

RAPID REPRODUCTIVE ANALYSIS AND LENGTH–WEIGHT RELATIONS FOR FIVE SPECIES OF CORAL-REEF FISHES (ACTINOPTERYGII) FROM PAPUA NEW GUINEA: *NEMIPTERUS ISACANTHUS*, *PARUPENEUS BARBERINUS*, *KYPHOSUS CINERASCENS*, *CTENOCHAETUS STRIATUS* (PERCIFORMES), AND *BALISTAPUS UNDULATUS* (TETRAODONTIFORMES)

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Longenecker K., Langston R., Bolick H., Crane M., Donaldson T.J., Franklin E.C., Kelokelo M., Kondio U., Potuku T. 2017. Rapid reproductive analysis and length–weight relations for five species of coral-reef fishes (Actinopterygii) from Papua New Guinea: *Nemipterus isacanthus*, *Parupeneus barberinus*, *Kyphosus cinerascens*, *Ctenochaetus striatus* (Perciformes), and *Balistapus undulatus* (Tetraodontiformes). Acta Ichthyol. Piscat. 47 (2): 107–124.

Background. We present length–weight relations (LWR) and describe the reproductive biology of five species of coral reef fishes from Papua New Guinea (PNG). Each of these species are targeted by artisanal- and small-scale commercial fisheries throughout the country. As such the purpose of this study was to provide baseline reproductive information that can be used for future evaluation and management of the fishery.

Materials and methods. We used recently developed methods for rapid, low-cost, on-site, histology-based reproductive analysis that requires minimal research infrastructure. These methods use standard techniques (e.g., plastic embedding) that have been modified such that work can be conducted in remote field settings without electrical service. We studied the following fish species: the teardrop threadfin bream, *Nemipterus isacanthus* (Bleeker, 1873); the dash-and-dot goatfish, *Parupeneus barberinus* (Lacepède, 1801); the blue sea chub, *Kyphosus cinerascens* (Forsskal, 1775); the striated surgeonfish, *Ctenochaetus striatus* (Quoy et Gaimard, 1825); and the orange-lined triggerfish, *Balistapus undulatus* (Park, 1797).

Results. In all species, length was an approximately cubic function of weight. Female *B. undulatus* mature at a smaller size than males; in the other four species males mature at a smaller size. *Nemipterus isacanthus*, *K. cinerascens*, *C. striatus*, and *B. undulatus* are gonochores. Females are rare or absent in the largest size classes of *N. isacanthus*, *P. barberinus*, *C. striatus*, and *B. undulatus*. For *P. barberinus* and *B. undulatus*, size classes well below maximum observed size are responsible for the majority of egg production.

Conclusion. Our results differed markedly from those of macroscopic and data-deficient approaches and may help to avoid unnecessary management actions. Further, an emergent pattern challenges a current paradigm in reef-fish conservation and management; contrary to a general assumption for many reef-fish species, the largest

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size classes of at least three species did not have the highest per-capita fecundity. Because females were rare or absent in the largest size classes, smaller fish are responsible for the majority of egg production. Thus, for coastal communities dependent upon reef fishes for sustenance, our results suggest it may be easier to balance food needs and conservation by fishing across a broad size range. Further, because of diminished concern about targeting the larger size classes commonly assumed to be responsible for the majority of egg production, doing so will simultaneously promote adequate reproductive output.

Keywords: size-at-maturity, sexual pattern, sex ratios, batch fecundity, Jungle Histology

INTRODUCTION

Subsistence and small-scale commercial fishing of shallow-water coral reef fishes are important sources of food and income throughout the Pacific region (Roberts and Polunin 1993, Tokeshi et al. 2013, Rhodes et al. 2015). However, with new technologies and increasing human populations, there is growing concern that coral-reef fisheries are being overexploited (Friedlander and DeMartini 2002, Pandolfi et al. 2003, McClanahan et al. 2008).

One of the most-easily understood concepts to promote sustainable fishing practices is to harvest coral reef fish only after they have grown large enough to reproduce, and thus allow each generation to “seed” the next (Froese 2004). However, basic reproductive information (e.g., size at maturity) is lacking for most exploited fishes (Froese and Binohlan 2000, Rhodes and Tupper 2007) and the deficiency is especially acute for coral-reef-associated fishes. The sheer diversity of coral-reef fishes and the supposed cost associated with the reproductive analysis of each species are often cited as barriers to obtaining this important information (Roberts and Polunin 1993, Johannes 1998). Further, many Pacific island nations, where many of the world’s coral reefs are located, have little or no capacity to conduct reproductive research (Dalzell 1998, Rhodes et al. 2008). The reason for the latter problem may be as simple as a lack of basic infrastructure (e.g., electrical service needed to operate laboratory equipment) in many parts of these developing countries. These problems hinder current abilities to effectively manage coral-reef fisheries in the Pacific.

Advances in knowledge of the reproductive biology of Indo-Pacific reef fishes are ongoing (DeMartini et al. 2014, Shimose and Nanami 2014, 2015, Taylor et al. 2014, Ohta and Ebisawa 2015, DeMartini and Howard 2016, Rhodes et al. 2016, Schemmel et al. 2016, Taylor et al. 2016). However, none of these studies use histological methods that can be readily employed in developing countries (with limited research infrastructure) and easily learned by local marine resource-management staff (who often may be inadequately trained).

To address these challenges, Longenecker et al. (2013a) developed a method for rapid, low-cost, on-site, histology-based reproductive analysis that requires minimal research infrastructure. The method has since earned the appellation “Jungle Histology”, which describes the environment for which the method was developed and hints at the “guerrilla” nature of the approach. The method focuses upon histological examination because other means of estimating reproductive parameters have well-demonstrated biases. The FishBase life history tool (Froese and Pauly 2016), built on the empirical relations of Froese

and Binohlan (2000), increasingly overestimates female size-at-maturity as the maximum size of a species increases (Longenecker et al., 2013b, Longenecker and Langston 2016). Visual (macroscopic) examination of gonads results in misclassification of sex and/or reproductive status in 43%–47% of specimens (Longenecker et al. 2013a, 2013c). These misclassifications tend to overestimate the number of mature females (Longenecker et al. 2013a, 2013c), underestimate female size-at-maturity (Grandcourt et al. 2006, 2011), and overestimate female spawning biomass (Vitale et al. 2006). Additionally, histological analysis is required to diagnose sex change (Sadovy and Shapiro 1987).

With the Jungle Histology method, reproductive information can be generated quickly and its low cost eliminates one of the arguments against broad-scale reproductive analysis surveys. Also, the simple methods can be easily learned by marine resource-management staff, thus increasing the capacity for “home grown” reproductive analysis in developing countries or in places with little or no research infrastructure or capability. The purpose of this article is to present the reproductive information generated during a Jungle Histology training workshop for natural resource professionals from US-associated Pacific Islands and Papua New Guinea. The goal of that workshop was to develop a cadre of fish reproductive biologists who can independently use the Jungle Histology method, thus increasing the rate at which new reproductive information is generated, and increasing the potential to effectively manage and conserve Pacific coral-reef fishes.

Workshop participants chose study species that were:

- Wide-ranging;
- Heavily exploited in some part of their range;
- Distinctive enough that the chance of misidentification is low; and
- Whose reproductive biology was studied little or not at all.

Published reproductive information is lacking or incomplete for the dash-and-dot goatfish, *Parupeneus barberinus* (Lacepède, 1801); the blue sea chub, *Kyphosus cinerascens* (Forsskal, 1775); the striated surgeonfish, *Ctenochaetus striatus* (Quoy et Gaimard, 1825); and the orange-lined triggerfish, *Balistapus undulatus* (Park, 1797). All four species are distinctive, widespread in the tropical Indo-West Pacific (Matsuura 2001, Randall 2001a, 2001b, Sakai 2001) and are heavily targeted reef-fishery species (McClanahan et al. 1999, Grandcourt 2002, Rhodes et al. 2008, Crawford et al. 2011, Feary et al. 2011, Ochavillo et al. 2011, Houk et al. 2012).

Additionally, a subset authors (KL, RL, HB, and UK) analysed a fifth species, the teardrop threadfin bream, *Nemipterus isacanthus* (Bleeker, 1873), for which there appears to be no published reproductive information. Until recently, *N. isacanthus* was reported to have a West Pacific distribution, including the Gulf of Thailand; waters surrounding the Philippine and Indonesian islands; and the north-western coast of Australia (Russell 1990). However, we studied a large, permanent population recently documented at Kamiali Wildlife Management Area (KWMA), Morobe Province, Papua New Guinea, representing the easternmost known limit of the species, where it forms a large part of the subsistence fish catch (Longenecker and Giamsa 2016). Reports from elsewhere indicate that *N. isacanthus* appears occasionally in small numbers in local markets, although there is no major fishery for the species (Russell 1990).

MATERIALS AND METHODS

We used methods modified from Longenecker et al. (2013a) to describe length–weight relations, size-at-maturity, sexual pattern, sex-ratios, and length-batch fecundity relations*. The modifications were designed to decrease the total volume of fixative used in histological processing and greatly decrease the time necessary for fecundity estimates.

Specimen acquisition and whole specimen processing.

Workshop specimens (*Parupeneus barberinus*, *Kyphosus cinerascens*, *Ctenochaetus striatus*, and *Balistapus undulatus*) were purchased from local fishers near Kavieng, New Ireland Province, Papua New Guinea (Fig. 1). All acquisitions were made in accordance with local laws and regulations. Special permits were not required because the specimens were purchased directly from markets and not exported out of the country. For each specimen, length from the front of the head with mouth closed to the end of the middle caudal ray, was measured to the nearest 0.1 cm. Whole body weight was measured with the smallest-possible of two hanging spring-scales (100 or 1000 g capacity, with 1 or 10 g increments, respectively), with the exception of seven juvenile *B. undulatus* that were weighed on a battery-powered jeweler's scale to the nearest 0.001 g. A mid-ventral incision was made from the vent through the pelvic girdle, sex and reproductive status (based on gross examination) were recorded, then gonads were excised and weighed to 0.001 g on a battery-powered jeweler's scale. For each ovary that appeared, macroscopically, to be at or nearing maturity (late vitellogenesis through hydration, stage IIIb through IVb, respectively), an approximately 1-cm thick transverse section was removed from one lobe, weighed to 0.001 g, and transferred to approximately 15 mL Gilson's fluid in a skirted 50-mL centrifuge tube for later batch fecundity analysis (below). For all gonads (regardless of sex or reproductive status) an approximately 3 mm × 3 mm × 3 mm subsample was excised, placed in one well of a tissue culture plate, and fixed in Dietrich's solution for at least 24 h.

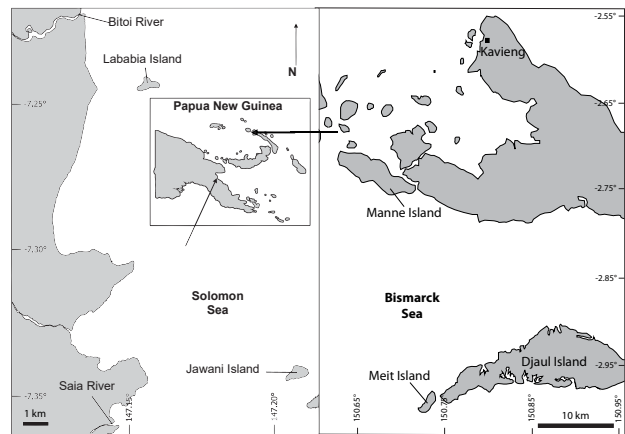


Fig. 1. Map of the sampling areas in Papua New Guinea; Specimens for the Jungle Histology workshop were obtained near Kavieng, New Ireland Province (right); Supplementary specimens of workshop species, and all *Nemipterus isacanthus* specimens, were obtained from Kamiali Wildlife Management Area, Morobe Province, located between the Bitoi and Saia Rivers (left)

Size-at-maturity and sexual pattern. The Dietrich's-fixed gonad subsamples were trimmed to a volume of approximately 8 mm³ (2 mm × 2 mm × 2 mm), then dehydrated in ethanol (30–60 min in each of 50%, and two changes of 95% ethanol). Using plastic embedding medium (JB-4, Electron Microscopy Sciences) and following kit instructions, gonad sections were infiltrated in two changes of infiltration solution (1 h and >8 h, respectively), transferred into embedding capsules (BEEM®, size 00), and embedded in catalysed JB-4 solution. Because high humidity in tropical locations often prevents tissue blocks from hardening completely, tissue blocks were dehydrated for 12 h in a “desiccating chamber” (a diver's dry box containing silica gel packets and placed in full sunlight). From each embedded gonad subsample, 10 tissue sections (approximately 7-μm thick), distributed evenly throughout each tissue block, were obtained by serial sectioning on an MT1 Porter-Blum microtome outfitted with a glass knife. The tissue sections were floated on water droplets distributed on microscope slides, and slides were dried on a “warmer” (a flat piece of thick metal placed in full sunlight). Tissue sections (now affixed to the slides) were stained in a 0.5% solution of Toluidine Blue in water for 15 s. Excess stain was removed with a gentle stream of water, and the slides were once again dried on the “warmer”. Ovary sections were examined at 100× and testis sections at 400× for evidence of reproductive maturity. Ovaries were classified according to Wallace and Selman (1981) and testes according to Nagahama (1983). Females were considered mature with the onset of vitellogenesis or when post-ovulatory follicles were present, and males mature when the testes contained visible spermatozoa (sperm cells with tails).

* Instructional videos used to prepare workshop participants for laboratory work are available at: <http://pbs.bishopmuseum.org/pacificfishes/modules.html>

Batch fecundity. Methods modified from Agger et al. (1974) were used to estimate batch fecundity. Ovarian samples reserved for batch-fecundity analysis (above) were hand-shaken at least once during each of 14 days. Batch fecundity estimates were generated for those that, based on the histological examination above, had reached the maturation or hydration stage (IVa or IVb, respectively). Oocytes were teased from the stroma, then the samples were diluted with water to a total volume of 400 mL. The diluted sample was stirred to distribute oocytes, and a Stempel pipette was used to obtain ten 1-mL subsamples. Counts of oocytes in the largest size-class in each subsample were recorded, and batch fecundity (BF) was estimated with the following equation:

$$BF = (N_o \cdot V)(W_o \cdot W_s^{-1})$$

where: N_o is the mean number of mature oocytes per mL, V is the total dilution volume in mL, W_o is the total ovary weight, and W_s is the sample weight.

Supplementary specimens. Poor weather at Kavieng prevented fishing during much of the time allotted for reproductive analysis, with the result that fishers caught the overwhelming majority of specimens in a single day. Very few ovaries from these specimens contained stage IV eggs, making fecundity analysis difficult. To address this shortcoming, a subset of authors (KL, RL, HB, and UK) targeted the same species in later field work at KWMA (Fig. 1), and hoped to increase the number of gravid females in our sample size while adding more specimens to the other analyses. Additionally, all *Nemipterus isacanthus* specimens were collected at KWMA. Village residents, through regular fishing activities from 4 February–4 June 2015, caught specimens by hook-and-line. These specimens were caught as many as four months before histological processing (i.e., they could not be fully processed as they were captured). Because there is no electrical service (and thus specimens could not be frozen) at KWMA, gonads were fixed immediately after capture, resulting in the following differences from Kavieng data:

- Whole-gonad weights are for fixed tissues;
- Ovarian subsample weights for fecundity analysis are for fixed tissues; and
- Fixed ovarian subsamples were placed into Gilson's fluid for oocyte counts.

All of these steps were accomplished using fresh tissues in Kavieng.

Data analysis. We constructed length–weight relations (LWR) using log-transformed data and following the guidance of Froese et al. (2011). Because of evidence that a scale used at KWMA was inaccurate, we excluded KWMA length–weight data from the analyses for workshop species (*Parupeneus barberinus*, *Kyphosus cinerascens*, *Ctenochaetus striatus*, and *Balistapus undulatus*). However, more-detailed analysis indicated the measurement error was restricted to specimens weighing < 40 g, thus the LWR for *Nemipterus isacanthus* (collected exclusively at KWMA) is restricted to specimens ≥ 40 g. For all

species, we considered any data point in the length–weight regression with a residual > 0.125 to be an outlier. We used analysis of covariance (ANCOVA) to evaluate whether the LWR varied between sexes. We report size at maturity (L_{50}) as the size at which a non-linear regression (3-parameter, sigmoidal) of percent mature individuals versus length (the mean length of individuals within a 2-cm size class) indicates 50% of individuals are mature. We assigned one-half of undifferentiated individuals to each sex. We used χ^2 analysis to test whether overall sex ratios differed from 1 : 1. We described size-specific sex ratios by determining the percent of mature females (of total mature individuals) in each size class, then plotting % mature females as a function of mean length within each size class. We used exploratory regression analysis to evaluate whether sex ratios of mature individuals varied predictably with length. We tested for relations between length and batch fecundity using linear regression analysis of log-transformed data. We evaluated which part of a population is responsible for the majority of egg production by first multiplying the result of the length–fecundity relation at a given length by the likelihood that an individual at that length is female (i.e., the size-specific sex ratio). We then plotted the product as a function of length. For comparative purposes, we assumed that the likelihood of an individual being female is constant across all lengths (using the overall sex ratio) and plotted the product as a function of length. Thus we standardized both curves to represent the expected batch fecundity of any randomly chosen individual of a given length (i.e., not just females).

RESULTS

Results are summarized in Table 1 and presented more fully in separate sections for each species, below.

***Nemipterus isacanthus*.** ANCOVA did not detect a significant sex-based difference in the length–weight relation for this population ($F = 0.28$, $DF = 1$, $P = 0.598$). Total body weight (W) in g was an approximately cubic function of fork length (FL) in cm (Table 1). The 95% CI of regression parameters a and b were 0.00726–0.0133 and 3.06–3.28, respectively ($r^2 = 0.977$, $n = 81$, FL range: 13.0–24.9, W range: 40–250).

We histologically examined gonads of 84 male and 23 female *Nemipterus isacanthus*. Figure 2 shows the size-frequency distribution of each sex and maturity stage. Figure 3 shows examples of immature and mature testes and ovaries. Ovaries of mature females contained several discrete stages of oocytes, indicating group-synchronous oocyte development (Wallace and Selman 1981). We therefore classified *N. isacanthus* as a batch spawner. Ovaries contained vitellogenic oocytes in females as small as 10.9 cm FL and all females ≥ 16 cm FL were mature. The smallest male with spermiated testes was 10.3 cm FL. Immature/inactive individuals were scattered throughout the male size range, with 90% of individuals ≥ 14.9 cm FL being mature. Figure 4 shows the relative frequency of reproductive states in each size class. Overall, 78.3% of females and 78.6% of males were mature. We had too few immature individuals to estimate L_{50} for either sex.

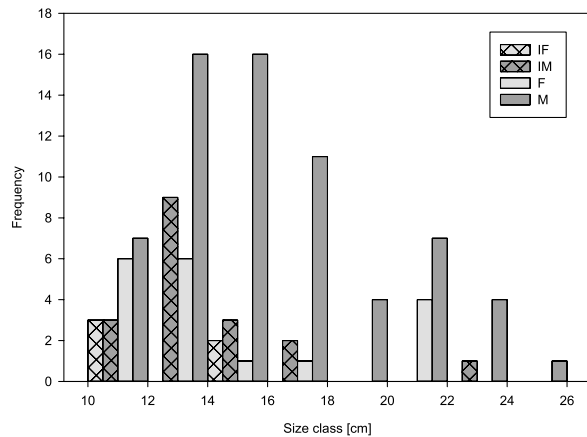


Fig. 2. Size-frequency plot of *Nemipterus isacanthus* specimens, with sex and maturity status determined by histological examination of gonads; IF = immature females, IM = immature males, F = mature females, M = mature males

We did not see evidence of sex change in *Nemipterus isacanthus*. A *t*-test did not detect a significant sex-based difference in mean length ($t = -1.69$, $DF = 105$, $P = 0.09$). We did not see a central membrane-lined lumen in testes, or any gonad containing a mixture of ovarian and spermatogenic tissue. Nor did we observe a dorsal accessory duct, associated with rudimentary hermaphroditism in some *Nemipterus* species (Takahashi et al. 1989, Lau and Sadovy 2001). Based on the above, we classified *N. isacanthus* as a gonochore.

Overall sex ratio in this *Nemipterus isacanthus* population was male-biased (Table 1, $\chi^2 = 34.8$, $DF = 1$, $P < 0.0001$). Considering only mature individuals, the observed sex ratio was also significantly male-biased ($\chi^2 = 27.4$, $DF = 1$, $P < 0.0001$). However, the sex ratio of mature individuals varied predictably with length; although all classes were male biased, the highest percentage of females was found in the smallest size class (Fig. 5). An equation describing the percent of mature females ($\%♀$), throughout the size range of mature specimens (Table 1, $r^2 = 0.965$), predicts that the population was exclusively male when body size ≥ 22.5 cm FL.

Our gross-level (macroscopic) evaluations of oocyte stage were usually incorrect. We judged all but two specimens as being in early vitellogenesis (stage IIIa) or earlier stages of oogenesis. Later histological evidence indicated only one of the two ovarian samples we collected for fecundity analysis had reached stage IV, and that four other specimens for which we did not collect fecundity-analysis samples had reached stage IV (maturation or hydration). This gross-level error left us with too few samples ($n = 1$) to construct a length–batch fecundity relation.

***Parupeneus barberinus*.** ANCOVA did not detect a significant sex-based difference in the length–weight relation for this population ($F = 0.27$, $DF = 1$, $P = 0.603$). Total body weight (W) in g was an approximately cubic function of fork length (FL) in cm (Table 1). The 95%

Table 1

Summary of length–weight relations (LWR) and reproductive information for five exploited species from Papua New Guinea

	<i>Nemipterus isacanthus</i>	<i>Parupeneus barberinus</i>	<i>Kyphosus cinerascens</i>	<i>Ctenochaetus striatus</i>	<i>Balistapus undulatus</i>
LWR	$W = 0.00983(FL)^{3.17}$	$W = 0.0140(FL)^{3.10}$	$W = 0.0294(FL)^{2.94}$	$W = 0.0339(FL)^{2.86}$	$W = 0.0252(FL)^{3.03}$ $W = 0.0660(FL)^{2.69}$ $W = 0.0346(FL)^{2.91}$
L_m	10.3	12.4	18.1	8.0	12.9
L_{50}	10.9	13.3	22.5	10.4	8.4
L_{90}	<10.3	<12.4	20.1	<8.0	—
L_{95}	<10.9	19.2	22.6	<10.2	15.7
Sexual pattern	Gonochore	See Table's footnote	Gonochore	Gonochore	Gonochore
Oocyte development	Group synchronous	Group synchronous	Group synchronous	Group synchronous	Group synchronous
Sex ratio (overall) ♂:♀	1:0.27	1:0.67	1:1.08	1:0.74	1:2.44
Sex ratio (mature) ♂:♀	1:0.27	1:0.28	1:0.48	1:0.64	1:2.39
Size-specific sex ratio	$\%♀ = 74.5 - 3.31(FL)$	$\%♀ = 132.83 - 5.60(FL)$	Not evident	$\%♀ = 69.79e^{\left(-0.5\left(\frac{FL-12.51}{1.86}\right)^2\right)}$	$\%♀ = \frac{100.80}{1+e^{\left(\frac{FL-10.62}{-1.36}\right)}}$ BF = 0.0093(TL) ^{0.02}
Batch fecundity	—	—	—	—	—

Longenecker et al. (2017) reported that some immature *P. barberinus* individuals are bisexual, but suggest the species is a functional gonochore; L_m = minimum mature length (the smallest mature individual observed during the presently reported study); L_{50} = length at 50% maturity (50% of all individuals at this length are expected to be mature).

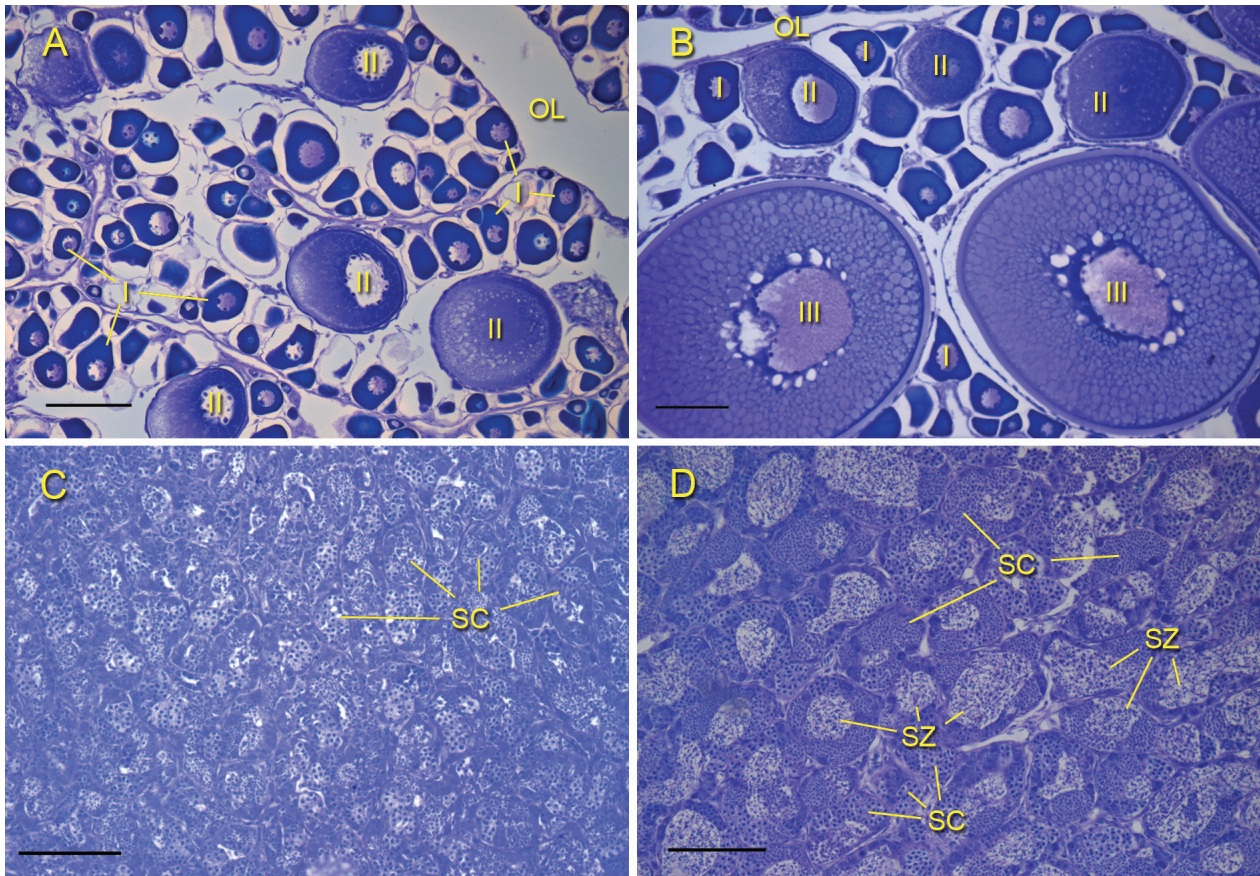


Fig. 3. Histological sections of gonads of teardrop threadfin bream, *Nemipterus isacanthus*, from the Kamiali Wildlife Management Area, Papua New Guinea: Ovary of immature female (A) (14.1 cm) containing primary-growth (I) and cortical vesicle (II) oocytes; Ovary of mature female (B) (21.5 cm) containing primary-growth (I), cortical vesicle (II), and vitellogenic (III) oocytes; Testis from an immature male (C) (13.6 cm) containing spermatogenic cysts (SC); Testis of a mature male (D) (12.8 cm) with spermatogenic cysts (SC) and tailed spermatozoa (SZ); OL = ovarian lumen; scale bars = 100 μ m

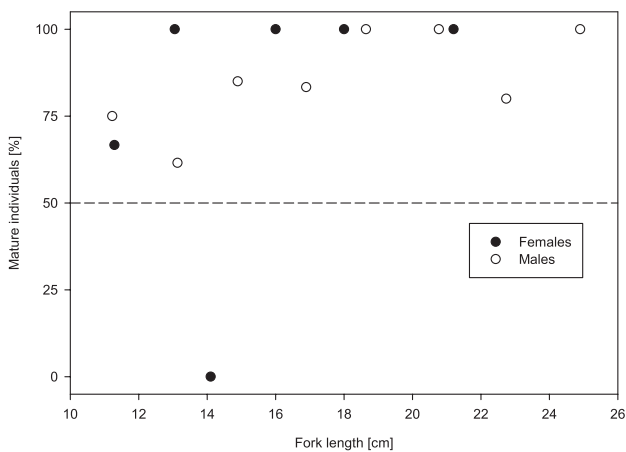


Fig. 4. Percent of mature individuals in each 2-cm size class for teardrop threadfin bream, *Nemipterus isacanthus*, from the Kamiali Wildlife Management Area, Papua New Guinea

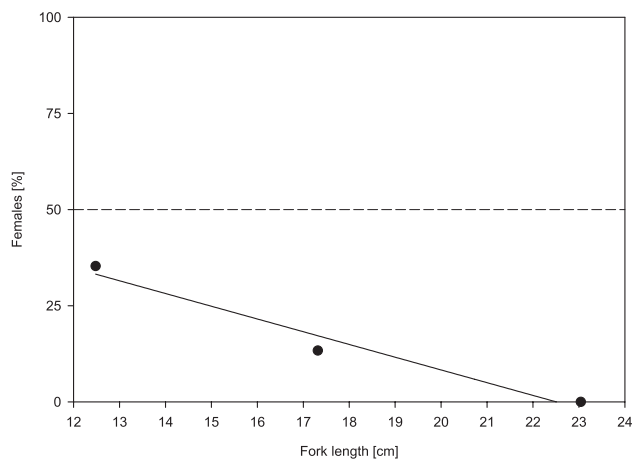


Fig. 5. Percentage of mature females, relative to all mature individuals, versus length for teardrop threadfin bream, *Nemipterus isacanthus*, from the Kamiali Wildlife Management Area, Papua New Guinea; The equation for the fitted line is given in Table 1 ($r^2 = 0.965$, $F = 27.4$, $P = 0.120$)

CI of regression parameters a and b were 0.0113–0.0174 and 3.02–3.17, respectively ($r^2 = 0.978$, $n = 158$, FL range: 12.5–25.3, W range: 40–335).

We histologically examined gonads of 94 male, 62 female, and 8 bisexual *Parupeneus barberinus*. Figure 6 shows the size-frequency distribution of each sex and maturity stage. Figure 7 shows examples of immature and mature testes and ovaries. Ovaries of mature females contained several discrete stages of oocytes, indicating group-synchronous oocyte development (Wallace and Selman 1981). We therefore classified *P. barberinus* as a batch spawner. Bisexual individuals ranged from 16.1–22.5 cm FL, did not show evidence of functioning as either sex, and were omitted from size-at-maturity analyses. Ovaries contained vitellogenic oocytes in females as small as 13.3 cm FL. Inactive and immature females (range 11.7–19.9) were scattered throughout the size range of mature females (range 13.3–22.0). We estimate female L_{50} at 19.2 cm FL (Fig. 8). The smallest male with spermiated testes was 12.4 cm FL. Inactive and immature males (range 12.8–24.8) were scattered throughout the size range of mature males (range 12.4–25.3). We could not generate a satisfactory L_{50} curve for males, the percent of mature individuals was greater than 50% for all size classes sampled and the mean was 78.7% (Fig. 8).

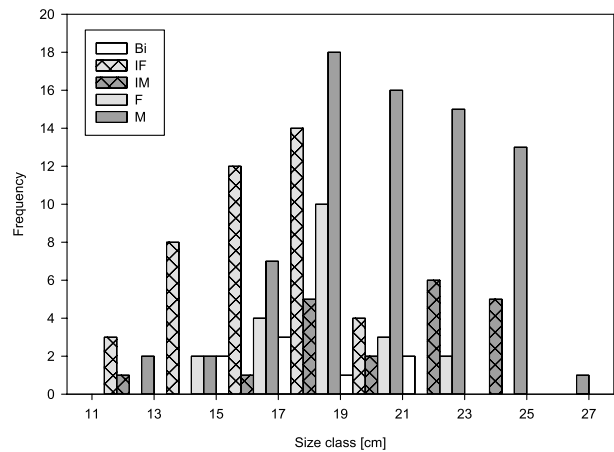


Fig. 6. Size-frequency plot of *Parupeneus barberinus* specimens, with sex and maturity status determined by histological examination of gonads; Bi = bisexual individuals, IF = immature females, IM = immature males, F = mature females, M = mature males

Overall sex ratio in this *Parupeneus barberinus* population was male-biased (Table 1, $\chi^2 = 6.564$, DF = 1, $P = 0.010$). Considering only mature individuals, the observed sex ratio was also significantly male-biased ($\chi^2 = 29.568$,

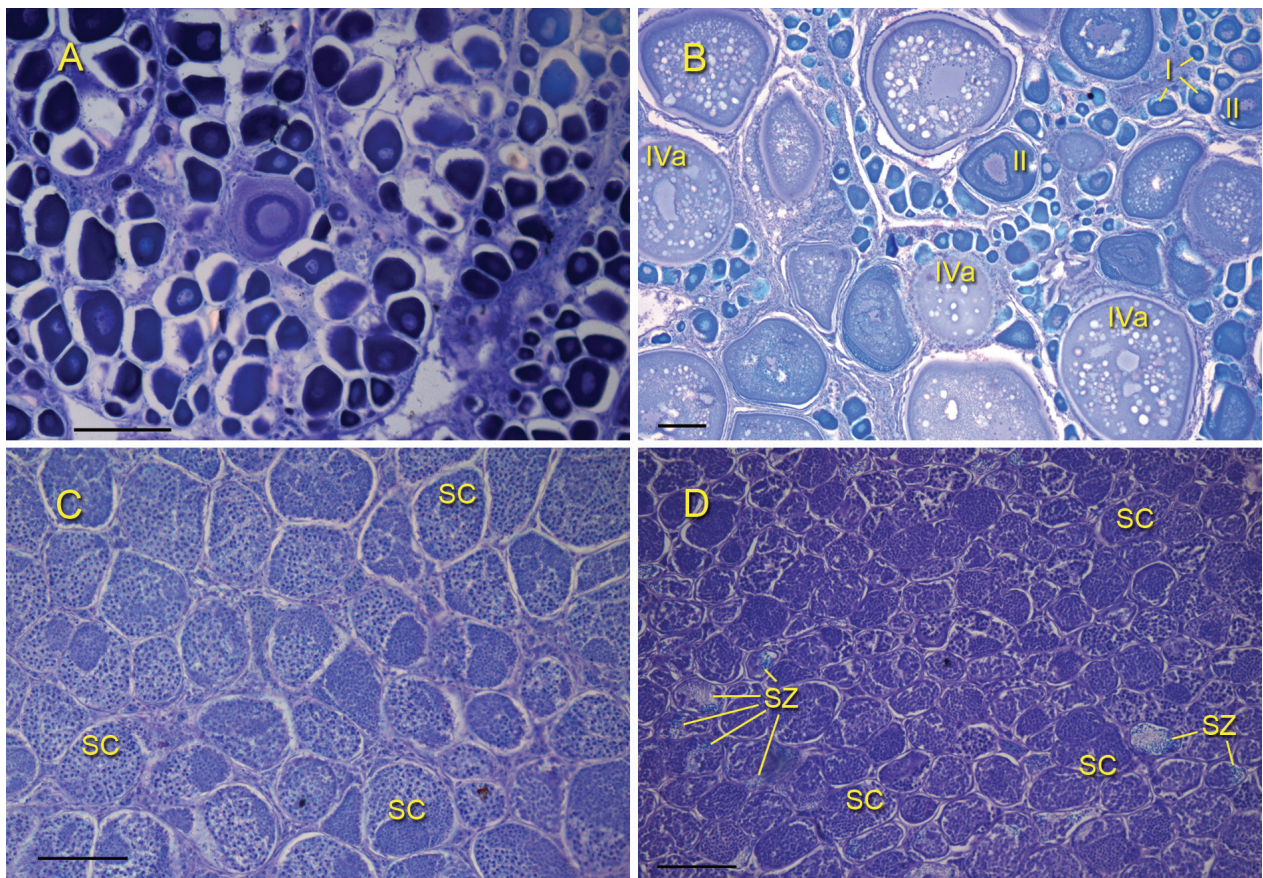


Fig. 7. Histological sections of gonads of dash-and-dot goatfish, *Parupeneus barberinus*, from Papua New Guinea: Ovary of immature female (A) (13.0 cm) containing only primary-growth oocytes; Ovary of mature female (B) (18.0 cm) containing primary-growth (I), cortical vesicle (II), and final maturation (IVa) oocytes; Testis from an immature male (C) (19.7 cm) containing spermatogenic cysts (SC); Testis of a mature male (D) (22.0 cm) with spermatogenic cysts (SC) and tailed spermatozoa (SZ); Scale bars = 100 μ m

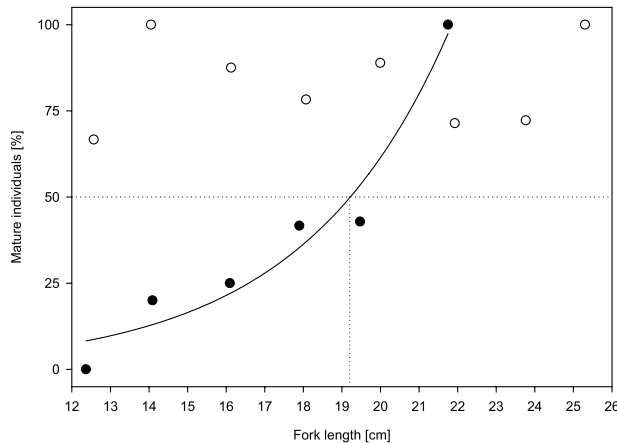


Fig. 8. Size-at-maturity (L_{50}) for *Parupeneus barberinus* from Papua New Guinea; Females are represented by closed circles and the solid line ($r^2 = 0.951$, $F = 28.9$, $P = 0.011$), males are represented by open circles

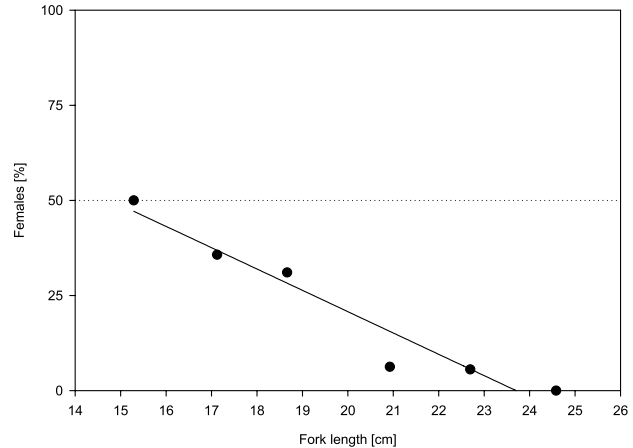


Fig. 9. Percentage of mature females, relative to all mature individuals, versus length for *Parupeneus barberinus* from Papua New Guinea; The equation for the fitted line is given in Table 1 ($r^2 = 0.938$, $F = 60.0$, $P = 0.002$)

DF = 1, $P < 0.001$). However, the sex ratio of mature individuals varied predictably with length; the percentage of females was inversely proportional to length (Fig. 9). An equation describing the percent of mature females ($\%♀$), throughout the size range of mature specimens (Table 1, $r^2 = 0.938$), predicts that the population was exclusively male when body size > 23.7 cm FL.

Linear regression analysis of log-transformed fecundity-at-length data was not significant ($F = 0.632$, $n = 7$, $P = 0.493$); batch fecundity did not increase with length. However, batch fecundity ranged from 5286 and 44 345 eggs in seven females ranging from 13.3 to 19.4 cm FL.

The curves in Fig. 10 show the relation between length and expected individual batch fecundity with observed overall sex ratios and with observed size-specific sex ratios (the curves incorporate the *Parupeneus barberinus* length-batch fecundity relation from Longenecker et al. 2011). With an assumed constant sex ratio, expected per individual egg production is greatest at maximum size. When size-specific sex ratios are considered, the small chance of an individual being a female eventually overwhelms increases in batch fecundity such that expected egg production per individual peaks at 18.2 cm FL, well below maximum observed length (Fig. 10, solid curve). Failing to account for size-specific sex ratios at the largest observed specimen size led to a 22 100-egg overestimate in expected individual batch fecundity.

***Kyphosus cinerascens*.** ANCOVA did not detect a significant sex-based difference in the length–weight relation for this population ($F = 1.20$, DF = 1, $P = 0.282$). Total body weight (W) in g was an approximately cubic function of fork length (FL) in cm (Table 1). The 95% CI of regression parameters a and b were 0.0187–0.0462 and 2.79–3.08, respectively ($r^2 = 0.979$, $n = 38$, FL range: 15.8–30.7, W range: 101–660).

We histologically examined gonads of two (2) undifferentiated, 26 male, and 28 female *Kyphosus cinerascens*. Figure 11 shows the size-frequency distribution of each sex and maturity stage. Figure 12

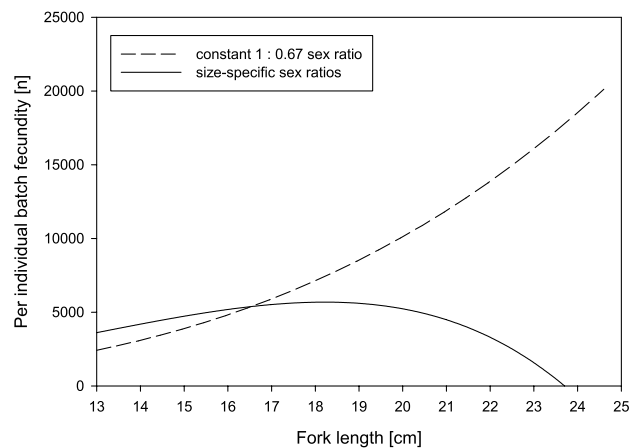


Fig. 10. The influence of size-specific sex ratios on population-level patterns of egg production: the overall sex-ratio (dashed curve) in *Parupeneus barberinus* makes it appear as though the largest individuals are responsible for the majority of egg production; however smaller individuals are responsible for the majority of egg production when size-specific sex ratios are considered (solid curve)

shows examples of immature and mature testes and ovaries. Ovaries of mature females contained several discrete stages of oocytes, indicating group-synchronous oocyte development (Wallace and Selman 1981). We therefore classified *K. cinerascens* as a batch spawner. Sexual differentiation occurs around 15 cm FL. The smallest male with spermiated testes was 18.1 cm FL. For males, the size at which 50% of individuals are mature (L_{50}) is 20.1 cm FL (Fig. 13). All males ≥ 22.4 cm were mature. Ovaries contained vitellogenic oocytes in females as small as 22.5 cm FL. We estimate female L_{50} at 22.6 cm FL (Fig. 13). All females ≥ 30.7 cm FL were mature.

We did not see evidence of sex change in *Kyphosus cinerascens*. A t -test did not detect a significant sex-based difference in mean length ($t = -1.338$, DF = 52, $P = 0.187$).

We did not see a central membrane-lined lumen in testes, nor did any gonad contain a mixture of ovarian and spermatogenic tissue. We classified *K. cinerascens* as a gonochore.

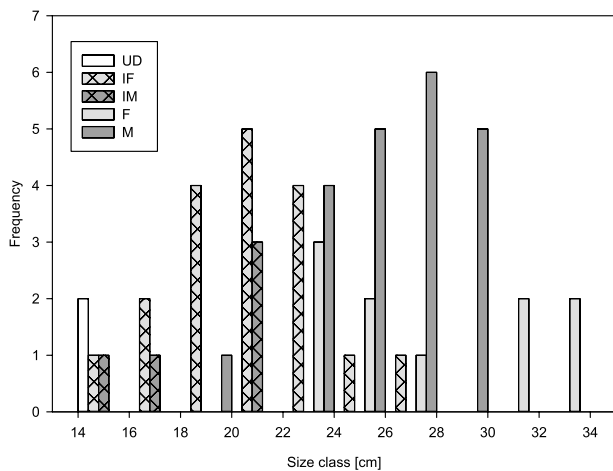


Fig. 11. Size-frequency plot of *Kyphosus cinerascens* specimens, with sex and maturity status determined by histological examination of gonads; UD=undifferentiated individuals, IF = immature females, IM = immature males, F = mature females, M = mature males

Overall sex ratio in this *Kyphosus cinerascens* population was not significantly different from a 1 male : 1 female sex ratio (Table 1, $\chi^2 = 0.074$, DF = 1, $P = 0.786$). Considering only mature individuals, the observed sex ratio was male-biased ($\chi^2 = 3.903$, DF = 1, $P = 0.048$). We saw no evidence that the sex ratio of mature individuals varied predictably with length.

We had too few females with stage IV oocytes to construct a length-batch fecundity relation. However, batch fecundity ranged from 4607 and 11 738 eggs in three females ranging from 23.0 to 32.0 cm FL.

***Ctenochaetus striatus*.** ANCOVA did not detect a significant sex-based difference in length–weight relations ($F = 0.46$, DF = 1, $P = 0.502$). Overall, total body weight (W) in g was an approximately cubic function of FL in cm (Table 1). The 95% CI of regression parameters a and b were 0.0243–0.0472 and 2.74–2.99, respectively ($r^2 = 0.953$, $n = 108$, FL range: 10.3–18.8, W range: 25–149).

We histologically examined gonads of 88 male and 64 female *Ctenochaetus striatus*. Figure 14 shows the size-frequency distribution of each sex and maturity stage. Figure 15 shows examples of immature and mature testes and ovaries. Ovaries of mature females contained several discrete stages of oocytes, indicating group-synchronous oocyte development (Wallace and Selman 1981).

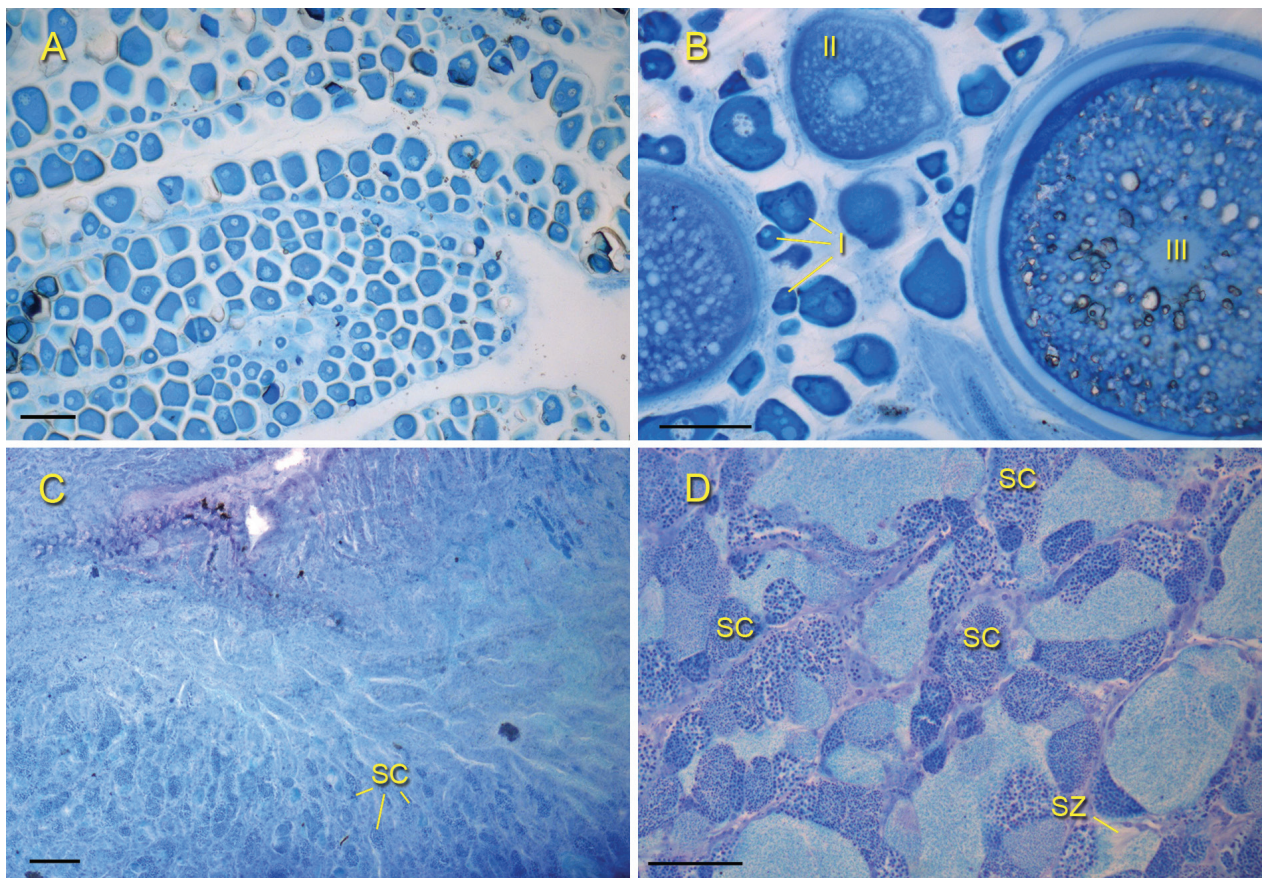


Fig. 12. Histological sections of gonads of blue sea chub, *Kyphosus cinerascens*, from Papua New Guinea: Ovary of immature female (A) (20.4 cm) containing only primary-growth oocytes; Ovary of mature female (B) (32.0 cm) containing primary-growth (I), cortical vesicle (II), and vitellogenic (III) oocytes; Testis from an immature male (C) (20.4 cm) containing spermatogenic cysts (SC); Testis of a mature male (D) (26.0 cm) with spermatogenic cysts (SC) and tailed spermatozoa (SZ); OL = ovarian lumen; scale bars = 100 μ m

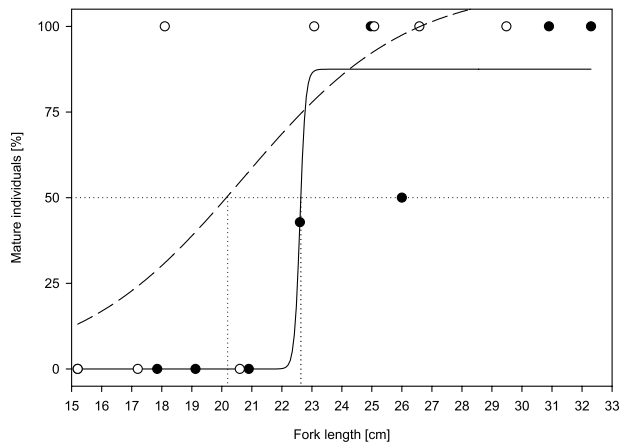


Fig. 13. Size-at-maturity (L_{50}) for *Kyphosus cinerascens* from Papua New Guinea; Females are represented by closed circles and the solid line ($r^2 = 0.891$, $F = 24.5$, $P = 0.001$), males are represented by open circles and the dashed line ($r^2 = 0.516$, $F = 2.67$, $P = 0.163$)

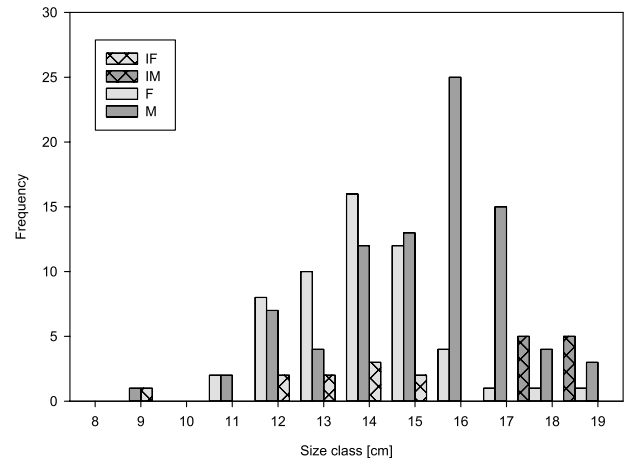


Fig. 14. Size-frequency plot of *Ctenochaetus striatus* specimens, with sex and maturity status determined by histological examination of gonads; IF = immature females, IM = immature males, F = mature females, M = mature males

We therefore classified *C. striatus* as a batch spawner. Ovaries contained vitellogenic oocytes in females as small as 10.4 cm FL, and 85.9% of the females we examined were mature. The smallest male with spermiated testes

was 8.0 cm FL, and 97.7% of the males we examined were mature. Because all 2-cm size classes of both sexes contained more than 50% mature individuals, we could not reliably estimate L_{50} .

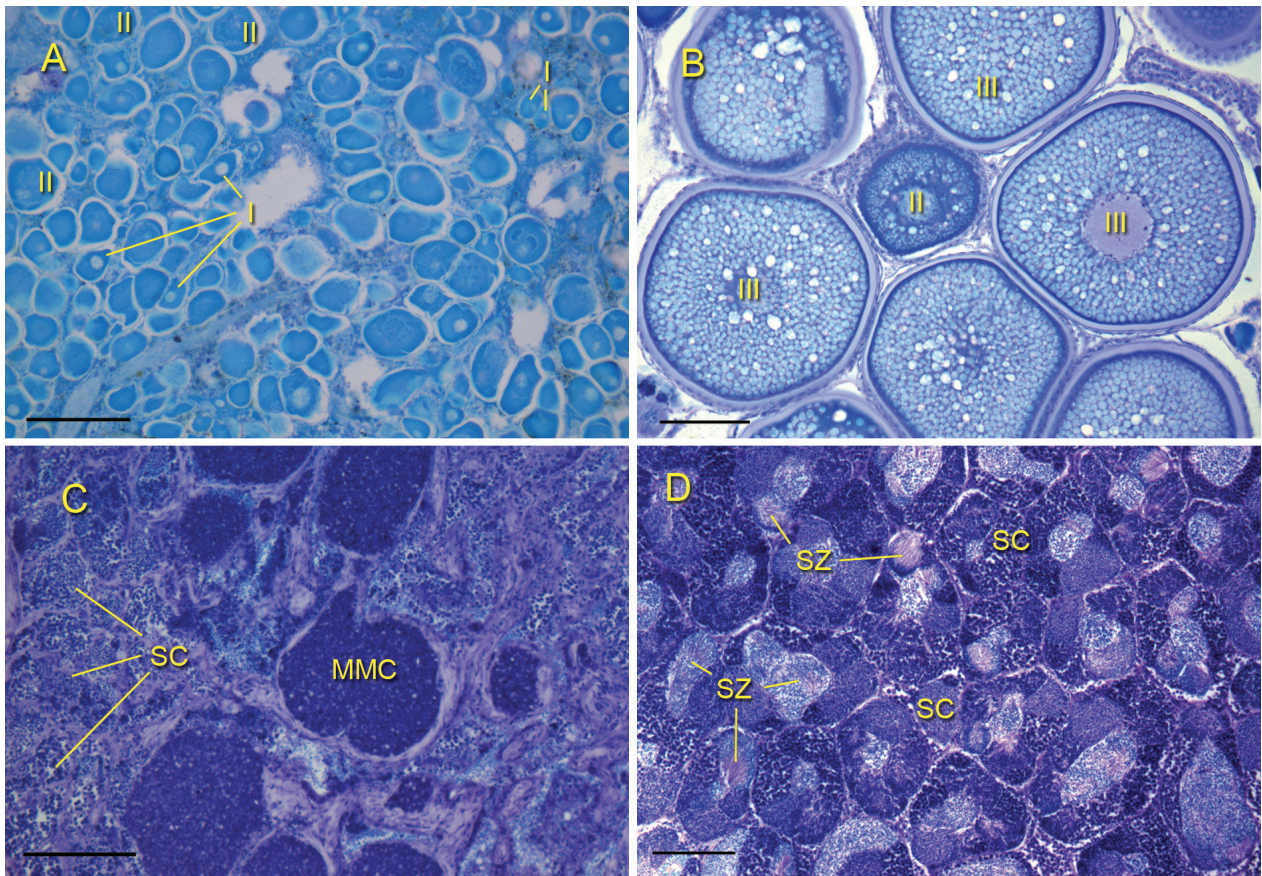


Fig. 15. Histological sections of gonads of striated surgeonfish, *Ctenochaetus striatus*, from Papua New Guinea: Ovary of immature female (A) (14.9 cm) containing primary-growth (I) and cortical vesicle (II) oocytes; Ovary of mature female (B) (14.9 cm) containing cortical vesicle (II) and vitellogenic (III) oocytes; Testis from an immature male (C) (17.4 cm) containing spermatogenic cysts (SC) and probable melano-macrophage centres (MMC); Testis of a mature male (D) (16.4 cm) with spermatogenic cysts (SC) and tailed spermatozoa (SZ); scale bars = 100 μ m

We saw a sex-based bimodal size distribution in *Ctenochaetus striatus*. A *t*-test indicated mean length of males was significantly greater than that of females ($t = -4.429$, $DF = 148$, $P < 0.001$). There was no other evidence of sex change; we did not see a central membrane-lined lumen in testes, nor did any gonad contain a mixture of ovarian and spermatogenic tissue. We classified *C. striatus* as a gonochore.

Overall sex ratio in this *Ctenochaetus striatus* population was not significantly different from 1 male : 1 female (Table 1, $\chi^2 = 3.227$, $DF = 1$, $P = 0.0724$). Considering only mature individuals, the observed sex ratio was male-biased ($\chi^2 = 7.111$, $DF = 1$, $P = 0.008$). Additionally, we saw size-specific sex ratios in the mature size classes; the sex ratio of mature individuals varied predictably with length (Fig. 16). Smaller size classes were male-biased, switched to a female-biased state as length increased, and the largest size classes were male-biased. An equation describing the percent of mature females (%♀), throughout the size range of mature specimens (Table 1, $r^2 = 0.80$), predicts that the population was female biased between 11.0 and 14.0 cm FL.

We had too few females with stage IV oocytes to construct a length-batch fecundity relation. However, batch fecundity ranged from 7904 and 37 975 eggs in two females ranging from 15.1 to 18.7 cm FL.

***Balistapus undulatus*.** ANCOVA detected a significant sex-based difference in length–weight relations ($F = 4.98$, $DF = 1$, $P = 0.029$). Overall and sex-based LWRs are presented in Table 1. Overall, total body weight (W) in g was an approximately cubic function of TL in cm. 95% CI of regression parameters a and b were 0.0224–0.0284 and 2.98–3.07, respectively ($r^2 = 0.990$, $n = 177$, FL range: 4.1–21.9, W range: 1.4–250). Males tended to weigh more per unit length than females. For females, the 95% CI of regression parameters a and b were 0.0217–0.0552 and 2.73–3.09, respectively ($r^2 = 0.958$, $n = 48$, FL range: 8.4–16.0, W range: 17–105). For males, the 95% CI of

regression parameters a and b were 0.0400–0.109 and 2.52–2.87, respectively ($r^2 = 0.982$, $n = 21$, FL range: 10.8–21.9, W range: 36–250).

We histologically examined gonads of 12 undifferentiated, 110 female, and 45 male *Balistapus undulatus*. Figure 17 shows the size-frequency distribution of each sex and maturity stage. Figure 18 shows examples of immature and mature testes and ovaries. Ovaries of mature females contained several discrete stages of oocytes, indicating group-synchronous oocyte development (Wallace and Selman 1981). We therefore classified *B. undulatus* as a batch spawner. Ovaries contained vitellogenic oocytes in females as small as 8.4 cm TL. We estimate female L_{50} at 15.7 cm TL (Fig. 19). The smallest male with spermiated testes was 12.9 cm TL. Because the percentage of mature individuals fell below 50% in all size classes (Fig. 19), we could not reliably estimate male L_{50} .

We saw a sex-based bimodal size distribution in *Balistapus undulatus*. A *t*-test indicated mean length of males was significantly greater than that of females ($t = -6.359$, $DF = 58$, $P < 0.001$). There was no other evidence of sex change in *B. undulatus*. We did not see a central membrane-lined lumen in testes, nor did any gonad contain a mixture of ovarian and spermatogenic tissue. We classified *B. undulatus* as a gonochore.

Overall sex ratio in this *Balistapus undulatus* population was female-biased (Table 1, $\chi^2 = 27.258$, $DF = 1$, $P < 0.001$). Considering only mature individuals, the observed sex ratio was also female-biased ($\chi^2 = 10.246$, $DF = 1$, $P = 0.001$). However we did see size-specific sex ratios in the mature size classes; the sex ratio of mature individuals varied predictably with length (Fig. 20). An equation describing the percent of mature females (%♀), throughout the size range of mature specimens (Table 1, $r^2 = 0.978$), predicts that the population was male biased at body lengths > 16.6 cm TL. The percentage of females dropped to 6.8% at maximum observed size of 20.2 cm TL.

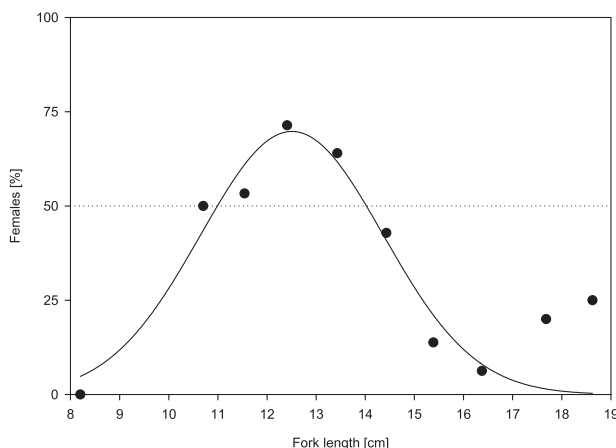


Fig. 16. Percentage of mature females, relative to all mature individuals, versus length for *Ctenochaetus striatus* from Papua New Guinea. The equation for the fitted curve is given in Table 1 ($r^2 = 0.796$, $F = 13.9$, $P = 0.004$)

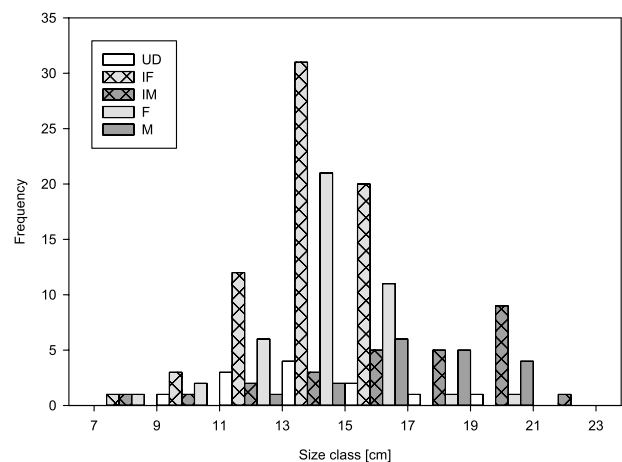


Fig. 17. Size-frequency plot of *Balistapus undulatus* specimens, with sex and maturity status determined by histological examination of gonads; UD = undifferentiated individuals, IF = immature females, IM = immature males, F = mature females, M = mature males

Linear regression analysis of log-transformed data produced a non-significant ($F = 2.520$, $n = 4$, $P = 0.253$), poorly descriptive ($r^2 = 0.558$) length-batch fecundity relation. Results suggest that batch fecundity is an exponential ($b > 6$) function of total length for females 13.2–16.2 cm (Table 1).

Figure 21 shows the relation between length and expected individual batch fecundity with observed overall sex ratios and with observed size-specific sex ratios. With an assumed constant sex ratio, expected per-individual egg production is greatest at maximum size. When size-

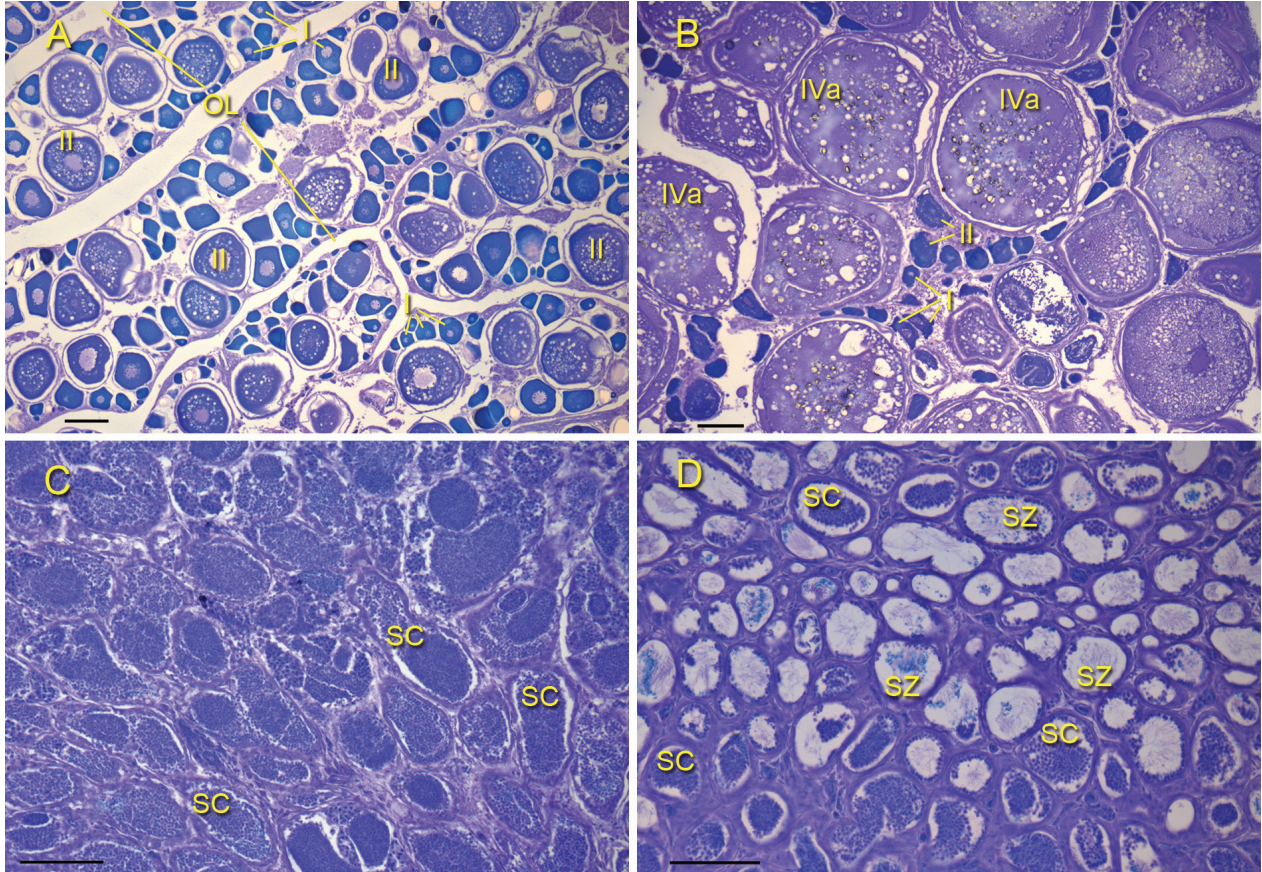


Fig. 18. Histological sections of gonads of orange-lined triggerfish, *Balistapus undulatus*, from Papua New Guinea: Ovary of immature female (A) (16.5 cm) containing primary-growth (I) and cortical vesicle (II) oocytes; Ovary of mature female (B) (18.2 cm) containing primary-growth (I), cortical vesicle (II), and final maturation (IVa) oocytes; Testis from an immature male (C) (20.4 cm) containing spermatogenic cysts (SC); Testis of a mature male (D) (16.8 cm) with spermatogenic cysts (SC) and tailed spermatozoa (SZ); OL = ovarian lumen; scale bars = 100 μ m

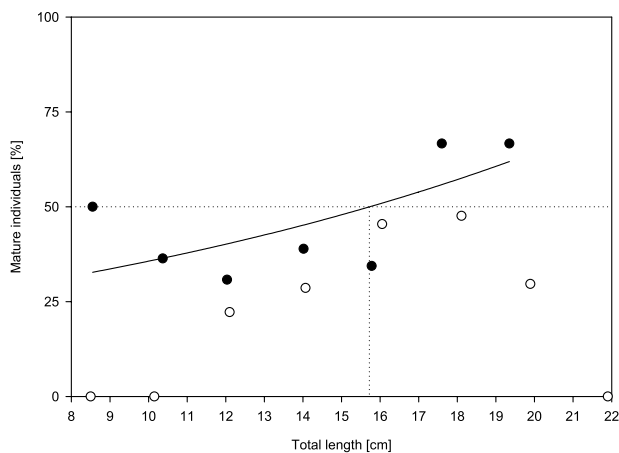


Fig. 19. Size-at-maturity (L_{50}) for *Balistapus undulatus* from Papua New Guinea; Females are represented by closed circles and the solid line ($r^2 = 0.409$, $F = 1.38$, $P = 0.394$), males are represented by open circles

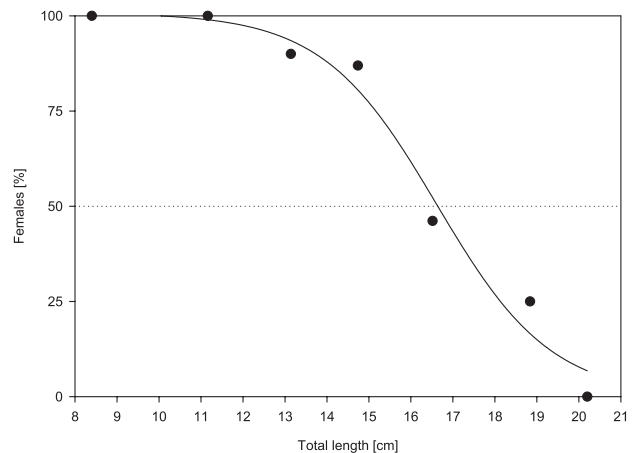


Fig. 20. Percentage of mature females, relative to all mature individuals, versus length for *Balistapus undulatus* from Papua New Guinea; The equation for the fitted curve is given in Table 1 ($r^2 = 0.978$, $F = 90.4$, $P < 0.001$)

specific sex ratios are considered, the small chance of an individual being a female eventually overwhelms increases in batch fecundity such that expected egg production per individual peaks at 16.6 cm TL, well below maximum observed length (Fig. 21, solid curve). Failing to account for size-specific sex ratios at the largest observed specimen size led to a 427 655-egg overestimate in expected individual batch fecundity.

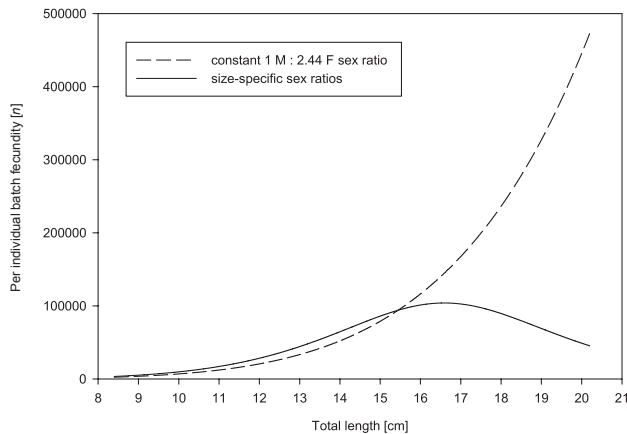


Fig. 21. The influence of size-specific sex ratios on population-level patterns of egg production: the overall sex-ratio (dashed curve) in *Balistapus undulatus* makes it appear as though the largest individuals are responsible for the majority of egg production, however smaller individuals are responsible for the majority of egg production when size-specific sex ratios are considered (solid curve)

DISCUSSION

Above, we presented a suite of reproductive parameters for five exploited reef-fish species, generated using Jungle Histology methods. These methods allow reproductive information to be generated quickly and at low cost, thus eliminating the perceived impediments to broad-scale reproductive analysis of Pacific coral-reef fishes. However, in the interest of rapidly increasing the amount of available reproductive information, Jungle Histology uses only one gonad subsample from the minimum number of specimens necessary to generate estimates of reproductive parameters. Therefore, results should be viewed as preliminary (Longenecker et al. 2014).

Despite the preliminary nature of these results, we think they are a useful contribution to the currently insufficient body of knowledge about reproduction in Pacific coral-reef fishes. Because microscopic examination of gametes yields a more-reliable evaluation of individual reproductive status than gross (i.e., macroscopic) or data-deficient methods (e.g., Froese and Binohlan 2000), we suggest that results of histological analysis are particularly necessary when evaluating the sustainability of reef-fishing practices. This is most convincingly demonstrated with our results for *Nemipterus isacanthus*, which were generated by the

subset of authors who developed the Jungle Histology methods, and were the most experienced at macroscopic evaluation of maturity. Our macroscopic evaluations were incorrect (wrong sex and/or reproductive status) for 62.6% of *N. isacanthus* specimens examined. Similar differences between histological and macroscopic results have been reported previously (Vitale et al. 2006, Grandcourt et al. 2011, Longenecker et al. 2013a, 2013c). Although Mackie and Lewis (2001) report that accuracy of macroscopic staging improves as personnel gain experience, they also state that misclassification rate exceeds 40% for many gonad stages, even when workers are experienced. In the case of *N. isacanthus*, our macroscopic misclassification errors would have led to L_{50} estimates of 18.9 cm FL for males and 20.9 cm FL for females. On the basis of our largest specimen (24.9 cm FL), the empirical equations of Froese and Binohlan (2000) predict that males mature at 14.5 cm FL and females mature at 16.8 cm FL. The results of our macroscopic analysis would indicate that 92.5% of the *N. isacanthus* catch we examined had not attained the size at female maturity, whereas the data-deficient approach (Froese and Binohlan 2000) would indicate that 75.7% of the catch had not attained female size at maturity. Thus, estimating L_{50} based on macroscopic analysis or the Froese and Binohlan equation would suggest that this *N. isacanthus* population is being unsustainably harvested (Froese 2004). However, histological analysis showed that only 21.5% of the catch was immature, and suggested that 100% of the catch was larger than female L_{50} . Thus, the results of our (presumably more-reliable) histological analysis suggest that this *N. isacanthus* population is being sustainably harvested.

These methods-based differences in the interpretation of fishing sustainability could have profound impacts on management strategies. The macroscopic and data-deficient approaches could lead fishery managers to impose fishing restrictions on the *Nemipterus isacanthus* population we studied, whereas the histological approach suggests that no management actions are necessary. Thus, the histological approach can help avoid the expense of unnecessary management actions that would make it more difficult for subsistence fishers to obtain food. Conversely, because of their increased accuracy, histological analyses may also enable managers to better identify stocks that are overexploited and institute effective management before the population declines to critical levels. For example, Vitale et al. (2006) found that macroscopic analysis of *Gadus morhua* stocks overestimated spawning stock by up to 35%, relative to histological analysis of gonads from the same population. For subsistence fishing communities in developing nations such as Papua New Guinea, the need to avoid unnecessary or inaccurate management action cannot be overstated.

Although several authors have shown that histological analysis is inherently more detailed/informative than macroscopic analyses (Mackie and Lewis 2001, Vitale et al. 2006), many continue to rely on macroscopic and gravimetric (gonadosomatic index) analyses because they

believe histological analysis to be too costly and labor intensive (Klibansky and Scharf 2015). We disagree. Using the simplified methods we have described (Longenecker et al. 2013a, 2013b, 2013c, 2014, 2016) histological analysis is fast and economical. Longenecker et al. (2013a) estimated the cost of consumable supplies at \$1.45 US per specimen. More recent record keeping demonstrates that total processing time is 30 min per specimen, including: whole-specimen length and weight measurements; excising and weighing whole gonads; and weighing, fixing, trimming, infiltrating, embedding, sectioning, staining, and microscopically evaluating gonad subsamples (note that these steps include the work necessary for both macroscopic and gravimetric analyses). The histological analysis portion requires only 17 minutes more, per specimen, than would be needed for macroscopic evaluation. When weighed against the possibility of instituting flawed management based on inaccurate macroscopic or data-deficient methods, we suggest the extra time and monetary investments are easily justified.

Another benefit of our rapid approach to reproductive analyses is the ability to identify emergent patterns. For instance, females of four of the five species in this study become less abundant as size class increases (*Nemipterus isacanthus*, *Parupeneus barberinus*, *Ctenochaetus striatus*, *Balistapus undulatus*). Similar patterns have been reported for confamilial species (Young and Martin 1985, Russell 1990, Lau and Sadovy 2001, Puentes Granada et al. 2004, Mant et al. 2006, Longenecker and Langston 2008, Langston et al. 2009, Boaden and Kingsford 2013, Amira et al. 2016, Pyle et al. 2016, Longenecker et al. 2016) and members of other families (Longenecker et al. 2014, 2016) subject to rapid reproductive analyses.

Although the pattern has been reported elsewhere, the impact of these size-specific sex ratios on which size classes are overwhelmingly responsible for population-level egg production is currently under-recognized (but see Longenecker et al. 2014, 2016). It has long been a common assumption that large fish are disproportionately responsible for population-level reproductive output because large females produce many more eggs than small females (see Roberts and Polunin 1993, Allison et al. 1998, Halpern 2003, Froese 2004, Birkeland and Dayton 2005, Sale et al. 2005). However, the assumption may not hold if females are rare in the largest size classes, and cannot hold if females are absent. Thus, the curves in Figs. 10 and 21 show the relation between length and expected individual batch fecundity with observed overall sex ratios and with observed size-specific sex ratios. Expected individual batch fecundity at a given length is the product of the result of the length-fecundity relation and the likelihood that a randomly chosen individual (at the given length) is a female. Thus the curves represent the mean batch fecundity of any individual at a given length and not just females. For both examples, when size-specific sex ratios are considered, the decreasing likelihood that an individual is female eventually overwhelms increases in batch fecundity

such that mean egg production per individual peaks well below maximum observed length (Figs. 10 and 21, solid curves).

The consequence of these patterns cannot be overstated. Assuming spawning frequency remains the same over the lifespan of an individual, considering size-specific sex ratios predicts that for *Parupeneus barberinus*, 50% of expected lifetime egg production occurs by the time an individual attains 17.6 cm FL, whereas if sex ratios are assumed to be constant, an individual would have to be 24 percentage points larger. More importantly, assuming that sex ratios are constant suggests that the largest individuals produce 22 100 eggs per spawning event, whereas when size-specific sex ratios are considered, none of the largest individuals are females and egg production would be expected to be zero. In fact, egg production would have stopped in all individuals 1.5 cm shorter than maximum observed specimen size. Similar results were obtained for *Balistapus undulatus*. When considering size-specific sex ratios, 50% of expected lifetime egg production occurs by the time an individual attains 16.2 cm TL, whereas an individual would have to be 13 percentage points larger to achieve the same reproductive output if sex ratios are assumed to be constant. Furthermore, failing to account for size-specific sex ratios at the largest observed specimen size led to a 10.4 X overestimate (473 003 vs. 45 348 eggs) in expected individual batch fecundity. The implications of size-specific sex ratios for fishery conservation and management are that, when females are rare or absent in the largest size classes, fishing at or near the maximal size for a species may have little impact on the overall reproductive output for the population. Furthermore, imposing slot limits that protect the largest size classes may actually redirect fishing pressure on the size classes of female fish that are collectively responsible for the majority of population-level reproductive output.

ACKNOWLEDGEMENTS

The Jungle Histology workshop was funded by a grant from NOAA's Saltonstall-Kennedy Grant Program pursuant to Project Number NA14NMF4270057. A private foundation, wishing to remain anonymous, provided financial support for work at KWMA. The Nago Island Mariculture and Research Facility provided in-kind support. We thank the fishers of Kavieng and the Kamiali Wildlife Management Area for collecting specimens (national-level approval included in wildlife export permit #015100). Shelley James provided logistical support, Sven Frijlink provided vessel support in Kavieng, and Bulisa Iova provided invaluable help obtaining an export permit. This is contribution 2017-001 of the Pacific Biological Survey, SOEST contribution 10021, and HIMB contribution 1687.

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Received: 23 December 2016

Accepted: 15 February 2017

Published electronically: 30 June 2017