

Evidence for protandry in *Polydactylus quadrifilis* in the Kwanza Estuary, Angola, and its implications for local fisheries

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Abstract A total of 141 *Polydactylus quadrifilis* were sampled from the Kwanza Estuary in Angola ranging in size from 436 to 1360 mm fork-length (FL). Of these, 124 were male, six intersex and 11 female. Female fish were significantly longer (mm, FL) and heavier (kg) than males and had significantly higher gonadosomatic indices (GSI's) than those of males and intersex fish. Transitional (intersex) gonads were delimited, with testicular and ovarian regions separated by connective tissue. The first signs of ovarian tissue appeared on the outer ventral surface of the gonad. A second layer of ovarian tissue was first noticeable at either end of the initial ovarian region and developed back towards the centre of the ventral wall to form a luminal space. Early-stage oocytes were commonly found in the outer area of male regions and residual late-stage spermatids and spermatozoa were found in the luminal space of ovarian regions, suggesting a process of sex change from the outside inwards. A loss of male function was noted with increased ovarian prevalence. Based on this evidence it is suggested that *P. quadrifilis* in the Kwanza Estuary are protandrous. Owing to the reliance of *P. quadrifilis* on large highly fecund females for egg production, it is

likely that they will be sensitive to fishing practices that target larger individuals within the population.

Keywords Sequential hermaphroditism · Polynemidae · Subsistence fisheries · Recreational fisheries · Sexual pattern · Histology

Introduction

Sequential hermaphroditism is a common trait in fishes (Ross 1990). It is a process that involves the sexual maturation of a fish as one particular sex before undergoing a change to the opposite sex at a certain age or size (Allsop and West 2003) or due to social cues (Avisé and Mank 2009). The evolutionary driver of this phenomenon is most likely related to the preferential selection for larger individuals to be a certain sex (Ghiselin 1969; Shapiro 1987; Warner 1988; Ross 1990). The diagnosis of such a reproductive strategy is essential in understanding the life history and biology of a fish species, particularly for important fishery species whereby the size-selective nature of fishing practices can drastically effect the population structure, and thereby sex-structure of fish stocks (Molloy et al. 2007). The result of this could be a reduction in recruitment through either sperm-limitation in protogynous species, or egg-limitation in protandrous species (Alonzo and Mangel 2004; Molloy et al. 2007).

The giant African threadfin, *Polydactylus quadrifilis* (Cuvier, 1829), is a member of the family Polynemidae (Motomura 2004). It is distributed from Angola to

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Senegal along Africa's west coast (Motomura 2004). *Polydactylus quadrifilis* is an important fishery species throughout its distribution and is captured in local subsistence and small-scale fisheries (trawl, gill-net and beach-seine) as well as a growing recreational fishery (Moses 2000; Motomura 2004; Carpenter and De Angelis 2016). This is particularly true in Angola, where the Kwanza Estuary is fast becoming popular with South African recreational anglers specifically targeting large *P. quadrifilis*. Despite their economic importance, there is limited biological information on the species, which is vital for promoting its sustainable management.

Polynemid reproduction is poorly understood although it is thought to occur in coastal areas or estuaries (Motomura 2004). Eggs have been recorded as small and pelagic (Motomura 2004) and the larval development of some species has been briefly noted (Santerre and May 1977). Protandrous hermaphroditism has been suggested for numerous species within the family (Longhurst 1965; Kagwade 1968, 1976; Motomura et al. 2002; Motomura 2004; Pember 2006), although the trait is unconfirmed due to a lack of conclusive evidence (Sadovy De Mitcheson and Liu 2008). A doctoral thesis (Pember 2006) suggested significant evidence for sex change in *P. macrochir* and *Eleutheronema tetradactylum* but was possibly not reviewed by Sadovy de Mitcheson and Liu (2008) due to the fact that the work had not undergone peer-review at the time. Since then, an additional paper has suggested protandry in *Eleutheronema tetradactylum* from India (Shihab et al. 2017).

A strict set of guidelines exists for the correct classification of species as functional hermaphrodites (Sadovy and Shapiro 1987; Sadovy de Mitcheson and Liu 2008). Initially, numerous lines of evidence were considered in the diagnosis of sequential hermaphroditism including the simple presence of a bisexual gonadal phase, age- and/or size-sex frequency histograms, sex ratios and sexual dimorphism (Longhurst 1965; Kagwade 1968, 1976; Sadovy and Shapiro 1987). More recently, many of these supporting features have been reconsidered following alternative explanations for the noted observations (Sadovy and Shapiro 1987). At present, it is suggested that the most significant aspects of diagnosing protandry in wild fish are the presence of degenerating testicular tissue and developing ovarian tissue in the gonads of transitional individuals as well as detailed histological descriptions of various stages of

sex reversal (Sadovy and Shapiro 1987; Sadovy de Mitcheson and Liu 2008). Additionally, controlled laboratory or field experiments that can be run over a period of years and using known individuals can provide undeniable evidence for sex change (Guiguen et al. 1994; Micale et al. 2002).

The aim of this study was to diagnose the sexual pattern of *P. quadrifilis* in the Kwanza River region of Angola. In doing so, particular attention was paid to the guidelines set out by Sadovy and Shapiro (1987) and Sadovy de Mitcheson and Liu (2008).

Materials and methods

Sampling took place in the mouth area and first four kilometres of the Kwanza Estuary in Angola between the 21st of June 2016 and the 21st of February 2017 (Fig. 1). Fish were either collected using conventional angling techniques or purchased from local subsistence gill-net and longline fishermen.

Fish were weighed (nearest 0.01 kg) and measured (FL, mm) before being dissected. Fish were sexed as male, female or intersex and the gonads were macroscopically staged according to the eight stages outlined by Laevastu (1965): I = virgin (males only); II = immature/resting; III = developing; IV = maturing; V = mature/pre-spawning; VI = spawning; VII = spent; VIII = recovering. The gonads were then removed and weighed (nearest 0.01 g). The eviscerated mass of the fish was noted (nearest 0.01 kg) and the gonadosomatic index (GSI) was calculated using the equation:

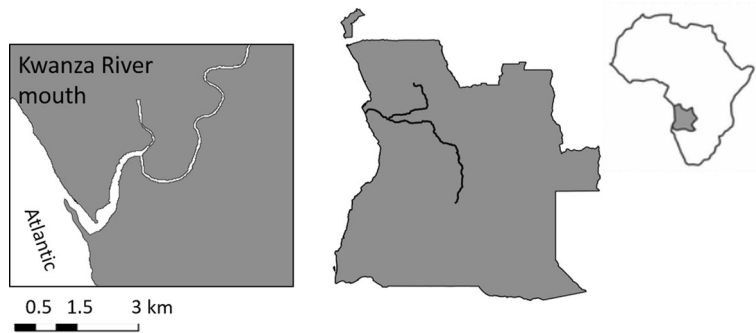
$$GSI = \frac{100 \times \text{gonad mass}}{\text{eviscerated mass}}$$

Mean monthly GSI values were calculated for males and females using non-juvenile individuals (stage II or above).

Representative male gonads belonging to each male macroscopic stage, all intersex and all female gonads were fixed in a 10% formalin solution for between one and four months before histological analysis.

Three 1 mm thick sections were taken transversely through the anterior, median and posterior regions of each gonad using a razor blade. These sections were directly embedded in paraffin wax, sectioned at 5–6 μm , and stained using haematoxylin and eosin (HE) (Austin and Austin 1989). Histological evidence was used to validate the sex of individuals and the macroscopic

Fig. 1 Study area displaying the mouth and first 4 km of the Kwanza Estuary in Angola where sampling took place



staging protocol. Validation of macroscopic staging was based on the histological characteristics of the various stage male and female gonads of *Eleutheronema tetradactylum* and *P. macrochir*, which were described by Pember (2006).

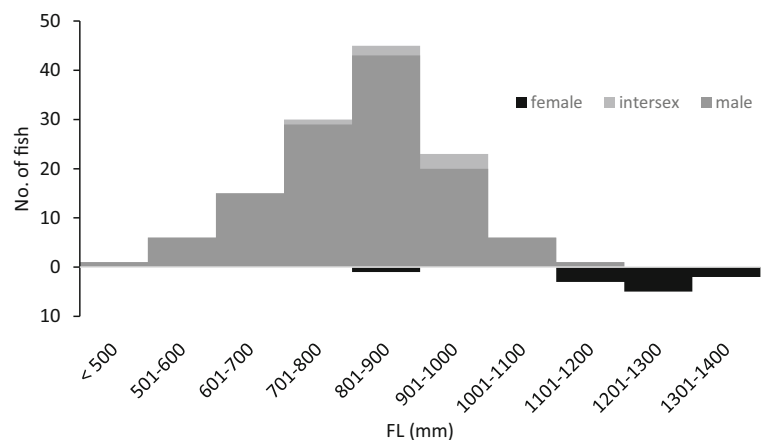
To diagnose sexual pattern, the histological sections of male and intersex fish were examined for any signs of testicular degeneration and ovarian development. Ovarian sections were analysed for the presence of residual testicular tissues. All intersex gonads were classified by their functional sex and the diameter of various stage oocytes were measured using the program Leica LAS EZ V3.3 (Leica Microsystems Inc.). From histological sections, the area of the testicular region was calculated as a percentage of the entire gonad using ImageJ free-ware (Schneider et al. 2012).

A non-parametric Kruskal-Wallis test with multiple comparisons was performed to assess for differences between the mean length (FL, mm), mass (kg) and GSI values of male, intersex and female fish. Vector images were created using free online graphics software Vectr (Vectr Labs Inc.).

Results

In total, 141 *P. quadrifilis* were sampled ranging in size from 436 to 1360 mm FL and weighing between 1.46 and 38.40 kg (Fig. 2). Of these, 124 were macroscopically sexed as male, 12 as female and five as intersex. Later histological analysis identified one fish that was initially classed as female to be intersex while all other macroscopic sex classifications were verified (ie: 124 male, 11 female and six intersex). The mean \pm SD fork-length (FL) was 813 ± 129 mm for males (range: 436–1120 mm), 894 ± 57 mm for intersex fish (range: 790–955 mm) and 1202 ± 147 mm for females (range: 825–1360 mm) (Fig. 2). The average weight was 8.23 ± 3.75 kg (range: 1.46–21.50 kg) for males, 10.37 ± 1.70 kg (range: 7.30–12.18 kg) for intersex fish and 27.45 ± 9.19 kg (range: 7.65–38.4 kg) for females. There was a significant difference between the FL (Kruskall-Wallis: $H(2) = 27.70$, $p < 0.01$) and weight ($H(2) = 27.54$, $p < 0.01$) of fish between sexes and female fish were significantly larger than males in terms of both FL ($p < 0.01$) and weight ($p < 0.01$).

Fig. 2 Sex-specific length frequency histogram of 141 *Polydactylus quadrifilis* sampled from the Kwanza Estuary, Angola



The majority of fish were captured during the winter months (late June, July, August & September), with reduced numbers from October onwards (Fig. 3). All female fish were encountered between the 1st of July and the 10th of September with only males and one intersex fish encountered outside of this time.

Macroscopic appearance of gonads & GSI

Macroscopic sexing of *P. quadrifilis* proved to be uncomplicated, as male, early- to mid-transitional intersex and female gonads were visually distinct. Late-transitional gonads were difficult to identify as very little testicular tissue was present.

Males

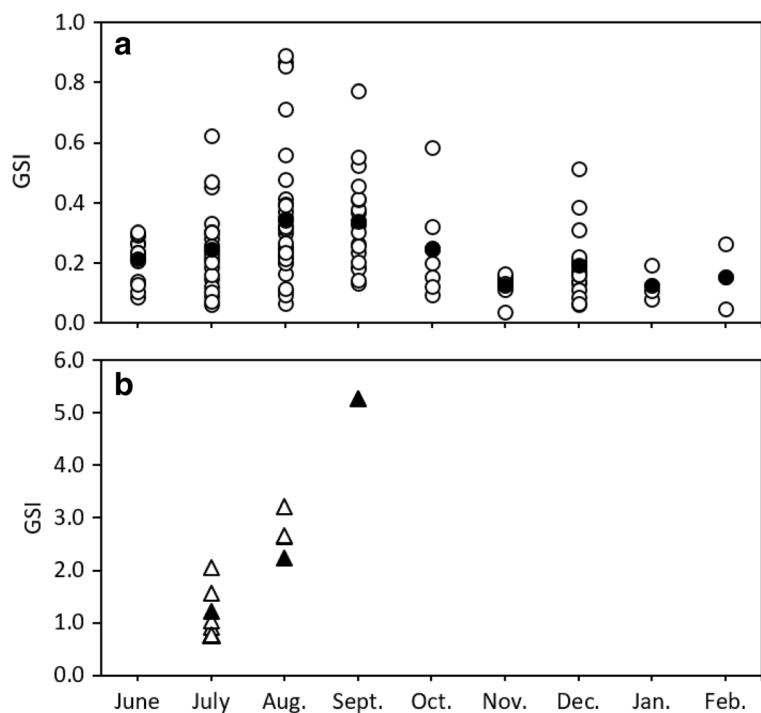
Males were observed throughout the sampling period and although developmental stages II through VIII were encountered, no virgin (stage I) fish were observed. Paired male gonads were white in colour and varied in length according to their level of development although they always occupied the majority of the length of the coelomic cavity forwards towards the head (Fig. 5a1, a2). Some well-developed testes (stage V-VI) had small lobes (1–4 cm) that branched rearward from the

urogenital opening. Early-stage testes (stage II-III) appeared flattened with an oval sectional (transverse) appearance. In contrast, the sectional appearances of more developed gonads (stage IV-VI) were triangular. Spent (stage VII) testes were flaccid and appeared more flattened than testes from the previous two stages. Recovering (stage VIII) testes appeared small and flaccid with a red/brown colouration.

Intersex

Early to mid-stage intersex gonads were noticeable macroscopically via the presence of a pink-orange translucent layer that seemed to cover the testicular tissue within (Fig. 4b1, b2). They also appeared more rounded compared to the triangular appearance of the well-developed male gonads. Milt was present in all of these individuals and was exuded spontaneously when fish were placed on a hard surface or when pressure was applied to the abdomen of the fish. Late-stage transitional gonads appeared similar to what a typical immature or early developing ovary would look like, bright orange in colour and cylindrical, but with a thin strip of noticeable white tissue on the inner dorsal surface of the gonad (Fig. 4c1, c2).

Fig. 3 Monthly gonadosomatic index (GSI) values for non-juvenile male (a) and female (b) *Polydactylus quadrifilis* from the Kwanza Estuary, Angola. Hollow points represent individuals and solid points represent the mean monthly value



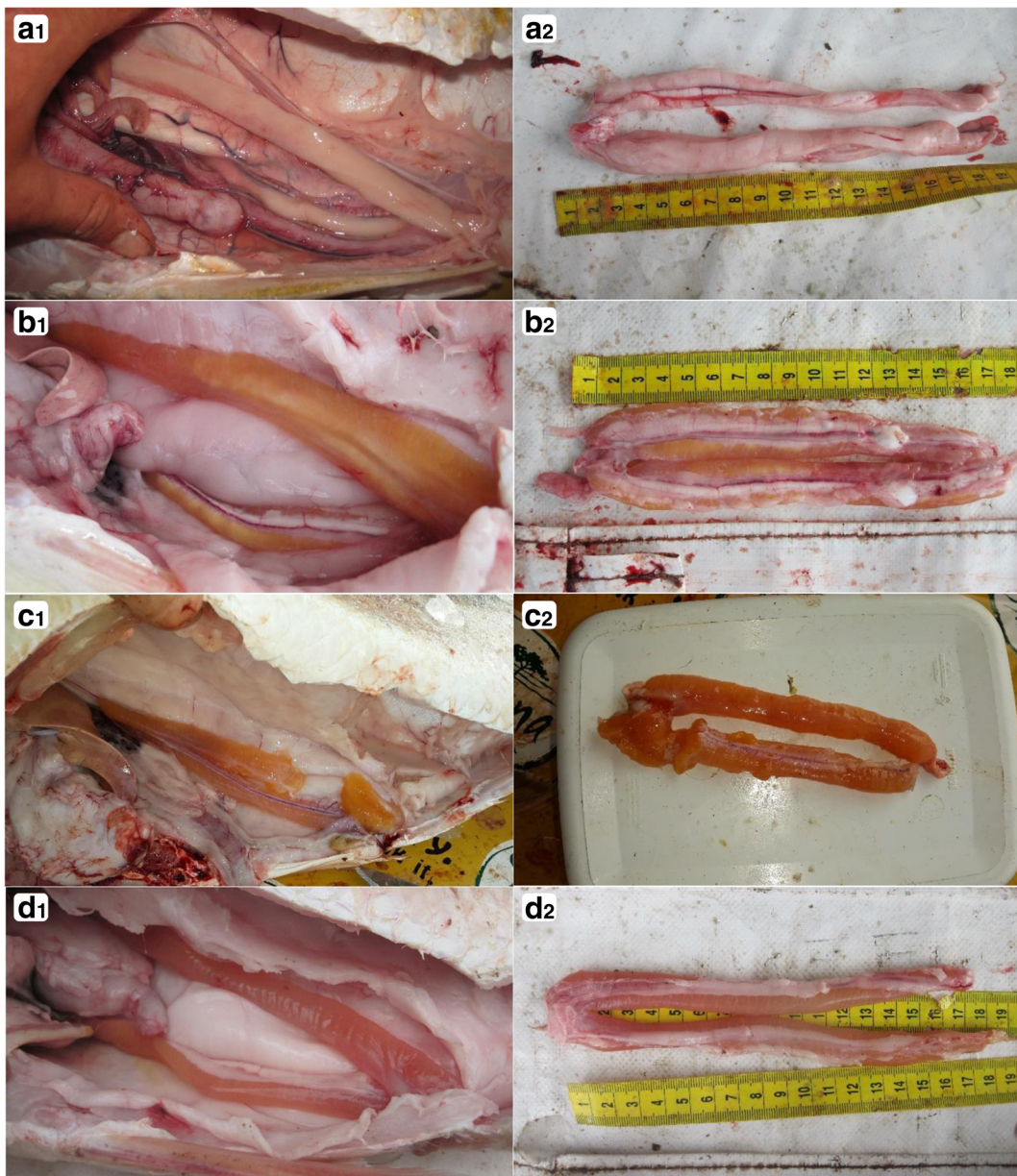


Fig. 4 Macroscopic appearance of various gonads of *Polydactylus quadrifilis* collected from the Kwanza estuary, Angola. Images display a mature (stage V) male testis (**a**) (August, 900 mm FL), **b**: an early/mid transitional gonad clearly displaying the testicular tissue (white) beneath the ovarian tissue (orange) and the developed sperm duct that runs along the inner dorsal area of the gonad (August, 880 mm FL), **c**: A late transitional gonad that

was initially described as female but later reclassified as intersex (note the thin line of white tissue on the inner dorsal surface where one would expect to find a sperm duct) (August, 955 mm FL), **d**: an immature/resting ovary (white tissue on the inner dorsal surface of the gonad is lipid) (August, 825 mm FL). Images display gonads inside the coelomic cavity (1) and post-dissection (2)

Females

Large female fish were mostly in a mature or spawning condition and only one small,

immature/resting female was observed (825 mm FL, 7.65 kg). Ovaries were varying shades of orange-yellow, with clearer orange colour indicative of early-stage ovaries and a creamy-yellow

colouration displayed in mature ovaries with oocytes clearly visible through the ovarian wall. A single immature/resting ovary (stage II) appeared fluid-filled and cylindrical in shape (Fig. 4d1, d2). Developing ovaries (stage III) were much larger than the single immature/resting ovary and possessed many folds. Maturing and mature ovaries (stages IV–V) were richly vascularised and extremely large, occupying a significant proportion (both forward and backward from the urogenital opening) of the body cavity.

Macroscopic staging was validated for 29 of 31 representative male histological samples. The two gonads that were staged incorrectly included a fish that was reclassified from mature (stage V) to developing (stage III) and another that was reclassified from developing (stage III) to resting (stage II). Of the 11 female fish, macroscopic staging was validated for 10 fish and corrected for one which was macroscopically staged as mature (stage V) but was reclassified as maturing (stage IV).

Mean monthly gonadosomatic indices were highest for both male ($n = 121$ - three male fish did not have values for gonad mass) and female ($n = 10$) *P. quadrifilis* in the months of August and September (Fig. 3). There was a significant difference between the mean GSI of males (0.27 ± 0.18 (SD)), intersex fishes (0.27 ± 0.08) and females (1.96 ± 1.43) ($H(2) = 28.81$, $p < 0.01$) (Fig. 5). Between the three sex classes, female GSI was significantly different from both male ($p < 0.01$) and intersex ($p = 0.01$) GSI, while the mean GSI for intersex fishes was similar to that of males ($p = 0.99$) (Fig. 5). The cumulative mass of all non-juvenile reproductive organs from

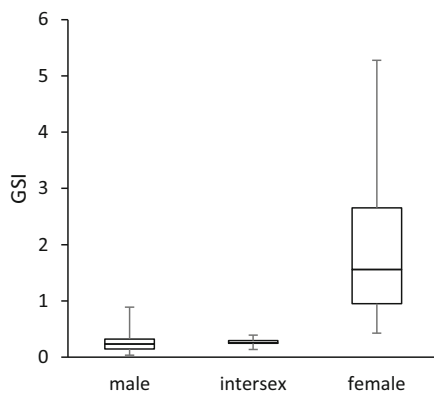


Fig. 5 Gonadosomatic indices (GSI) displayed for male, intersex and female *Polydactylus quadrifilis* sampled from the Kwanza Estuary, Angola

121 males was 2778.8 g when compared to 5867.7 g from the 10 females.

Histological characteristics of gonads

Males

The main histological characteristics used to differentiate between the developmental stages of males included the development of the sperm duct and sperm sinuses, the amount of connective tissue present and the composition of specific male germ cells and their location (Fig. 6a1, a2). Stage II and III testes were characterized by large amounts of connective tissue. Stage II testes contained spermatocytes, spermatids and spermatozoa which were present in numerous small crypts throughout the testis with no sperm sinuses yet present. Sperm ducts were visible in stage III testes, although they were not always filled with sperm cells. Sperm sinuses that contained spermatids and spermatozoa were noticeable close to the sperm duct. The outer region of the gonad was characterised by crypts (Fig. 6a1) that contained cells at various stages of spermatogenesis (as seen in Fig. 6a2). In relation to the sperm sinuses and sperm duct, these crypts were located on the outer area of the gonad in all testes belonging to stages III–VII. Stage IV testes could be differentiated from stage III testes by the increased development of the sperm sinuses and sperm duct, which were filled with spermatids and spermatozoa. The sperm sinuses of mature (stage V) and spawning (stage VI) testes extended the majority of the distance towards the gonad wall (Fig. 6a1). The spermatids and spermatozoa in spent (stage VII) testes were arranged less densely while recovering (stage VIII) testes were characterised by large amounts of connective tissue and a lack of early-stage spermatogonia.

Intersex - transitional gonads

Transitional gonads were delimited (Sadovy and Shapiro 1987) and characterised by well-defined testicular and ovarian regions that were separated by connective tissue (Fig. 6b–e). Ovarian regions were located on the outer ventral surface of the gonad while testicular regions were located on the inner dorsal area (Fig. 6b1, c1, d1, e1). Small numbers of early-stage oocytes were often noted within the outer areas of male regions (Fig. 6b2) and late-stage spermatids and spermatozoa were present in parts of the ovarian region (Fig. 6c2). Ovarian

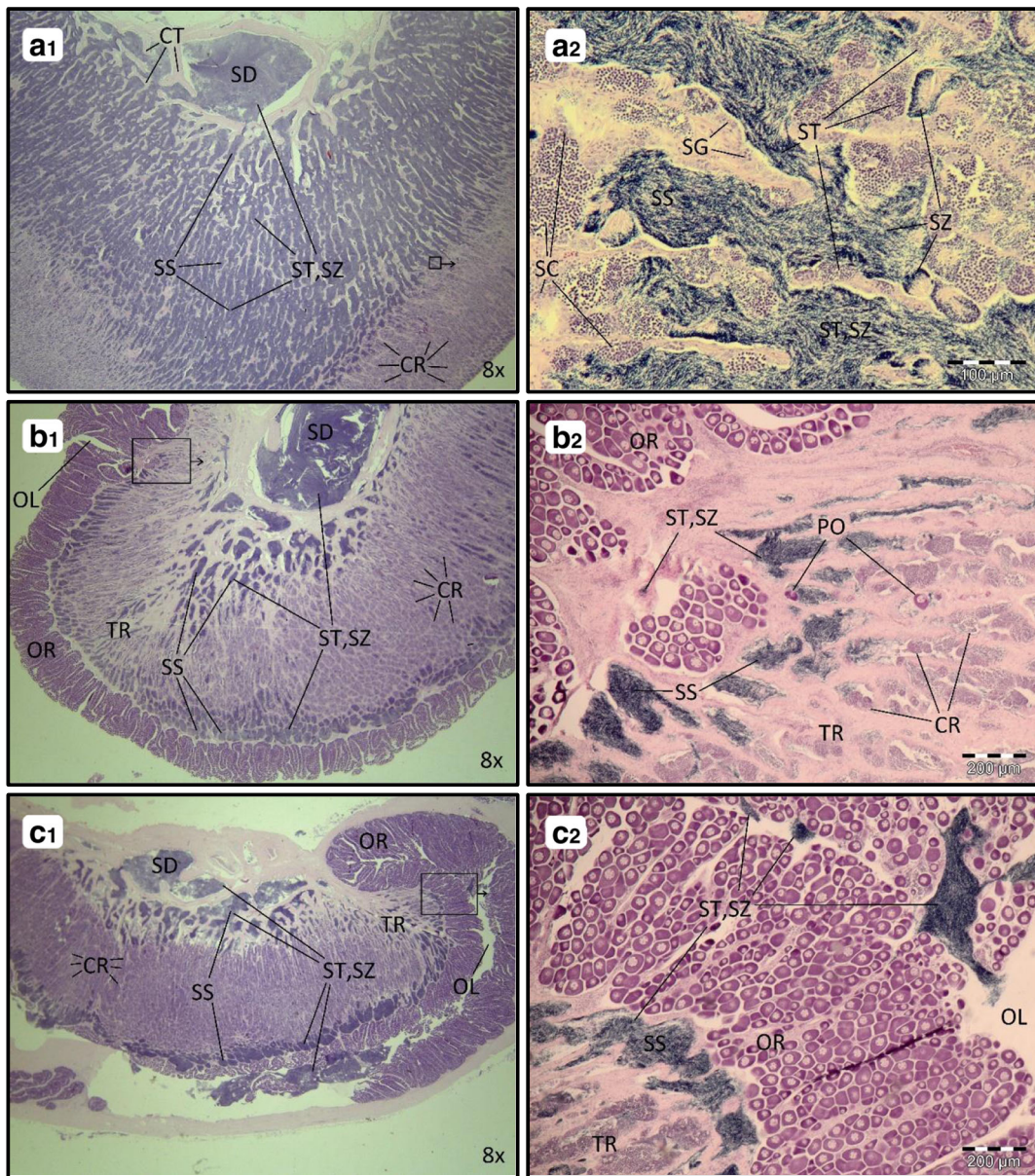


Fig. 6 Low (1) and high (2) power images of transverse microsections through gonads at various stages of sex change in *Polydactylus quadrifilis* including: **a** a mature (stage V) testis (August, 890 mm FL); **b** an early-transitional intersex gonad with ovarian tissue on the outer ventral surface (1) and early-stage oocytes in the outer testicular region (2) (August, 925 mm FL); **c** a mid-transitional intersex gonad displaying ovarian tissues developing from either side of the gonad (1) and residual male tissues in amongst the developing ovarian space (2) (July, 897 mm FL); **d** the first late-transitional gonad displaying the greatly reduced male region (1) and degenerated sperm duct (2) (August, 915 mm FL); **e**

the second late-transitional gonad displaying the remnant male region (1) comprising residual spermatids and spermatozoa in pockets of connective tissue (2) (August, 955 mm FL); **f** an immature/resting ovary showing extensions of connective tissue which resemble those of the second late transitional gonad (1) and what may be the remnant degenerated sperm duct* (2). (August, 825 mm FL) BV – blood vessel; CR – crypts; CT – connective tissue; OL – ovarian lumen; OR – ovarian region; PO – perinucleolar oocyte; SC – spermatocytes; SD – sperm duct; ST – spermatids; SZ – spermatozoa; TR – testicular region

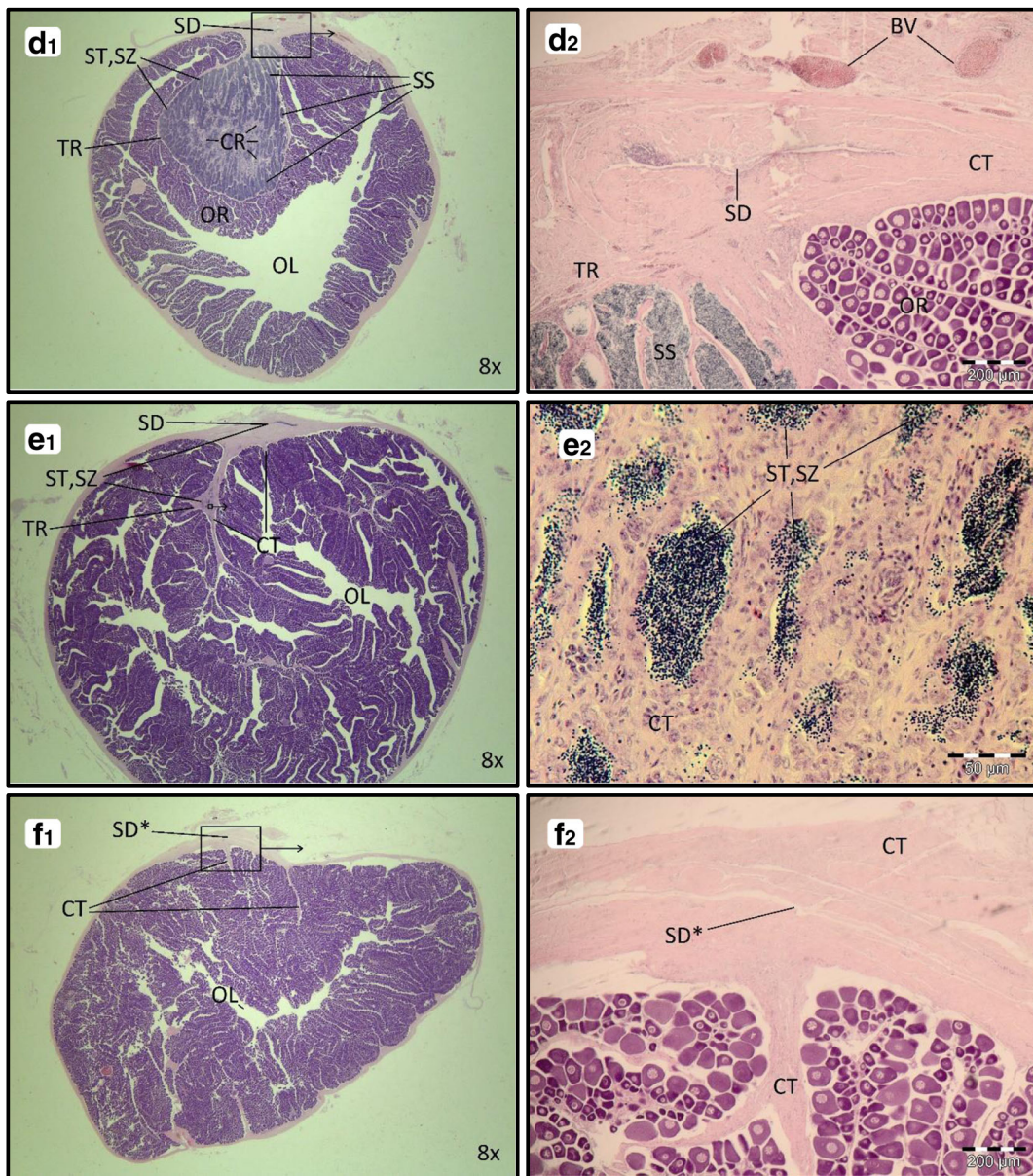


Fig. 6 (continued)

regions were comprised of previtellogenic oocytes. Testicular regions were made up of various male germ cells in crypts, sperm sinuses and the sperm duct. In all transitional gonads, a layer of late-stage spermatids and spermatozoa (in sinuses) was present around the periphery of the male region (Fig. 6b1, c1, d1, e1).

Of the six intersex gonads, four were classified as functionally male due to the presence of large functional sperm ducts and testicular cells at various stages of

spermatogenesis (Fig. 6b1, c1). The remaining two intersex gonads were classified as late transitional with no clear functional sex due to the undeveloped nature of the sperm duct and the presence of few early-stage male cells (Fig. 6d1, e1).

Early to mid-transitional gonads (functional males) For functional male transitional gonads, the ratio of male to female tissue was not always uniform throughout the

length of individual gonads and in some cases the transverse sections from one region comprised 90% male tissue while other regions comprised only 40 to 50% male tissue. In two of these cases male tissue dominated the posterior region of the gonad and was least prevalent in the anterior region. However, there was no noticeable difference in the ratio of male to female tissue between the different sections of the other two individuals.

Histological examination of the four functional males revealed well-developed testicular tissue which occupied between 40% and 90% of the gonad (Fig. 6b1, c1). This contained obvious sperm ducts and well-defined sperm sinuses that were filled with mature spermatids and spermatozoa (Fig. 6b1, c1). Numerous crypts were present that encased male germ cells at various stages of spermatogenesis. The testicular region appeared similar to that of a maturing (IV) or mature (V) male testis (as seen in Fig. 6a). However, a layer of sperm sinuses (that were filled with spermatids and spermatozoa) was apparent in the outer area of the testicular region (Fig. 6b1, c1). Early-stage oocytes were often located in this outer male region (Fig. 6b2).

The ovarian region was largely comprised of early-stage perinucleolar ($\pm 50 \mu\text{m}$) and chromatin nucleolar oocytes ($<20 \mu\text{m}$). In sections that comprised small percentages of female tissue ($<15\%$), a single layer of ovarian tissue was present (Fig. 6b1). In further developed sections that comprised more ovarian tissue (20–60%), a second layer of female tissue was present on either side of the ovarian region (Fig. 6c1). The second layer appeared to develop from both ends of the ovarian region, eventually meeting in the centre of the ventral surface of the gonad. The gap between the first and second layer formed a luminal space. Residual testicular tissue, in the form of late-stage spermatids and spermatozoa, was commonly found within areas of the developing ovarian lumen (Fig. 6c2). No early-stage spermatogenic tissue was apparent in the ovarian component.

Late transitional The gonads of two intersex individuals were categorised as late transitional and appeared to have lost their male function due to the prevailing ovarian tissue which dominated the gonadal area (Fig. 6d1, e1). There was no noticeable difference in the ratio of male to female tissues between the anterior, median and posterior regions of either of these gonads.

Testicular tissue comprised 16% of the gonadal space in the first individual (Fig. 6d1). This tissue was

dominated by late-stage spermatids and spermatozoa which were found in sinuses around the periphery of the testicular region (Fig. 6d1). Some early-stage or developing male germ cells were present in the centre of the testicular region and the sperm duct was not well developed (Fig. 6d2). The ovarian component of the gonad was characterised by early-stage oocytes, primarily perinucleolar and chromatin nucleolar, of small size ($\pm 50 \mu\text{m}$). Early-stage oocytes were, as in the functional male gonads, observed in the outer testicular region of the gonad (see Fig. 6b2). There was evidence of residual testicular tissue in some areas of the ovarian component of the gonad (see Fig. 6c2).

The gonads of the second late transitional fish were dominated (98%) by female tissue (Fig. 6e1). Ovarian tissue was at a very early stage of development although stage-one perinucleolar oocytes appeared larger (70–80 μm) than those in the early to mid-transitional gonads and in the previous late transitional gonad. Stage two perinucleolar oocytes (130 μm in diameter) were also visible and were characterised by clear amphophilic vacuoles within the cytoplasm. Male tissue was comprised of residual spermatids and spermatozoa which were situated in pockets of connective tissue (Fig. 6e2) and in the degenerated sperm duct (Fig. 6e1). No early-stage spermatocytes or spermatogonia were present in the gonad and, like in the first specimen, some residual spermatids and spermatozoa were evident within the luminal space of the ovarian region (see Fig. 6c2).

Females

The ovary of the single immature/resting female (825 mm, FL) (Fig. 6f1) was similar to that of the ovarian section of the second late transitional gonad (see Fig. 6e1) and was dominated by small (50–70 μm) perinucleolar oocytes, although a few larger late-stage perinucleolar oocytes (120–140 μm) were also present. The walls of the gonad appeared thin in comparison to the more developed ovaries, with arms of connective tissue projecting inwards from the ovarian wall in a similar fashion to those observed in the late transitional gonad (see Fig. 6e1). A small area of weakly formed connective tissue was present in the gonadal wall where one would expect to find a sperm duct (Fig. 6f2).

Developing (stage III) ovaries were histologically characterised by the presence of cortical alveolar oocytes in relatively high numbers along with

perinucleolar and chromatin nucleolar oocytes. Thick well-vascularised connective tissue was present in the walls of these ovaries and also extended across regions of the ovary. Stage IV ovaries were similar to those of stage III with the additional presence of yolk granule oocytes. The ovaries of mature females (Stage V) were dominated by yolk granule oocytes with cortical alveolar and perinucleolar oocytes also present.

Discussion

Several lines of histological evidence for classifying protandry in *P. quadrifilis* were identified in this study. In transitional gonads, residual late-stage spermatogenic tissue within the ovarian component suggests previous male function. The outer testicular region of transitional gonads only contained late-stage spermatogenic tissues, similar to those found within the ovarian component. This is unlike the normal testicular anatomy of mature male fish, whose testes are characterised by the presence of late-stage spermatids and spermatogonia in sinuses near the sperm duct while the outer region is comprised of crypts containing cells at various stages of spermatogenesis. It is therefore likely that this outer testicular region is in the process of degeneration. In addition, the presence of early-stage oocytes in the outer testicular region suggests that this area is in a process of cellular reorganisation towards female function. The degeneration of the sperm duct with increased prevalence of ovarian tissue also suggests that there is a loss of male function that corresponds with ovarian development.

The process of sex change and the development of the ovarian luminal space in *P. quadrifilis* can be explained via a number of steps. An overall reshaping of the gonad from triangular (Fig. 7a) to circular (Fig. 7e) takes place. A layer of degenerating male tissue, in the

form of late-stage spermatids and spermatozoa, is present on the outer testicular region of all intersex gonads (Fig. 7b-d). A single layer of ovarian tissue is first noticeable on the ventral surface of the gonad (Fig. 7b). Thereafter a second layer develops from either end of the ovarian region to form the luminal space (Fig. 7c). Residual male tissue within the luminal space of the (outer) ovarian component and developing early-stage oocytes in the (inner) male portions, suggest a process of development from the outside inwards. There is a loss of male function with increasing ovarian prevalence shown first by the degeneration of the sperm duct (Fig. 7d) and then by a loss of all early-stage male germ cells (Fig. 7e). This sex change process appears to follow a similar process to that of *Inegocia japonica* described by Fujii (1971), whereby the first signs of ovarian tissue appeared on the outer region of the gonad and developed inwards, displacing the testicular tissue over time. The luminal space was formed through a reshaping of the gonad to form a second layer of ovarian tissue on the outer surface of the gonad (Fujii 1971).

The characteristics of the bisexual gonads of *P. quadrifilis* display many similarities to those of other polynemids. In many species, including *P. macrochir* and *Eleutheronema tetradactylum* (Pember 2006), *Leptomelanosoma indicum* (Kagwade 1976), *Galeoides decadactylus* (Longhurst 1965) and *P. microstoma* (Dorairaj 1963), ovarian tissue is located on the outer ventral region of the gonad and testicular tissue on the dorsal inner region. In fact, while working on *Leptomelanosoma indicum* (previously known as *Polydactylus indicus*), Kagwade (1976) noted that “It may be said that it (testicular tissue) is not noticeable anywhere on the outer surface of the gonad. It appears that the proliferation of the ovarian lamellae extends so much over the greatly reduced testicular part that the latter which remains concealed inside the former cannot

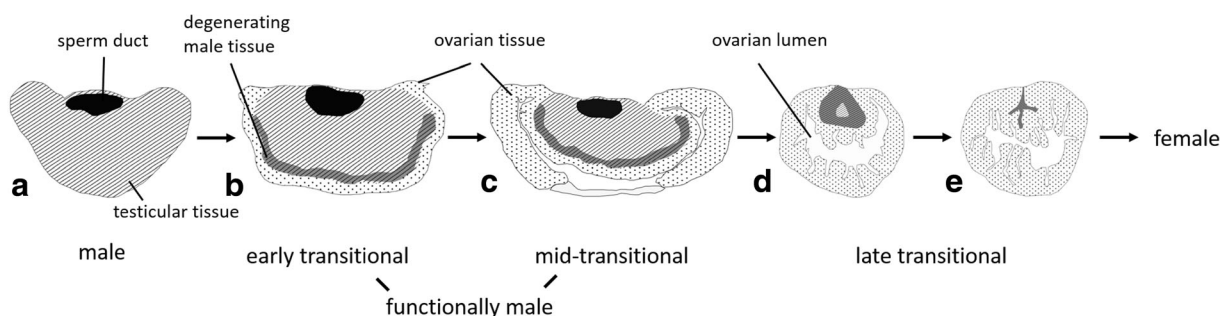


Fig. 7 Schematic diagram showing the process of sex change in *Polydactylus quadrifilis*, from male (A) through intersex (transitional gonads) (B-E) to female

be taken notice of". Furthermore, the male and female regions of bisexual gonads have been consistently reported as being separated by connective tissue (Dorairaj 1963; Kagwade 1976) and gonads comprising various amounts of testicular and ovarian tissues have often been suggested to be at different stages of transition (Longhurst 1965; Pember 2006). Thus, it is possible that protandry may feature in other polynemid species and reproductive studies that use conclusive histological approaches are necessary to appropriately diagnose the trait.

With regard to the population structure of *P. quadrifilis* in this study, the presence of large male fish can be explained using two separate lines of reason, either they are yet to undergo sex change (monogyny) or they are not going to. Both scenarios are common in confirmed protandrous species (Moore 1979). Some protandrous species display a wide size range at which sex change can occur (Pember 2006). For *P. quadrifilis*, intersex fish were observed over a fairly broad size range (790–955 mm FL, 7.30–12.18 kg). Despite this, male fish were observed at sizes up to 1120 mm FL and 21.50 kg, far larger than that of the observed intersex fish. Therefore, it is possible that some proportion of male fish do not undergo sex change, although age and growth information is necessary to rule out the possibility that differential individual growth may explain the observed patterns, as sex change may be more closely correlated with age and not size. For example, in the rudimentary hermaphrodite, *Diplodus cervinus hottentotus*, age was found to be a more meaningful predictor for juvenile bisexual individuals than size (Winkler et al. 2014). Similarly, age was found to be a better predictor of sex change in the protandrous *Pagellus bogaraveo* (Micale et al. 2002).

The relatively low occurrence of intersex fish sampled is worth noting. The fact that sampling was not undertaken over the space of a year may have led to a disproportionate observation of intersex individuals. In a study on *Galeoides decadactylus*, Longhurst (1965) found female sex ratios of less than 16% (compared to 7.8% for *P. quadrifilis* in the present study) in any given monthly sample while the ratio of intersex fish varied between 0.3 and 39.9% depending on the month. Furthermore, several studies have observed a higher proportion of intersex fish following the reproductive period (Fujii 1971; Moore 1979) and in a study on *Polynemus heptadactylus* (Polynemidae), the occurrence of intersex fish in monthly samples varied

between 2 and 45% depending on the time of year (Nayak, 1959 cited by Kagwade 1968). Although the reproductive period for *P. quadrifilis* in this area is undocumented, the GSI results from this study suggest that it may peak in August and September (see Fig. 3). This is also when the large mature females were observed in the area. Seeing as the majority of fish were sampled during this period, it is possible that intersex individuals may have been underrepresented in the sample.

Diagnosing the sexual pattern of *P. quadrifilis* is crucially important due to the value of the species towards local fisheries. Size selective fishing on sequentially hermaphroditic species has been known to lead to recruitment overfishing and a reduction of the age and size at which sex inversion takes place (Molloy et al. 2007; Moore et al. 2011). Although no quantitative fishing effort data are available for the region, anecdotal evidence suggests that the number of people fishing in the area has increased substantially over the past 10 years and that fishermen, including local gillnet fishermen, have begun using selective fishing methods, such as the use of artificial plastic lures, to target large *P. quadrifilis*. The result of this may be that the species has already begun to shift towards a smaller size at which sex change occurs, causing the observed gap between the size range of intersex individuals and larger males. It may also partially explain the highly male-dominant sex ratio as increasing levels of exploitation remove the larger (in this case female) individuals first from the population (Law 2000).

This same line of reasoning may also help to explain the presence of a single small female of 825 mm FL, and 7.65 kg, well below the size of the next largest female (1110 mm FL, 18.75 kg). The gonads of this fish appeared extremely similar to those of the late transitional fish, whose transitional state only became apparent after histological examination. Histologically, the gonads of these two fish appeared similar, both displaying a near-identical composition of ovarian cells as well as projections of connective tissue which, in the transitional fish, were filled with residual spermatogenic tissue. In the female, an area of weakly formed connective tissue was also noticeable close to where one would expect to find a residual sperm duct. Therefore, it is likely that this fish may have only recently been derived from a transitional fish, with the testicular tissue not apparent. The fact that it is of a smaller size

may be a result of slow growth or due to an early change driven by social or environmental cues.

Alternatively, it is also possible that this small fish is a primary female in an immature or resting state. If we consider the possibility that it may be an immature primary female, it would indicate that primary female maturation occurs at a far larger size compared to that of males due to the fact that no immature male fish were observed, even at relatively small sizes (smallest male – 436 mm FL, 1.46 kg). It is also unlikely that this fish would be resting as the ovaries of all other female fish were categorized as stage-III or higher during what appears to be their peak reproductive period (based on monthly GSI values). Despite this, we cannot rule out the possibility that primary females are present within the population. Indeed, digyny (where females have dual pathways of development) has been observed in other confirmed and suggested protandrous species (Longhurst 1965; Fujii 1971; Moore 1979).

Polydactylus quadrifilis in the Kwanza Estuary are renowned for their large size and all of the International Game Fish Association world recreational angling records come from this location (IGFA 2017). Anecdotal evidence from the local community suggests that the largest fish are always female and the results from this study support this notion. Female *P. quadrifilis* displayed significantly higher GSI values (mean – 1.96, max – 5.28) when compared to those of mature males (mean – 0.27 and maximum of 0.89). The higher GSI of females combined with their larger average body size indicates that they hold high reproductive potential. The fact that the population structure is dominated by many smaller male fish may be balanced by the high fecundity of large female fish. To further illustrate this, the 10 developed females observed in this study held more than two-fold more cumulative gonadal mass than all 121 non-juvenile males combined. The observation is striking and the population of *P. quadrifilis* in this region are undoubtedly highly dependent on large females for successful reproduction and recruitment. Furthermore, through natural selection, females that have survived long enough to reach large sizes are likely to be the most well-adapted individuals in the population and therefore hold immense genetic value.

Protandrous fishery species are becoming increasingly acknowledged as highly susceptible to overexploitation (Molloy et al. 2007). The stock collapse of *Polydactylus sexfilis*, a suspected protandrous polynemid species from the Indo-Pacific, has largely

been attributed to recruitment overfishing (Friedlander and Ziemann 2003). The reliance of *P. quadrifilis* on large highly-fecund female fish for reproductive success is likely to make the species sensitive to overfishing. Appropriate management strategies need to be implemented and should aim to protect large adult females through the use of either maximum legal limits or slot sizes. The fact that many *P. quadrifilis* fisheries make use of gill-nets would seemingly make the targeting of specific sized individuals fairly uncomplicated. However, the large individuals captured using less selective gears, such as beach seines and hook and line should be revived and released rather than harvested. The seasonal nature of the fishery for large females may reduce the difficulty of implementation and enforcement of these regulations. Unfortunately, the compliance with regulations in the artisanal and subsistence sectors may be complicated due to their reliance on fish for their livelihoods. However, emphasis on compliance in the recreational sector, whose participants are not dependent on the capture of fishes for their livelihoods, may be the best way forward for the species in the short-term.

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