Maturation and sexual ontogeny in the spangled emperor
*Lethrinus nebulosus*


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The reproductive development and sexual ontogeny of spangled emperor *Lethrinus nebulosus* populations in the Ningaloo Marine Park (NMP) were investigated to obtain an improved understanding of its evolved reproductive strategy and data for fisheries management. Evidence derived from (1) analyses of histological data and sampled sex ratios with size and age, (2) the identification of residual previtellogenic oocytes in immature and mature testes sampled during the spawning season and (3) observed changes in testis internal structure with increasing fish size and age, demonstrated a non-functional protogynous hermaphroditic strategy (or functional gonochorism). All the smallest and youngest fish sampled were female until they either changed sex to male at a mean $277.5 \text{ mm}$ total length ($L_T$) and 2.3 years old or remained female and matured at a larger mean $L_T$ (392.1 mm) and older age (3.5 years). Gonad masses were similar for males and females over the size range sampled and throughout long reproductive lives (up to a maximum estimated age of c. 31 years), which was another correlate of functional gonochorism. That the mean $L_T$ at sex change and female maturity were below the current minimum legal size (MLS) limit (410 mm) demonstrated that the current MLS limit is effective for preventing recreational fishers in the NMP retaining at least half of the juvenile males and females in their landed catches.

Key words: age estimation; functional gonochorism; gonadal development; Lethrinidae; non-functional protogynous hermaphrodite.

INTRODUCTION

The emperors (Lethrinidae) are a family of tropical reef fishes that comprise an important component of commercial, recreational and subsistence fisheries throughout the tropical and subtropical waters of the Indo-Pacific Ocean (Carpenter & Allen, 1989). Sex change in the form of functional protogynous hermaphroditism has been demonstrated for the majority of lethrinids (Young & Martin, 1982; Ebisawa, 2006; Sadovy de Mitcheson & Liu, 2008). Sequential hermaphroditism in exploited fish stocks can be of great importance to their management, because size-selective...
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Harvest could also be sex selective, which could affect operational sex ratios and overall reproductive output (Bannerot *et al.*, 1987; Coleman *et al.*, 2000; Levin & Grimes, 2002).

A proto-hermaphroditic condition has been postulated for teleosts because of the wide range of sexual strategies exhibited, including variation within families such as the Lethrinidae. Consequently, understanding the phylogenetic patterns separating functional *v.* non-functional hermaphroditic groups has been useful for generating hypotheses concerning the evolution of different sexual ontogenies by different species (Sadovy de Mitcheson & Liu, 2008). Since female-first sex change is predicted to be adaptive when relative reproductive success increases at a faster rate with body size for males than for females (Warner, 1975; Leigh *et al.*, 1976), studies of the relative reproductive output between sexes have been useful as an interspecies correlate for exploring the validity of this theory (Sadovy, 1996; Molloy *et al.*, 2007). These approaches could also be applied to single species analyses for determining whether the patterns of relative reproductive output with body size are consistent with those typified by Molloy *et al.* (2007) for either functional gonochorism or functional protogyney.

The spangled emperor *Lethrinus nebulosus* (Forsskål) is exploited throughout its distribution as a food fish by commercial (Ebisawa, 1990; Salem, 1999) and artisanal (Grandcourt, 1999) fisheries. In some countries, it is also targeted frequently and can be caught in large numbers by recreational fishers, particularly when line fishing in the vicinity of coral reefs, such as those found within the Ningaloo Marine Park (MP) in north-western Australia (Westera *et al.*, 2003; Fig. 1). The management of fishing on *L. nebulosus* populations in the Ningaloo MP and wider Gascoyne region of western Australia is currently reliant on reliable estimates of the average size at maturity within the exploited stock, as this is used directly for setting an effective minimum legal size limit, and for other purposes, such as when used as an input variable in stock assessment models.

The reproductive development of *L. nebulosus* has previously been investigated in several countries, and results on sexual ontogeny have differed among studies. Previous research in north-western Australia, including the North-West Shelf, Gulf of Carpentaria and Ningaloo MP, described sex-specific length-frequency distributions for the species, which failed to demonstrate any evidence of sex change (Young & Martin, 1982; M. Moran, unpubl. data). In Okinawan waters, Ebisawa (1990) described and characterized oogenesis, analysed sampled sex ratios and the proportion of ovarian development stages with fish length and identified intersexual gonads in juveniles from histology to conclude juvenile protogynous hermaphroditism for *L. nebulosus*. Grandcourt (1999) also found evidence of juvenile protogyney from histological analysis of samples from the Great Barrier Reef, although Salem (1999) failed to detect any evidence of sex change from sampling populations off the Sinai Peninsula in the Red Sea. Importantly, the juvenile hermaphroditism reported by Ebisawa (1990) and Grandcourt (1999) is regarded as a non-functional sex change and if it is not followed by post-maturational sex change later in life, could instead be referred to as functional gonochorism (Sadovy de Mitcheson & Liu, 2008).

The aim of this study was to investigate the reproductive development and sexual ontogeny of *L. nebulosus* populations in the Ningaloo MP. Histological analysis of sampled ovaries and testes from a large size and age range of *L. nebulosus* specimens was done to determine the ontogenetic strategy and define reproductive stages of
Fig. 1. Location of the study area and the management zones within the Ningaloo Reef Marine Park.
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Analyses of the relative reproductive output of males and females with size were done to provide some further insight into its evolved reproductive and sexual ontogenetic strategy.

**MATERIALS AND METHODS**

**SAMPLING METHODS AND LOCALITY**

Collections of *L. nebulosus* were obtained using fishery-dependent (*n* = 150) and fishery-independent (*n* = 194) sampling strategies from August 2006 to November 2007. Various sampling methods were used to selectively target individuals of different sizes in order to obtain a more complete representation of the size and age range of *L. nebulosus* populations residing on the northern section of the Ningaloo Reef, from Point Maude (23° 07′ S; 113° 35′ E) northwards to include the North-West Cape and Muiron Islands (Fig. 1). Fork lengths (*L*<sub>F</sub>) were measured to the nearest mm. Sagittal otoliths and gonads were dissected from fresh-filleted carcasses of the landed catch of recreational (*n* = 129) and charter (*n* = 21) fishers and from whole fish collected by research trapping (*n* = 55) and line fishing (*n* = 139). Baited fine mesh (10 mm) fish traps and research line fishing with small hooks were used to target small (<410 mm total length, *L*<sub>T</sub>) juveniles from 14 to 19 November 2006 (*n* = 53) and from 3 to 4 April 2007 (*n* = 2). Research line fishing was undertaken to specifically target larger adults spawning in deeper waters over the new and full moon periods from 8 to 13 October 2007 (*n* = 55) and 21 to 26 November 2007 (*n* = 76).

Samples collected from up to 340 km south of the study region (southern Gascoyne; *n* = 201) were also analysed histologically for improved confidence in describing and categorizing stages of ovarian and testicular development for the species but were not used in the present analysis. The dataset was also supplemented with otolith data from the southern Gascoyne and subsequent sample collections to 31 November 2008 for the purpose of validating the age estimation method using a marginal increment analysis based on classification of edge types (*n* = 813).

**AGE ESTIMATION**

Thin (250–300 μm) transverse sections of sagittal otoliths were prepared using the methods of Newman & Dunk (2002) for age estimation. Opaque increments were counted along the dorsal sulcus from the primordium to the proximal margin when viewed with reflected lighting at ×40 magnification against a black background (Fig. 2). The proximal margin was categorized for the formation of newly deposited opaque increments accordingly: (1) opaque where an opaque increment was visible on the proximal margin, or (2) narrow translucent where the width of translucent material on the margin was judged to be <50% of the penultimate translucent zone width, or (3) wide translucent. An analysis of the relative frequency of proximal margin types sampled per month demonstrated that peak periods of opaque increment deposition occurred on an approximately annual cycle (peaks in January 2007, December 2007 and November 2008; Fig. 3), indicating that counts of opaque increments could be used to estimate fish ages in years. Furthermore, deposition of the first opaque increment was assumed to occur at c. 1 year of age because the timing of opaque increment formation coincided with the period of peak spawning (September to November; R. J. Marriott, unpubl. data). Fish that were sampled during the period of peak opaque increment deposition (1 October to 31 December) that had wide translucent otolith margins were assumed to have late forming opaque zones and the estimated age classes of these fish were the number of opaque increments counted plus one.

**GONAD PROCESSING AND HISTOLOGY**

Gonads were incised transversely several times with a clean scalpel before placing in pH neutral 4% phosphate-buffered formaldehyde as soon as practicable following capture to
Fig. 2. Transverse section of a *Lethrinus nebulosus* otolith estimated to be in the 9 year age class (scale bar: 2 mm).
ensure adequate penetration of the fixative into the tissue (Nieland & Wilson, 1995). For smaller individuals (<250 mm LF), a portion of the abdomen that contained gonad material was excised from the whole fish using cutting shears. Medial transverse sections of gonad lobes and abdominal sections were embedded in paraffin wax, sectioned at 5 μm, mounted on slides and stained with Mayer’s haematoxylin and eosin. Sagittal, anterior transverse and posterior transverse histological sections of some testes were prepared using the same method to explore the efferent transport pathways for spermatozoa.

Ovarian development stages were assessed microscopically from the identification of the most developed oocytes (Yamamoto et al., 1965; Wallace & Selman, 1981) and other relevant structures, such as the structural appearance of lamellae, thickness of the ovarian wall, intralamellar stromal tissue, atretic oocytes, brown bodies and postovulatory follicles (Ferreira, 1995; Burton et al., 1997; Bean et al., 2003; Mackie, 2006; Erisman et al., 2008). Testes were classified as mature when spermatozoa were visible in either the intact spermatocysts of lobules (continuous germinal epithelia: Grier & Taylor, 1998; Brown-Peterson et al., 2002) or released from ruptured spermatocysts, within lobules or central efferent ducts (Murphy & Taylor, 1990). Mature testes were classified into reproductive stages based on the relative location and density of spermatozoa within histological transverse sections. These stages were judged to approximately align with those defined for other teleosts based on internal spatial patterns of the structural integrity of germinal epithelia (Grier & Taylor 1998; Brown-Peterson et al., 2002). Stages based on germinal epithelia could not be confidently determined for all specimens because the conventional preservation and paraffin-embedding techniques used resulted in some distortion of cellular structure (H. Grier, pers. comm.). Intersexual gonads were identified as those containing both male and female gametes (Yamamoto, 1969).

**ANALYSES**

The logistic model of the form: \( P_x = P_\infty \left(1 + e^{aL_F + b}\right)^{-1} \) was fitted separately to \( P_x \), the percentage of males (for the assessment of sex change), mature females or mature males...
in each $L_F$ (or age) group, and $a$ and $b$ are model coefficients. $P_\infty$ was fixed at 100% for maturity analyses (all data) and was estimated for sex change analyses constrained to fish $<450$ mm $L_F$ (or 5 years old). The $L_F$ (or age) at which the population was 5, 50 and 95% of the $P_\infty$ value was estimated using the inverse form of the fitted models. Slightly lower numbers were analysed for age classes than for $L_F$ classes as sex change analyses were constrained to different bin groupings for small ($L_F < 450$ mm) and young (age $<6$ years) fish and as age estimates for some specimens were not available. For male maturity analyses, data for the two smallest $L_F$ bins ($P_{201-250}$ mm and $P_{251-300}$ mm) were combined because of low sample numbers and assumed poor sample precision, and the percentage of mature males was recalculated for a corresponding weighted average $L_F$ (based on $n = 1, 4$, for each $L_F$ bin, respectively) chosen for model fitting. For sex change analyses, one datum ($P_{281-320} = 87.5\%$) was regarded as an outlier and excluded from the fit to proportion of males at age due to low replication ($n = 5$) and assumed poor sampling precision.

To explore the validity of postulated mechanisms for the observed sexual pattern, the relative gonad mass data were analysed following the methods described in Molloy et al. (2007). Relative reproductive output for $L. nebulosus$ was quantified separately for each sex as the highest value of mean monthly gonado-somatic index ($I_G$) values, where $I_G$ were calculated as the gonad mass ($W_G, g$) divided by the total body mass ($W_B, g$) for each specimen. In instances where $W_B$ data were not available, $W_B$ was estimated from $L_F$ measurements (mm) according to the following formula: $W_B = 6.002 \times 10^{-5}L_F^{2.810}$ generated from fitting the power function to observed $W_B$ on $L_F$ data ($n = 204; r^2 = 0.975$). The relationship of $\log_{10}(W_G)$ with $\log_{10}(W_B)$ was then plotted for males and females sampled in the peak spawning period (September to November) in order to visually compare the relationship between males and females, as undertaken by Molloy et al. (2007). Trend lines were generated, which represent the best-fit geometric-mean regressions (Ricker, 1984) along the reduced major axis for male and female data separately.

RESULTS

OVARIAN DEVELOPMENT

Gonads of the smallest individuals sampled were characterized by the presence of undifferentiated gonia in developing germ tissue (presumably oogonia), which resembled ovarian lamellae in structure, and a membrane-lined central lumen, which opened into a ventral cavity along the length of each lobe. Since the formation of an ovarian cavity can be used as a sign of early sex differentiation (Nagahama, 1983), gonads at this early stage of development were classified as rudimentary females [$n = 23$; Fig. 4(a)]. The appearance of chromatin nucleolus oocytes defined progression to the immature female stage and the lamellae developed to become relatively elongate and densely packed with previtellogenic (chromatin nucleolus, perinucleolus or cortical alveoli) oocytes [$n = 49$; Fig. 4(b)].

Mature inactive ovaries were characterized by a marked thickening in the gonad wall and the conspicuous presence of thicker and more prevalent eosinophilic intralamellar stromal strands [$n = 105$; Fig. 4(c)]. Asynchronous oocyte development was evident. Previtellogenic oocytes became less aligned and less densely packed within lamellae, which became less elongate than observed in immature female ovaries. Yellow-brown bodies (Sadovy & Shapiro, 1987) were only observed in some ($n = 37$) mature inactive ovaries.

The onset of vitellogenesis was marked by the appearance of lipid droplets within the cytoplasm of cortical alveoli oocytes, which defined the onset of the mature developing stage ($n = 19$). Mature developed ovaries were characterized by the presence
Fig. 4. Representative stages of ovarian development during the reproductive cycle of *Lethrinus nebulosus*: (a) rudimentary female, (b) immature female, (c) mature inactive, (d) mature developed, (e) prespawning and (f) postspawning. AO, atretic oocytes; BB, brown bodies; DBV, dorsal blood vessel; GW, gonad wall; HY, hydrated oocytes; I, intestine; ILS, intra-lamellar stromal strands; L, lumen; LD, lipid droplets within cortical alveoli stage oocytes; M, mesentery; PN, perinucleolus stage oocytes; POF2, stage 2 (old) postovulatory follicle; UG, undifferentiated gonia; YG, yolk globule stage (scale bar: 200 μm).

of yolk globule oocytes within the lamellae \( n = 76; \) Fig. 4(d)]. Prespawning ovaries were identified by the presence of migratory nucleus and hydrated oocytes within the lamellae \( n = 9; \) Fig. 4(e)]. Spawning ovaries were identified by the presence of hydrated oocytes in the lumen following ovulation and stage 1 (new) postovulatory follicles (POF) in the lamellae, often beneath the membrane that lined and was adjacent to the lumen \( n = 1 \). That this stage was rarely sampled demonstrated that
spawning events were rarely encountered during sampling. In postspawning ovaries, yolk globule oocytes were present in the lamellae with stage 2 (old) POF and possibly some atretic oocytes \( n = 23; \) Fig. 4(f) (Ferreira, 1995; Mackie, 2006; Erisman et al., 2008).

**SEX CHANGE AND TESTIS DEVELOPMENT**

Change from immature ovary to testis involved the separation of lamellar tissue from the gonad wall and some splitting of the gonad wall dorsally to form a peripheral sperm sinus. This sinus was approximately one half of the circumference of the gonad wall in length and terminated on either side of, but encompassing, the vestigial lamellar tissue, dorsal to the ventral lumen cavity [Fig. 5(a), (b)]. Intersexual gonads were classified as type 2 undelimited (Sadovy & Shapiro, 1987), with residual previtellogenic oocytes interspersed among developing male tissue and germ cells \( n = 4; \) Fig. 5(a), (b)). Intersexual gonads were either non-functional immature testes (no spermatozoa; \( n = 2 \)) or functional young mature testes (with spermatozoa; \( n = 2 \)). The vestigial ovarian lamellae were observed to fuse and underwent complete structural and cellular transformation into testis tissue of unrestricted lobular type (Parenti & Grier, 2004). Both stages were observed to have accumulations of leucocytes predominantly in central locations within the vestigial lamellae, which were observed to be the sites of presumed vasa efferentia (Hoar, 1969), seminiferous tubule (Rhodes & Sadovy, 2002) or efferent duct (ED) (Murphy & Taylor, 1990) formation in more developed mature testes [Fig. 5(c)]. All testes were observed to have a vestigial, membrane-lined lumen, which was further evidence of sex change (Sadovy & Shapiro, 1987).

Mature testes were observed to develop with seasonal spawning activity, with spermatozoa forming within intact spermatocysts lining the lobules (continuous germinal epithelia, CGE) and then rupturing from the spermatocysts for release into the lobules (discontinuous germinal epithelia, DGE), which drained into the sperm sinus via the ED during spawning. In the best-preserved testes, changes in germinal epithelia throughout their transverse sections were observed with reproductive development. From early to mid-maturation, DGE appeared near the ED only. At late maturation, DGE were observed throughout the testis, from the ED to the periphery [Fig. 5(e)]. During regression, spermatozoa occurred throughout the testis, and spermatogenesis was greatly reduced, with spermatogonia and occasionally spermatocytes visible at the periphery [Fig. 5(f)].

It was not possible to use these criteria to confidently discern changes in DGE throughout the testis for all specimens, although it was possible to identify the relative proportions of spermatozoa in the sperm sinus, ducts and lobules as an indication of seasonal reproductive development. Mature resting testes were classified as having little (\( \leq 10\% \)) or no spermatozoa in the sperm sinus and ducts \( n = 34 \). Mature developed testes were classified as having a greater density \( (\geq 10\% \) and \( \leq 90\% ) \) of spermatozoa within sperm sinuses and ducts; \( n = 41 \); Fig. 5(d)]. Mature spawning testes were classified as having sperm sinuses and ducts full (\( >90\% \) density) of spermatozoa [Figs 5(e), (f) and 6]. Mature spawning testes were further categorized into mature spawning A \( n = 142 \), where spermatozoa were not predominant (\( \leq 75\% \) density) in testis tissue outside sperm sinuses and ducts \[i.e. within lobules; Figs 5(e]\)
Fig. 5. Representative stages of testis development during the reproductive cycle of *Lethrinus nebulosus*: (a) intersexual, (b) intersexual (mature, functional male), (c) immature, (d) mature developed, (e) mature spawning A (late maturation) and (f) mature spawning B (regression). DBV, dorsal blood vessel; DGE, discontinuous germinal epithelia; DSS, dorsal sperm sinus; GW, gonad wall; L, lumen; Le, leucocytes in areas of developing ducts; PSS, peripheral sperm sinus; RO, residual oocytes (degenerating); SC, spermatocytes; SG, spermatogonia; ST, spermatids; SZC, spermatozoa in spermatocyst; SZD, spermatozoa in duct; SZL, spermatozoa in lobule lumen; SZS, spermatozoa in sperm sinus (scale bar: 200 μm).

and 6] to mature spawning B \(n = 43\); Fig. 5(f)], where spermatozoa were predominant (>75% density) outside sperm sinuses and ducts.

In larger and older males with mature spawning A testes, the relative circumferential length of the sperm sinuses decreased to be smaller than observed in smaller younger males, restricting the extent of the sperm sinus. Efferent duct regions full of spermatozoa formed centrally within the lobular tissue, on either side of the vestigial
lumen in mature spawning A testes and adjoined the dorsal sperm sinus. In the transverse sections of some testes, mainly of the younger and smaller males, there were several of these central ED regions. As the lobular tissue fused with development, there became fewer central ED regions. In testes of the oldest and largest males, there were only two central ED regions; one on either side of the vestigial lumen [Fig. 6(a)]. These central ED regions were observed (from sagittal sections) to run the length of each testis lobe, on either side of the vestigial lumen, to link with the sperm sinus dorsally along this length, and also posteriorly near the position of the gonoduct and urogenital papilla [Fig. 6(b), (c)].
Maturity and sex change relationships

The $L_F$ frequency distributions of the different sexes of $L. nebulosus$ demonstrated that the smallest individuals sampled were females and included rudimentary females, which were sampled over the smallest $L_F$ range (170–211 mm; Fig. 7). Immature females ranged from 174 to 443 mm. Males were not detected in samples until a larger size, approximating the narrow size range within which intersexual fish were detected in samples (251–274 mm). Immature and mature males were detected over broad $L_F$ ranges (269–482 and 220–601 mm, respectively), demonstrating that mature males were present across the entire size range of males that were sampled. Many of the testes that were classified into the immature stage were likely to have been from males that had previously spawned (i.e. were functionally mature) but were not reproductively active at the time they were sampled. This is because there was no reliable histological criterion established for distinguishing between functionally immature testes and functionally mature testes without spermatozoa. Mature males and females were sampled over broad and overlapping size ranges, with the minimum and maximum size of mature males being shorter than the minimum and maximum size of mature females sampled. Over most of the sampled $L_F$ range (251–600 mm; Fig. 7), there was an approximately even number of mature male and female $L. nebulosus$ sampled, although there were progressively fewer males in the largest $L_F$ bins. The total sampled sex ratio (M:F) for mature fish of 1.29:1 was slightly male biased.

The $L_F$ and age at which 50% of females had changed sex to male [Fig. 8(c), (d)] were less than the respective $L_F$ and ages at which females reached maturity [Fig. 8(a), (b) and Table I]. The $L_F$ at which 50% of females had changed sex to

![Graph showing percentage contribution of development stages for each fork length class.](image-url)
male was less than the $L_F$ at which 50% of males reached maturity [Fig. 8(e)], as expected (Table I). The age at which 50% of females had changed sex to male, however, was greater than the age at which 50% of males reached maturity [Fig. 8(f) and Table I]. Importantly, the age at which 50% of males reached maturity was an extrapolated estimate, so this result was equivocal. These estimates for maturity and sex change were relatively young compared with the maximum age class fitted for females [18 years; Fig. 8(b)] and males [25 years; Fig. 8(f)] in this study and the oldest estimated age for this species sampled from c. 200 km south of the study area (30-75 years; R. J. Marriott, unpubl. data).

The occurrence of intersexual gonads with spermatozoa present in sperm sinuses strongly suggests that male maturity occurs rapidly after sex change, and that some

![Graphs showing observed percentages and fitted logistic curves](image)

**Fig. 8.** Observed percentages and fitted logistic curves of (a), (b) mature females, (c), (d) males (reflecting sex change), (e), (f) and mature males in relation to (a), (c), (e) fork length ($L_F$) class and (b), (d), (f) age class for *Lethrinus nebulosus*. 


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**Table I.** Coefficient estimates for fit of the logistic function to per cent mature or per cent sex change data for *Lethrinus nebulosus*

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Coefficient estimates</th>
<th>Female maturity</th>
<th>Sex change</th>
<th>Male maturity*</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>$L_F$ class</td>
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<td>$n$</td>
<td>182</td>
<td>139</td>
<td>157</td>
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</tr>
<tr>
<td>$a \pm$ s.e.</td>
<td>7·19 ± 2·08</td>
<td>12·16 ± 2·82</td>
<td>2·95 ± 0·66</td>
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<tr>
<td>$b \pm$ s.e.</td>
<td>−0·02 ± 0·01</td>
<td>−0·05 ± 0·01</td>
<td>−0·01 ± 0·00</td>
<td></td>
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<tr>
<td>$P_\infty \pm$ s.e.</td>
<td>100†</td>
<td>50·30 ± 1·72</td>
<td>100†</td>
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</tr>
<tr>
<td>$n \ (L_F \text{ classes})$</td>
<td>11</td>
<td>7‡</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>$r^2$</td>
<td>0·92</td>
<td>0·98</td>
<td>0·94</td>
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<tr>
<td>$L_{05} \ (P_\infty)$</td>
<td>206·75</td>
<td>187·21</td>
<td>0·67§</td>
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<tr>
<td>$L_{50} \ (P_\infty)$</td>
<td>350·23</td>
<td>247·03</td>
<td>224·56</td>
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<tr>
<td>$L_{95} \ (P_\infty)$</td>
<td>493·70</td>
<td>306·85</td>
<td>448·46</td>
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<tr>
<td>Age class (years)</td>
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<tr>
<td>$n$</td>
<td>178</td>
<td>130</td>
<td>156</td>
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<tr>
<td>$a \pm$ s.e.</td>
<td>4·53 ± 0·48</td>
<td>9·30 ± 4·67</td>
<td>0·79 ± 0·32</td>
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<td>$b \pm$ s.e.</td>
<td>−1·25 ± 0·13</td>
<td>−4·00 ± 2·25</td>
<td>−0·59 ± 0·10</td>
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<tr>
<td>$P_\infty \pm$ s.e.</td>
<td>100†</td>
<td>53·64 ± 4·16</td>
<td>100†</td>
<td></td>
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<tr>
<td>$n \ (age \ classes)$</td>
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<td></td>
</tr>
<tr>
<td>$r^2$</td>
<td>0·99</td>
<td>0·98</td>
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<tr>
<td>$t_{05} \ (P_\infty)$</td>
<td>1·27</td>
<td>1·59</td>
<td>−3·64§</td>
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<tr>
<td>$t_{50} \ (P_\infty)$</td>
<td>3·62</td>
<td>2·33</td>
<td>1·33§</td>
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<tr>
<td>$t_{95} \ (P_\infty)$</td>
<td>5·97</td>
<td>3·06</td>
<td>6·31</td>
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</table>

*Due to the low number of replicates in the first age and fork length ($L_F$) class groupings, data were combined for $L_F$ classes 151–200 and 201–250 mm and weighted mean $L_F$ was assigned as the independent datum to those combined data.

†Value fixed at 100%. Slightly lower numbers were analysed for age classes than for $L_F$ classes as sex change analyses were constrained to different bin groupings for small ($L_F < 450$ mm) and young (age <6 years) fish and as age estimates for some specimens were not available.

‡An outlier of 87·5% males in the $L_F$ class 281–320 mm was excluded from this fit, given the assumed logistic trend.

§Extrapolated estimate.

of these intersexual gonads were obtained from functional males. The approximately uniform sampled sex ratio, and the fact that all the intersexual individuals sampled were within a narrow $L_F$ range approximating the modelled $L_{50}$ for sex change based on percentages of males per $L_F$ bin collected, further supports the finding of pre-maturational (non-functional) protogynous sex change in this species, or alternatively, functional gonochorism. According to the above evidence, a logical explanation of this strategy is that *L. nebulosus* initially develop sexually as immature females, then either change sex to male at an average age of 2·3 years and mature shortly later or remain female to mature at an average age of 3·6 years.

**Relative Gonad Masses**

The relationships of ovarian and testis masses with $W_B$ are shown in Fig. 9. The large overlap of the linear-transformed $W_G$ with $W_B$ relationships between males and females and the close alignment of the reduced major axes for both data groups.
Fig. 9. Relationship between body mass (log₁₀\(W_B\)) and gonad mass (log₁₀\(W_G\)) for male (○) and female (●) *Lethrinus nebulosus* sampled during the spawning season, with lines representing the best-fit geometric-mean regression for male (—) and female (—) data groups, respectively.

demonstrate that the relative mass of prespawning testes are approximately the same as those of prespawning ovaries during the spawning season. This indicates the potential for relatively high volumes of reproductive output (sperm production) by males. There appears to be a different slope to these trends, however, with ovaries being noticeably heavier than testes for smaller (and younger) *L. nebulosus* on average. The peak in monthly mean ± s.e. \(I_G\) for males (3·42 ± 0·36%) was also not significantly different from that observed for females (3·84 ± 0·88%; Welch two-sample \(t\)-test, d.f. = 17·70, \(P > 0·05\)).

**DISCUSSION**

The findings of pre-maturational protogynous sex change in this study for *L. nebulosus* corroborated those reported by Ebisawa (1990) for Okinawan populations and by Grandcourt (1999) for populations on the Great Barrier Reef. The average length at maturity for females in this study closely approximated the length previously estimated from an earlier study done in the Ningaloo region that used more rudimentary analyses, including trends in relative ovarian masses and mean oocyte diameters with fish lengths (M. Moran, unpubl. data). The results from this earlier study were used as evidence for setting the current minimum legal size limit for this species (410 mm \(L_T\)) in 1994. The current minimum legal size limit, which is equivalent to 366·8 mm \(L_F\), is larger than the \(L_{50}\) for sex change and female maturity estimated in this study and therefore would be effective for preventing the
retention of at least half of the immature *L. nebulosus* in fishing catches. In addition, *L. nebulosus* demonstrated several developmental correlates of functional gonochorism, which indicate an intrinsic robustness to effects of fishing for this species relative to other lethrinids that demonstrate functional protogyny.

The relatively early protogynous sex change for males suggests that there may be some selective advantage for young males in *L. nebulosus* populations. This result contrasts to those reported for many other species of Lethrinidae that are functionally protogynous and only have relatively few large, old males that participate in spawning events (Young & Martin, 1982; Bean et al., 2003; Ebisawa, 2006). Interestingly, some males were capable of producing spermatozoa in their testes at a size and age approximating that of sex change. The presence of some intersexual gonads with spermatozoa present in their sperm sinus suggests that the onset of sex change is triggered in the second year of life, during the spawning season, when they become functional males. If so, it is possible that it could be a socially mediated process for the regulation of operational sex ratios within spawning aggregations. It is also of note that there was a relatively high proportion of mature males identified across the sampled *L* and age range. This suggests that those testes classified as immature might have been functionally mature but were not identified as such as they were not in the mode of producing or storing spermatozoa at the time of sampling.

The sampling of *L. nebulosus* throughout the adult size range demonstrated an approximately uniform sex ratio, which was also consistent with a functionally gonochoristic strategy (Young & Martin, 1982; Sadovy & Shapiro, 1987). The reproductive contribution of males, indicated by relative gonad mass, was also relatively high, evidenced by *I* values for males and females during peak spawning that were approximately the same. A relatively large *I* for males of another functionally gonochoristic lethrinid, *Lethrinus obsoletus* (Forsskål), has also been reported (Ebisawa, 2006), which further corroborates the findings of Molloy et al. (2007) that males of functionally gonochoristic species have relatively large gonads. This contrasts with results reported for other functionally protogynous lethrinids, such as *Lethrinus miniatus* (Forster), which was reported to have a much larger peak *I* for females (c. 6-0%) than for males (c. 1-2%; Williams et al., 2006).

It has been suggested that juvenile sex change, which has also been reported to occur in some species of Sparidae (Francis & Pankhurst, 1988; Hesp & Potter, 2003), represents secondary development from ancestral hermaphroditism towards gonochorism (Alekseev, 1983). Furthermore, central efferent duct-like tissues have also been observed to be a prominent histological feature in the testes of other functionally gonochoristic teleosts (Murphy & Taylor, 1990; Marriott et al., 2007). Therefore, it is probable that *L. nebulosus* evolved a functionally gonochoristic reproductive strategy, in response to adaptive constraints, by increasing the spawning capacity of males throughout life, facilitated to a large extent by the central ducts within the lobular tissue of the testes. It is apparent that these networks of ducts are important structures for draining high volumes of spermatozoa released from the lobules in relatively large testes, such as found in males of this species.

Research into, and the synthesis of, the spawning behaviour of species such as *L. nebulosus* is a key requirement for gaining an improved insight into the evolved life-history trade-offs that explain the reproductive strategy of this species, including the reproductive contribution of the smallest, youngest males. Both male and female *L. nebulosus* are capable of repeat spawning over relatively long reproductive lives,
and according to bet-hedging theory, this may indicate an evolved strategy to counteract a high mean or high variance in juvenile mortality (Stearns, 1976). Although the per capita relative reproductive output of small males appears to be limited by relatively small testis size (and therefore potential volume of sperm spawned per fish), these younger males are numerically abundant in the population and the combined reproductive output from small males could potentially be significant. Observations of the sex-specific size structures of _L. nebulosus_ within spawning aggregations are required to determine whether, and to what extent, small males participate in spawning events.

On the basis of these findings, it seems likely that the functionally gonochoristic life-history strategy evolved by _L. nebulosus_ populations could impart a greater robustness for resisting fishing impacts than the functionally protogynous strategy present in populations of other lethrinids. Sequential hermaphroditism, including functional protogyyn, could lead to sex-selective effects from fishing because line fishing is size selective and typically removes the larger, older fish from populations first. If these larger, older fish are primarily comprise one sex (because of sequential hermaphroditism), then fishing would probably deplete that sex more heavily. This could affect operational sex ratios within spawning aggregations and therefore limit overall reproductive output from the population (Bannerot et al., 1987; Coleman et al., 2000; Levin & Grimes, 2002). That _L. nebulosus_ does not demonstrate functional protogyyn suggests that such sex-specific effects are less likely for this species.

There are other potentially important aspects of the population dynamics of _L. nebulosus_ to consider, however, which could also contribute to the inherent risk of fishing effects on its populations. Although male and female _L. nebulosus_ mature at a relatively young age, this species has demonstrated a relatively high longevity with an estimated maximum age >30 years. This high longevity predicts a relatively low rate of natural mortality (\(M\)) (Hoening’s (1983) \(\hat{M}<0.15\)) and susceptibility for _L. nebulosus_ populations to rapid overexploitation (Parent & Schriml, 1995; Jennings et al., 1998). Furthermore, this species is known to form spawning aggregations that can be targeted by commercial or recreational fishers to achieve high catch rates (Salem, 1999) and so future monitoring and regular assessments of exploited _L. nebulosus_ populations will continue to be important.

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References


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