lengths of 6.9 cm and 6.4 cm, respectively. Magnification: 50X.

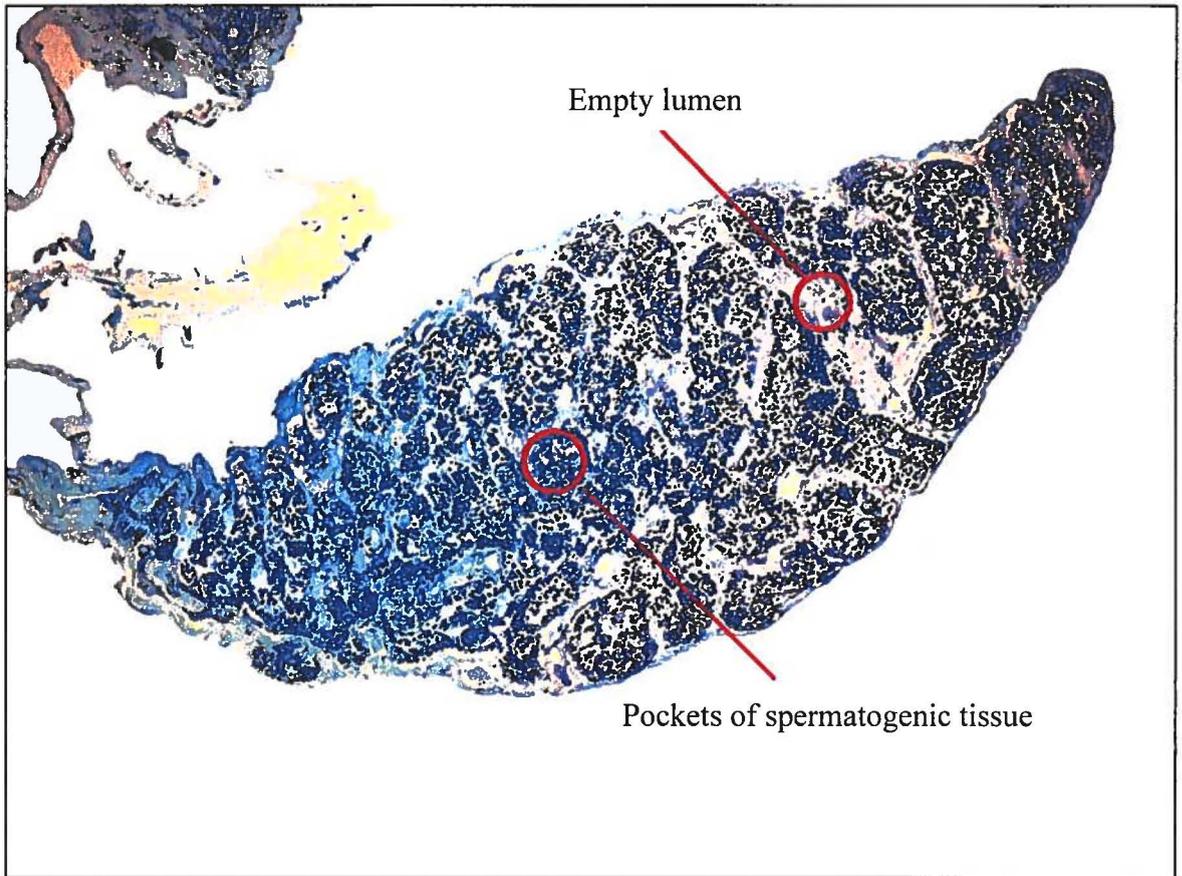


Figure 6: A mature male of *Paracirrhites arcatus* with a total body length of 7.8 cm. Magnification: 100X.

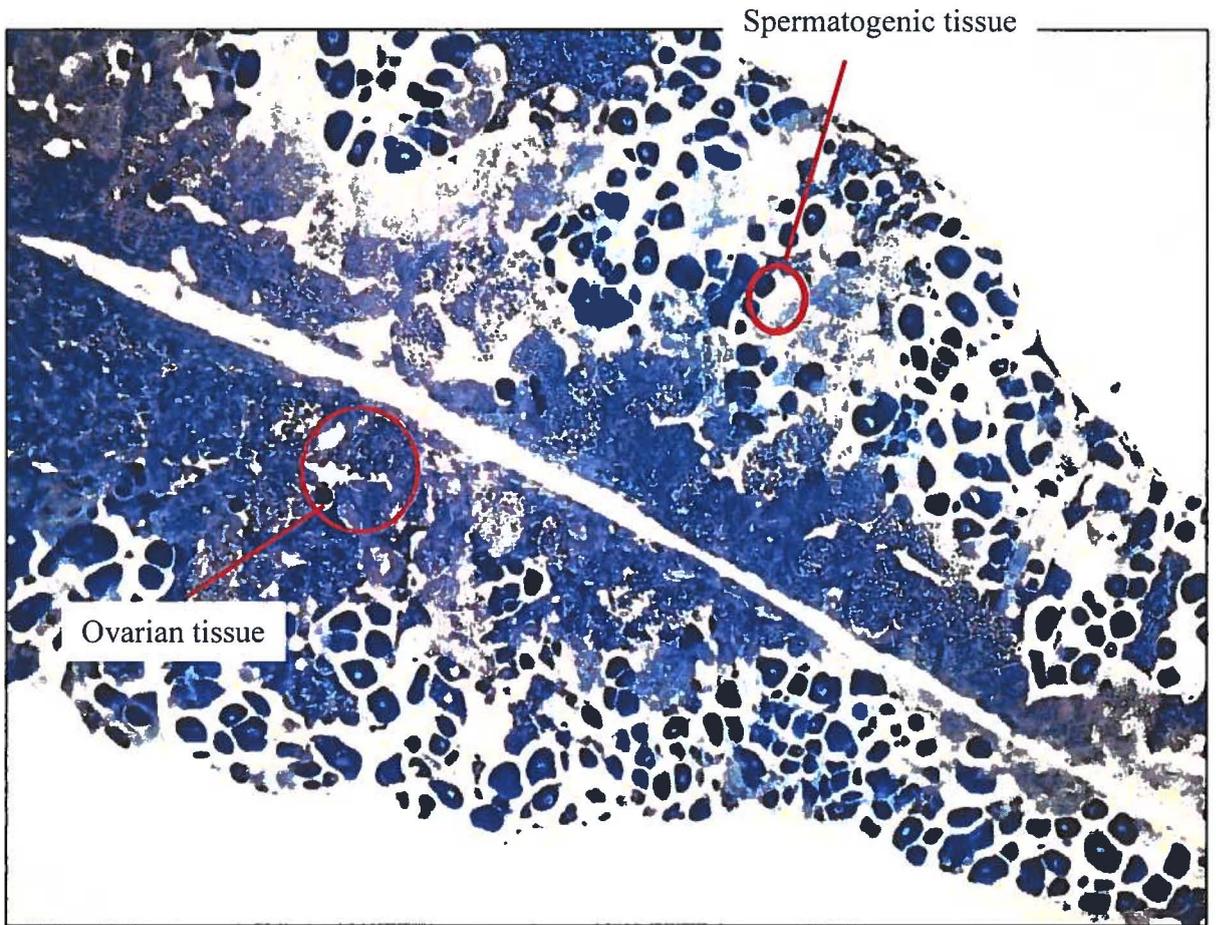


Figure 7: Image showing a transitional of *Paracirrhites arcatus* with a total body length of 6.8 cm. Magnification: 100X.

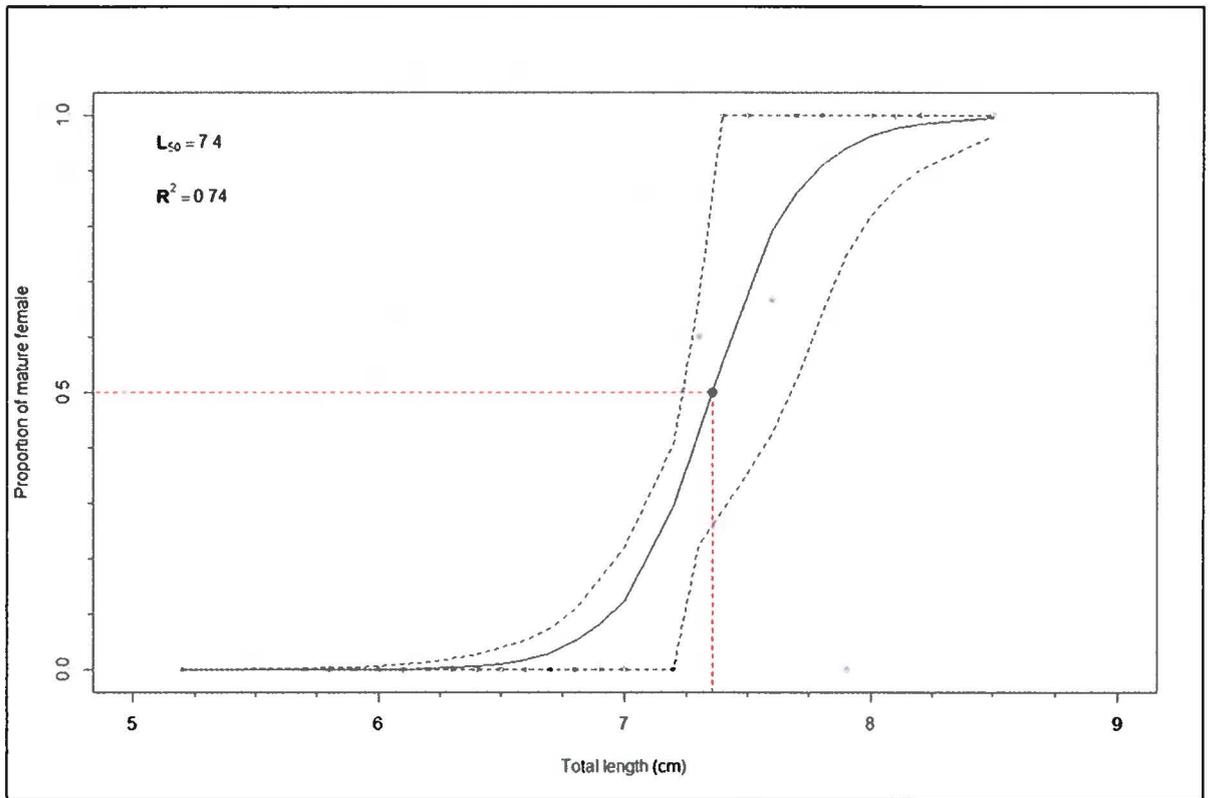


Figure 8: Size comprising 50% female mature individuals (L_{50}) of *Paracirrhites arcatus*, $n = 63$.

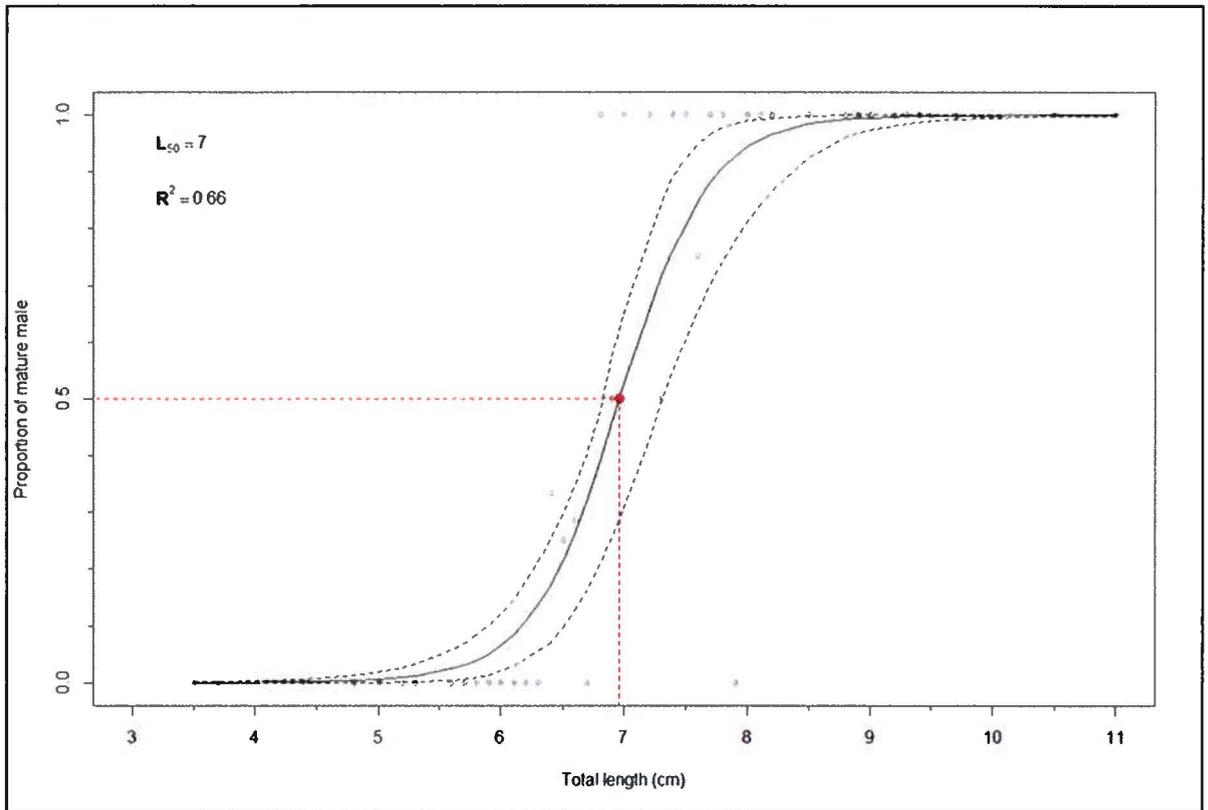


Figure 9: Estimated body size (length, TL) (L_{50}) at sexual transition from female to male of *Paracirrhites arcatus*, where $n = 86$.

Discussion

Protogynous hermaphroditism has been suggested for hawkfishes because of two features described by Sadovy and Donaldson (1995). The first being the mating system of harem polygyny composed of large dominant male and several smaller females, a characteristic common among protogynous hermaphrodites. The second being the presence of sexual dimorphism which is not exclusive to protogynous hermaphrodites (Sadovy and Shapiro 1987). Most hawkfishes studied thus far have a harem mating system composed of male dominated social groups and several females with a female-biased sex ratio suggesting a protogynous sexual pattern (Donaldson 1987; 1990). Sexual dimorphism in body sizes is also evident among all species of hawkfishes, with males having larger body sizes than adult females within a harem or pair. This suggests that body size differences are a trait that is consistent with protogynous hermaphroditism. Sexual patterns in hawkfishes have been studied both in natural conditions and captivity with few species studied and have been confirmed protogynous hermaphrodites for *Amblycirrhitus pinos*, *Cirrhitus pinnulatus*, *Cirrhichthys aprinus*, *C. falco*, and *C. oxycephalus* (Thresher 1984; Kobayashi et al. 1985; Kobayashi and Suzuki 1992; Sadovy and Donaldson 1995; Sadovy and Liu 2008, Sakai et al., 2011a). Although suggested to be protogynous hermaphrodites, some species of hawkfishes studied thus far are confirmed to be bidirectional sex changers (the ability to switch sex back and forth as the need arises). Bidirectional hermaphroditism is reported in *Cirrhichthys aureus* and *Cirrhichthys falco* (Kobayashi and Suzuki 1992; Kadota et al. 2011a). Other species which have been reported to simultaneously contain both male and female reproductive tissues, but not yet confirmed, include *C. falco*, *C. aprinus*,

Cirrhitops hubbardi, and *Cyprinocirrhites polyactis*. Results from this study suggest *P. arcatus* is a protogynous hermaphrodite on the basis of transitional individuals present in the sampled population, differences in size frequency distribution of sexes, and a sex biased distribution with a predominance of female individuals.

Gonadal structure of *P. arcatus* shows a distinct difference between male and female in sexual function, and there is a shift from female to male with increased body size (Figure 3). The presence of transitional individuals strongly suggests sex change, as well. This sexual pattern was proposed during behavioral studies by Donaldson (1990), where groups of hawkfish species, including *P. arcatus*, were reported inhabiting coral heads where there were several females (usually the smaller individuals) guarded by a large male. The presence of transitional individuals (female to male) within the sampled population also suggests protogynous sex change. Both sexes had a distinct lumen, and gonadal lamellae containing spermatogenic and ovarian tissue (Figure 7). Protogyny is suggested for *P. arcatus* on the basis of testes exhibiting evidence of previous female function, particularly the presence of degenerating vitellogenic oocytes and, in mature ovaries, the presence of scattered cysts and islets of spermatogenic tissue. A diagnosis of protogyny is further supported by larger male sizes, female bias towards smaller body size, and by the absence of smaller males within the sampled population.

Estimation of size at sex change and maturity has not been analyzed in previous studies of hawkfishes, therefore in this study it was attempted for *P. arcatus*. Sex change for *P. arcatus* where 50% of the population would have fully changed from female to male was 7 cm, while size at maturity for females where 50% would be mature in the sampled population was 7.4 cm. This variation in size and maturity is probably from

social control within the harem rather than from the overall population because every harem (social group) is different. Also, this result is due to sampling as a population rather than as a social group where changes are clearer. The sex ratio for *P. arcatus* was not equal, 0.33: 1 (M: F) and indicated a female-biased population. This is consistent with findings from studies of other protogynous species where there are more females than males within mating groups and populations (Warner 1975; 1984). This pattern is similar also to that found in other hawkfishes that have been confirmed to be protogynous hermaphrodites where the females outnumber males within the group (Donaldson 1990).

Although not addresses in this study, courtship and spawning of hawkfishes have been observed to take place at dusk, just after sunset and continuing into darkness (Thresher 1984; Donaldson 1986; 1987; 1990; Donaldson and Colin, 1989; Kadota et al. 2010). Spawning of hawkfishes is pelagic, and cirrhitids studied thus far have a pelagic egg and larval phase (Thresher 1984; Donaldson 1986b; 1987; Donaldson and Colin 1989). Although aquarium observations by Lobel (1974) of *O. typus* suggested that this species was a demersal spawner, Donaldson and Colin (1989) demonstrated that it spawned pelagically in the wild. Courtship and spawning of Indo-West Pacific hawkfishes including *P. arcatus* are suggested to occur year-round at low, tropical latitudes, and seasonally in higher latitudes where water temperatures during cooler parts of the year limit reproductive activities (Donaldson 1989).

Based upon the findings of this study, protogynous hermaphroditism is confirmed for *P. arcatus*. To date, 13 species in six hawkfish genera have been confirmed to exhibit protogynous and bidirectional hermaphroditism based upon similar histological studies with support from observations of courtship behavior in captivity and the wild

(Kobayashi and Suzuki 1992; Sadovy and Donaldson 1995; Sadovy de Mitcheson and Liu 2008; Sakai et al. 2011a; 2011b). A long-term study could be undertaken to fully understand and evaluate the reproductive biology of *P. arcatus* and other hawkfishes. Fecundity and spawning periodicity are important aspects of reproductive biology of coral reef fishes and should also be studied.

Conclusions

The results of this study confirm that *P. arcatus* is a protogynous hermaphrodite on the basis of histological analysis confirming sex change, the presence of a female-biased population (common for protogynous species), and the presence of transitional individuals in the sampled population. Female *P. arcatus* mature at an estimated total length of 7.4 cm, where this may be when large female individuals within a harem are highly fecund and focus energy towards egg production. Through future studies of fecundity in *P. arcatus*, this aspect can be fully understood.

Transitional individuals were observed in between 4 – 8 cm TL suggesting that sex change occurs at a smaller size. Because not all female individuals would contribute towards egg production during spawning periods, sex change may help them to gain more status within the harem and most likely increase their reproductive success. With sex change occurring at very small sizes, additional studies should investigate the sexual pattern of *P. arcatus* to determine if other sexual patterns, including bidirectional sex change under unusual circumstances, exists within this species. For male individuals sampled, all but one was considered mature due to the presence of sperm tails. The estimated total length of mature male individuals ranged from 5.9 – 11.0 cm, which is a

high range of values and suggests that sex change for *P. arcatus* is most likely occurring at a smaller size, or that some individuals change sex, and some do not.

Reproductive studies on small coral reef fishes including hawkfishes provide an understanding of some important aspects of sexual patterns in these fish species. Understanding the social systems of harem fish such as hawkfishes, including *P. arcatus*, through studies of their social behavior coupled with histological analysis of gonads provides a clear understanding of their sexual pattern. Future studies could focus more upon sexuality at the level of the harem rather than at the population level because there is the opportunity to obtain a better understanding of sexual patterns in such groups and determine how sex change structures them within a population.

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