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BRIEF COMMUNICATION

First report of intersex in a lutjanid: the goldband snapper *Pristipomoides multidens*

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This is the first report of intersex in a lutjanid species, the goldband snapper *Pristipomoides multidens*, in which the gonads of a male fish contain multifocal oocytes scattered among testicular tissue. The incidence rate of intersex was low ($< 1.0\%$), with oocytes observed in the testes of only two of 206 male fish examined. The capacity for *P. multidens* to develop an intersex condition suggests that future monitoring of this species should include histological analysis of gonads.

The goldband snapper *Pristipomoides multidens* (Day 1871) is a marine species belonging to the large and widespread family Lutjanidae. It is broadly distributed throughout the central Pacific and western Indian Oceans, and as far south as northern Australia to depths of 80–150 m (Newman and Dunk, 2003). It is a long-lived species (up to 30 years) that reaches sexual maturity at fork lengths (L_F) of 470 mm (8.0 years; males) and 473 mm (8.2 years; females) (Newman *et al.*, 2016).

Like all other lutjanids that have been studied to date, *P. multidens* is considered to be gonochoristic, whereby sex as either male (with testes) or female (with ovaries) is determined *in ovo*, and there is no evidence of sex change, secondary gonochorism or sequential hermaphroditism (based on no distinct differences in size classes of males and females and no sex skew in sampled populations; Newman *et al.*, 2016). Intersex is the condition whereby a gonad shows simultaneous presence of both male and female gonadal characteristics (Jobling *et al.*, 1998). To date, intersex has not been reported in any wild collected members of the Lutjanidae (Bahamonde *et al.*, 2013).

As part of a larger project investigating the health of commercially important fisheries in the Timor Sea, specimens of *P. multidens* were collected in October 2011 using baited traps (Gagnon and Rawson, 2012). Thirteen (13) sampling locations were centred on the Montara oil field in the Timor Sea, and are described in Gagnon & Rawson (2012). Distances of sampling locations from the oil field varied from *c.* 2 to 200 km. Fish were captured by baited traps deployed at depths of 50 to 200 m. Traps were slowly brought to the surface and fish were kept in live tanks until killed by *ikejime* (severing the spine at the base of the skull), according to the Curtin University Animal Ethics No. N50-09. All fish were killed and processed within 30 min of capture. Fish were weighed (M_T) and externally examined for any signs of anomalies, then a range of tissue samples were collected for analysis, as described in Gagnon and Rawson (2012).

Gonads were isolated and weighed (M_G) prior to being immersed in a fixative solution of glutaraldehyde in phosphate buffer, pH 6.5. Later, fixed samples were washed and transferred to a 50% ethanol preservative for longer term storage. Upon laboratory processing, samples were cut into 10 mm transverse sections and dehydrated in a series of progressively increasing ethanol concentrations over 4 h. The tissue was cleared in xylene for a further 3 h and embedded in paraffin wax overnight. The embedded samples were sectioned (5 μ m) using a microtome (Leitz 1512; www.leitz.com) and gently heated and dried overnight prior to staining. Three slides were prepared for each gonad sample, the slides were stained using standard haematoxylin and eosin methods (Lillie, 1965), then examined under an Olympus BX51 microscope equipped with a DP70 camera (www.olympus.com), and

photographed at magnifications x40, x200 and x400. A sample of 206 male fish and 183 female fish were histologically examined.

Male gonads were macroscopically staged as described in Barnett & Pankhurst (1999), female gonads were macroscopically staged as described in Marshall *et al.* (1993), and both sexes were microscopically staged using criteria described in Johnson *et al.* (2009) and Dietrich & Krieger (2009; Table 1). An alternative set of criteria for describing the phases of the fish reproductive cycle as described by Brown-Peterson *et al.* (2011) are shown in Table 1.

Male *P. multidentis* testes display typical lobular morphology (Billard, 1986), wherein each lobule contains multiple spermatocysts and mature spermatozoa are released into the lobule lumen before moving to central collecting ducts [Figure 1 (a),(b)]. Female *P. multidentis* displayed typical group-synchronous teleost ovaries (Wallace & Selman, 1981), with distinct populations of oocytes developing at the same rate, thus enabling their release in batches [Figure 1 (c),(d)]. No anomalies were found in any of the female gonads examined. Intersex testes were observed in two male *P. multidentis* from two different collection sites, and occurred as individual or small clusters of oocytes scattered among testicular tissue [Figure 1 (e),(f)]. Both primary and secondary oocytes were observed and no distinct membrane or connective tissue layer could be seen separating the oocytes from the testicular tissue, as would be expected if the fish was a transitioning, hermaphrodite (Devlin and Nagahama, 2002).

One male that displayed intersex captured at 13.2712° S, 124.8492° E was 347 mm L_F and M_T 700 g and another captured at 12.5017° S, 124.3632° E was 466 mm L_F and M_T 1590

g. Both fish had testes that were at an early stage of gonad development (macroscopic stage 2; Table 1).

As far as is known, this study is the first to describe the presence of intersex gonads in a species of the family Lutjanidae. The incidence rate was very low ($< 1.0\%$), and in both cases the male gonad comprised mostly of normal spermatogenic tissue with just a few scattered clusters of oocytes. There was no apparent structure or pattern in their appearance, nor was there any separation of the male and female germ cells by membranes, somatic cell layers or connective tissue, as would be expected if the fish were hermaphroditic (Devlin and Nagahama, 2002).

Hermaphroditism has been reported in about 2% of all teleost fish species (Avisé & Mank, 2009) and it appears to be an uncommon trait in fishes closely related to lutjanids, such as pomacanthids (as defined by Betancur-R. *et al.*, 2013). There are only a few species of pomacanthids, which are protogynous hermaphrodites, being reported as having any deviation from primary gonochorism (Avisé & Mank, 2009). It is unlikely, therefore, that the isolated occurrence of intersex individuals reported here is the result of a phylogenetic predisposition to hermaphroditism.

Non-anthropogenic factors have the potential to disrupt the normal development of some germ cells, leading to the production of oocytes instead of spermatogenic cells. Even in primary or secondary gonochorists, gonadal sex determination in many fishes is plastic and can be altered post-hatch in response to density, hypoxia, pH, temperature and exposure to endocrine disrupting chemicals (reviewed by Devlin & Nagahama, 2002). Any one of these

factors could be responsible for the observed production of a few scattered oocytes among spermatogenic tissue in the male *P. multident* in this study.

Given the importance of *P. multident* to commercial fisheries in northern Australia, knowledge of its reproductive biology, including descriptions of any anomalous findings, such as the two cases of intersex gonads reported here, is important. The demonstration of the capacity for *P. multident* to develop intersex gonads suggests that environmental and fisheries monitoring using this species, in which changes to environmental conditions or anthropogenic pollution are suspected, should include histological analysis of gonads and not rely solely on external gonad morphology.

The ecological consequences of the rare observation of multifocal oocytes among testicular tissue in *P. multident* are probably insignificant, since the remainder of the testis in the two affected individuals appeared normal, with no disruption of the testicular architecture and apparently normal spermatogenesis. Importantly, the low occurrence of the condition (*e.g.*, first reported occurrence, and only two observations out of 206 male gonads examined) supports the notion that these are isolated incidences and not indicative of a consequence of exposure that might lead to population-level effects.

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Figure Captions

FIG 1. Histological appearance of *Pristipomoides multidens* gonads, following haematoxylin and eosin staining: (a,b) Male testis at a mid-stage of development – the lobular testes contain distinct spermatocysts and a central sperm collecting duct; (c,d) female ovaries at early stages of ovarian development – ovaries contain predominantly perinucleolar and cortical alveolar stages, as well as some larger, early vitellogenic oocytes; (e,f) intersex gonads, based on the presence of testicular oocytes (testis-ova); spermatocysts of all developmental stages still present.

Figure Captions

FIGURE 1 Histological sections of *Pristipomoides multidentis* gonads, following haematoxylin and eosin staining. (a),(b) Testis at a mid-stage of development; the lobular testes contain distinct spermatocysts and a central sperm collecting duct. (c),(d) Ovaries at early stages of development; ovaries contain predominantly perinucleolar and cortical alveolar stages, as well as some larger, early vitellogenic oocytes. (e),(f) Intersex gonads, showing the presence of testicular oocytes (testis-ova); spermatocysts of all developmental stages still present.

Typesetter – change A, B etc to (a), (b) etc.

TABLE I Criteria used for staging male and female goldband snapper *Pristipomoides multidens* gonads, modified from Barnett & Pankhurst (1999), Marshall *et al.* (1993), Johnson *et al.* (2009), and Dietrich & Krieger (2009). Reproductive phases listed as described in Brown-Peterson *et al.* (2011)

Stage	Classification	Macroscopic Appearance	Microscopic Appearance	Phase (based on updated classification system) ^D
Male ^{A,B}				
0	Undeveloped	Immature; translucent threads.	Exclusively immature phases (spermatogonia to spermatids); no spermatozoa.	Immature
1	Early spermatogenic	Spermatogenic; small firm white threads.	Immature phases predominate, but spermatozoa may also be observed.	Developing
2	Mid-spermatogenic	Partially spermiated) firm, white and viscous milt expressible under pressure.	Spermatocytes, spermatids and spermatozoa present in roughly equal proportions.	Spawning capable
3	Late spermatogenic	Fully spermiated; plump, firm, white and milt flows freely under gentle pressure.	All stages may be observed, but mature sperm predominate. Immature phases may be present throughout or restricted to small nests of cells.	
4	Spent	Spent; bloody and flaccid, no milt expressible. Small, threadlike testes.	Loose connective tissue with some remnant sperm. No spermatocysts. Proliferation of spermatogonia, residual spermatozoa may be present in lumen of lobules or sperm duct.	Regressing Regenerating
Female ^{B,C}				
1	Virgin	Ovary pink, firm texture.	Exclusively immature phases (oogonia to perinucleolar oocytes); no cortical alveoli.	Immature
2	Maturing virgin	Ovary pink and glassy. Recovering- bloodshot and flabby at posterior.	Predominantly perinucleolar through cortical alveolar oocytes present; yolk vesicles may be apparent.	Developing
3	Developing	Ovary opaque and becoming yellow; ova not discernible.	Some early vitellogenic follicles; yolk vesicles apparent in cytoplasm, increasing in size and number with development; zona radiata thickens.	
4	Late Developing	Ovary opaque and yellowish pink; ova discrete.	At least half of observed follicles are early and mid-vitellogenic; evenly distributed and uniform appearance of yolk vesicles, oil vesicles and eosinophilic yolk granules in cytoplasm.	Spawning capable
5	Ripe	Ovary and ova becoming transparent.	Majority of developing follicles are late vitellogenic; migration of nucleus to periphery of cytoplasm, visible nuclear material and dissolution of nuclear membrane.	
6	Running ripe	Eggs express with slight pressure; ovary pinkish, clear and granular.	Majority of follicles are late vitellogenic and mature–spawning follicles; follicles are larger as compared to Stage 3; hydration of cell, uniform pink staining yolk.	

7	Spent	Ovary slack and bloodshot; few residual oocytes present. Small ovaries, blood vessels reduced but present.	Predominately post-ovulatory follicles, remnants of theca externa and granulosa. Oogonia and primary growth oocytes present; atretic follicles or degenerating post ovulatory follicles may be present.	Regressing Regenerating

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A, Macroscopic testis descriptions based on Barnett and Pankhurst (1999); B, microscopic testis and ovary descriptions based on Johnson et al. (2009) and Dietrich and Krieger (2009); C, macroscopic ovary descriptions based on Marshall et al. (1993); D, classification of phases of fish reproductive cycles as described in Brown-Peterson et al. (2011)

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