Rapid Reproductive Analysis of Four Heavily Exploited Reef Fishes from Pohnpei State, Federated States of Micronesia

Ken Longenecker and Ross Langston

Honolulu, Hawaii
November 2016

COVER

One of approximately 30 fish markets operating on the island of Pohnpei, Federated States of Micronesia. Photo: Ken Longenecker.

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Pacific Biological Survey
Bishop Museum
Honolulu, Hawaii 96817, USA

Bishop Museum Technical Report 68

Honolulu, Hawaii
November 2016

Bishop Museum Press
1525 Bernice Street
Honolulu, Hawaiʻi

Printed in the United States of America

ISSN 1085-455X

Contribution No. 2016-004 to the Pacific Biological Survey

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EXECUTIVE SUMMARY

We used rapid, histology-based methods (*i.e.*, Jungle Histology) to describe length-weight relationships and reproductive parameters of four heavily exploited reef fishes from Pohnpei State, Federated States of Micronesia, for which reproductive information is scant or completely lacking: lined surgeonfish, *Acanthurus lineatus* (Linnaeus, 1758); epaulette surgeonfish, *Acanthurus nigricauda* Duncker & Mohr, 1929; humpback red snapper, *Lutjanus gibbus* (Forsskål, 1775); and humpnose big-eye bream, *Monotaxis grandoculis* (Forsskål, 1775). The weight of all four species is an approximately cubic function of length, and only in *M*. *grandoculis* did the relationship differ between sexes. All four species are batch-spawning gonochores with females maturing at a larger size than males. The fork length (FL) at which 50% of females are mature (L_{50}) is 16.8 cm for *A*. *lineatus*, 18.4 cm for *A*. *nigricauda*, 21.5 cm for *L*. *gibbus*, and 27.5 cm for *M*. *grandoculis*. Overall, the population of *L*. *gibbus* was female biased, whereas the sex ratio of the other species was not significantly different from 1 : 1. Considering only mature individuals, *L*. *gibbus* was again female biased, *A*. *lineatus* and *A*. *nigricauda* were male biased, and the *M*. *grandoculis* sex ratio was not significantly different from 1 : 1. For all species, the sex ratio of mature individuals changed with length. Females were rare or absent in the largest size classes of *A*. *nigricauda*, *L*. *gibbus*, and *M*. *grandoculis*, whereas the proportion of *A*. *lineatus* females increased with increasing length. Our histology-based results often greatly differed from those obtained by data-poor or gross (macroscopic) methods. In most cases, the latter methods would lead to a conclusion that all species are being overexploited. However, our histology-based results often suggested otherwise. Thus our Jungle Histology approach can help avoid the expense of unnecessary management actions that would make it more difficult for subsistence fishers to obtain food. We offer the following suggestions and observations should Pohnpei fishery managers deem regulations necessary for any of our study species:

- A 17-cm FL minimum-size restriction for *Acanthurus lineatus* would insure most females and virtually all males had the chance to reproduce. Adding a maximum-size limit may help to protect individuals responsible for the majority of population-level egg production. This is the only one of our study species for which a slot limit seems appropriate.
- A 19-cm FL minimum-size restriction for *Acanthurus nigricauda* would insure that most females and virtually all males had the chance to reproduce.
- A recently enacted minimum-size limit for *Lutjanus gibbus* corresponds to our histology-based L_{50} estimate of 21.5 cm FL and insures that most females and virtually all males had the chance to reproduce. If additional protection is deemed necessary, a minimum-size limit of 28-cm FL would protect virtually all reproductive females.
- A 28 cm FL minimum-size restriction for *Monotaxis grandoculis* would insure that most females and virtually all males had the chance to reproduce; however, a slightly larger minimum-size limit (31 cm FL) would protect a larger percentage of reproductive females.

INTRODUCTION

Problem

Subsistence and small-scale commercial fishing are important sources of food and income throughout the Pacific region (Roberts & Polunin 1993), and shallow-water coral-reef fishes form the majority of landings (*e.g.*, Tokeshi *et al.* 2013). However, with increases in human populations and more-efficient fishing technologies there is growing concern that coral-reef fisheries are being overexploited (Friedlander & DeMartini 2002, Pandolfi *et al.* 2003, McClanahan *et al.* 2008).

One of the most-easily understood concepts in fishery management and conservation is to harvest individuals 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 fishes (Froese & Binohlan 2000). This problem is especially acute for coral-reef fishes. The sheer diversity of coral-reef fishes, the supposed cost associated with the reproductive analysis of each species, and the lack of expertise and research infrastructure in developing Pacific Island nations (where most coral reefs are located) are often cited as barriers to obtaining this important base-line information (Roberts & Polunin 1993, Johannes 1998, Froese & Binohlan 2000). These problems hinder current abilities to effectively manage coral-reef fisheries in the Pacific.

Our Approach

To address these issues, Longenecker *et al.* (2013a) developed a portable method for rapid, low-cost, on-site, histology-based reproductive analysis that requires minimal research infrastructure. The method was developed while working in a subsistence fishing village in Papua New Guinea where we did not have access to many of the conveniences of a western-style laboratory (*e.g.*, because there was no electrical service in the village, we could not use most of the equipment typically found in a histology laboratory). The method has since become known by the epithet "Jungle Histology", to indicate the environment in which it was developed. With this method, reproductive information can be generated quickly, and its low cost eliminates one of the arguments against broad-scale reproductive analysis surveys. Importantly, the histological aspect allows for a high degree of certainty when assigning sex and maturity stages to individuals.

Other Approaches

Other methods designed to address the reproductive-information gap existed before our Jungle Histology approach. For instance, Froese & Binohlan (2000) developed empirically derived equations to estimate reproductive size. They report that maximum length is highly predictive ($r^2 = 0.905$) of size-at-maturity for the set of species used to develop the regression. The appeal of the this "data-poor" method, hereafter called F&B (for Froese & Binohlan, 2000), is that one only need to know the maximum size of a species to estimate its reproductive size. Thus, the F&B approach is extremely fast and inexpensive. F&B is increasingly being used to evaluate and model fisheries (Jennings *et al.* 2001, Cope & Punt 2009, Houk *et al.* 2012, Cengiz 2013, ElGanainy & Amin 2012).

Another common approach to reproductive analysis is to use gross (or macroscopic) gonad morphology to evaluate the reproductive status of an individual. Here, whole gonads are examined with the naked eye and compared to a standardized index. The advantage of this method is that it requires less equipment and is faster than histological analysis.

A Critique of Data-Poor and Macroscopic Techniques

We focus on histological examination because the F&B approach and gross (macroscopic) evaluation of gonads can introduce excessive error into estimates of reproductive parameters. We fully recognize the value of the F&B equation; it is far better to have an approximate reproductive size based on empirical evidence than to devise fishery management and conservation plans without reference to reproductive biology. However, the F&B equation tends to overestimate reproductive size for a small set of exploited reef fishes that we studied in Papua New Guinea (Longenecker *et al.* 2014a). Further, the degree of overestimation systematically increases with increasing maximum length (*i.e.*, the bigger the fish can get, the more its reproductive size is overestimated). Figure 1 demonstrates this phenomenon by comparing estimates of female L_{50} (the length at which 50% of individuals are mature) produced by microscopic examination of gonads to estimates produced by the F&B equation.

The impact to fishery management of the systematic error in the F&B equation is clearly demonstrated by our recent work (Longenecker *et al.* 2014a). Based on a maximum length of 50 cm (Carpenter 1998, Allen & Swainston 1993, Randall 2005), the estimated female reproductive size for

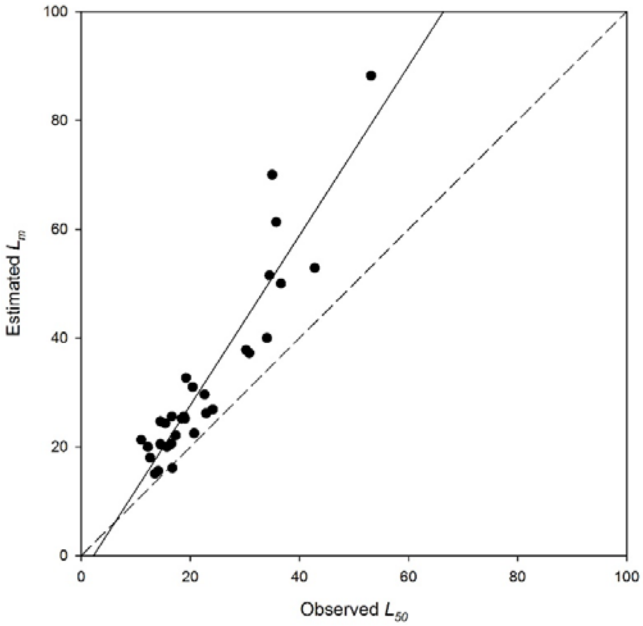

Figure 1. The data-poor method of Froese & Binohlan (2000), solid line, systematically overestimates female size-at-maturity of exploited reef fishes at Kamiali Wildlife Management Area, Papua New Guinea. The dashed line is a 1:1 reference. Sources (and species): Barba, 2010 (*Cetoscarus bicolor*, *Scarus niger*); Chan & Sadovy 2002 (*Cephalopholis boenak*); Cole 2008 (*Mulloidichthys vanicolensis*); Davis & West 1993 (*Lutjanus vitta*); Grandcourt *et al.* 2011 (*Diagramma pictum*); Howard 2008 (*Scarus rubroviolaceus*); Kritzer 2004 (*Lutjanus carponotatus*); Longenecker & Langston 2008 (*Parupeneus multifasciatus*); Longenecker *et al.* 2011 (*Lutjanus semicinctus*, *Siganus lineatus*); Longenecker *et al.* 2013a (*Lutjanus biguttatus*); Longenecker *et al.* 2013b (*Lutjanus fulvus*); Longenecker *et al.* 2013c (*Myripristis adusta*); Longenecker *et al.* 2014b (*Caesio cuning*, *Lethrinus erythropterus*); Longenecker *et al.* 2016 (*Parupeneus indicus*, *Rhinecanthus verrucosus*, *Scolopsis lineata*); Longenecker *et al.* in preparation (*Balistapus undulatus*, *Parupeneus barberinus*, *Kyphosus cinerascens*); Marriott *et al.* 2007 (*Lutjanus bohar*); Murty 2002 (*Epinephelus merra*); Rhodes *et al.* 2013 (*Plectropomus areolatus*); Russell & McDougall 2008 (*Lutjanus argentimaculatus*); Sudekum *et al.* 1991 (*Caranx melampygus*); Taylor & Choat 2014 (*Chlorurus microrhinos*); Taylor *et al.* 2014 (*Naso lituratus*, *N. unicornis*).

Caesio cuning is 27 cm. This estimate would lead to the conclusion that only 0.2% of the individuals in a study population had attained reproductive size and suggests that the population is acutely overfished (Froese 2004). However, histological analysis of *Caesio cuning* indicates the size at which 50% of females are mature (L_{50}) is 15 cm, and L_{50} for males is 13 cm (Longenecker *et al.* 2014). In reality, average length of the population (16 cm) is larger than female size-at-maturity and 77% of individuals had attained female L_{50}. These results suggest the *Caesio cuning* population is being sustainably fished (Froese 2004).

We saw similar results for *Nemipterus isacanthus* (Longenecker *et al.* in preparation). On the basis of our largest specimen (24.9 cm FL), the empirical equations of F&B predict that males mature at 14.5 cm FL and females mature at 16.8 cm FL. These results suggest that 75.7 % of the catch had not attained female size-at-maturity and thus that the population is being unsustainably harvested (Froese 2004). However, histological analysis indicates that 100% of the catch was larger than female L_{50}. The results of our (presumably more-reliable) histological analysis suggest that this *N. isacanthus* population is being sustainably harvested.

Gross (or macroscopic) evaluation of gonads can over- or underestimate reproductive size. Longenecker *et al.* (2013a) compared results from gross and histological reproductive analysis and found that reproductive status and/or sex was misclassified in 47% of *Lutjanus biguttatus* specimens examined. This level of error appears consistent; in a later study, 43% of *Lutjanus fulvus* specimens were misclassified (Longenecker *et al.* 2013b). Importantly, in both studies gross examinations led to overestimates of the number of mature females and underestimates of the number of mature males. These systematic errors underestimate female and overestimate male size-at-maturity. For instance, gross examination of *Diagramma pictum* gonads (Grandcourt *et al.* 2006) underestimated female size-at-maturity by 11% compared to results of histological examination of the same population (Grandcourt *et al.* 2011). If size-at-maturity is used as the basis for establishing a minimum-size limit, gross gonad examination can potentially lead to potentially unsustainable fisheries.

Gross gonad evaluation can also lead to overestimates of female size-at-maturity. In the case of *Nemipterus isacanthus* (Longenecker *et al.* in preparation), our macroscopic misclassification errors would have led to L_{50} estimates 20.9 cm FL for females, suggesting that 92.5 % of the *N. isacanthus* catch we examined had not attained the size at female maturity. Thus, estimating L_{50} based on gross analysis would suggest that this *N. isacanthus* population is being unsustainably harvested (Froese 2004). However, as indicated above, histological analysis suggests 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-poor approaches could lead fishery managers to impose fishing restrictions on the *Caesio cuning* and *Nemipterus isacanthus* populations 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 the Federated States of Micronesia, the need to avoid unnecessary or inaccurate management action cannot be overstated. The errors inherent in data-poor methods and gross (macroscopic) reproductive analysis highlight the urgent need for histology-based reproductive studies of Pacific reef fishes.

Purpose

The purpose of this study is to generate histology-based reproductive information for four heavily exploited reef fishes from Pohnpei State, Federated States of Micronesia, for which reproductive information is scant or lacking (Figure 2): *Acanthurus lineatus* (Linnaeus, 1758); *Acanthurus nigricauda* Duncker & Mohr, 1929; *Lutjanus gibbus* (Forsskål, 1775); and *Monotaxis grandoculis* (Forsskål, 1775). All four species are widespread in the Indo-Pacific region. The lined surgeonfish, *A. lineatus*, occurs from the east coast of Africa to the Marquesas Islands, French Polynesia; and from southern Japan to central New South Wales, Australia (Randall 2001). The epaulette surgeonfish, *A. nigricauda*, occurs from the east coast of Africa to the Tuamoto Archipelago, French Polynesia; and from the Ryukyu Islands, Japan, to the Great Barrier Reef, Australia (Randall 2001). The humpback red snapper, *L. gibbus*, occurs from the east coast of Africa to the Society Islands, French Polynesia; and from southern Japan to the Great Barrier Reef, Australia (Allen 1985). The humpnose big-eye bream, *M. grandoculis*, occurs from the east coast of Africa to Hawaii and southeastern Oceania; and from southern Japan to northern Australia (Carpenter & Allen 1989). A market-based survey of the Pohnpei coral-reef fishery (Rhodes *et al.* 2008) indicates that, by frequency of occurrence, *L. gibbus* ranks 2nd, *M. grandoculis* ranks 9th, *A. nigricauda* ranks 12th, and *A. lineatus* ranks 13th. All four species are considered to be common or very common in Pohnpei fish markets.

MATERIALS AND METHODS

We used methods modified from Longenecker *et al.* (in preparation) to examine length-weight relationships, size-at-maturity, sexual pattern, sex-ratios, and length-batch fecundity relationships. Additionally, we used methods described in Longenecker *et al.* (2014) to search for patterns of spawning periodicity.

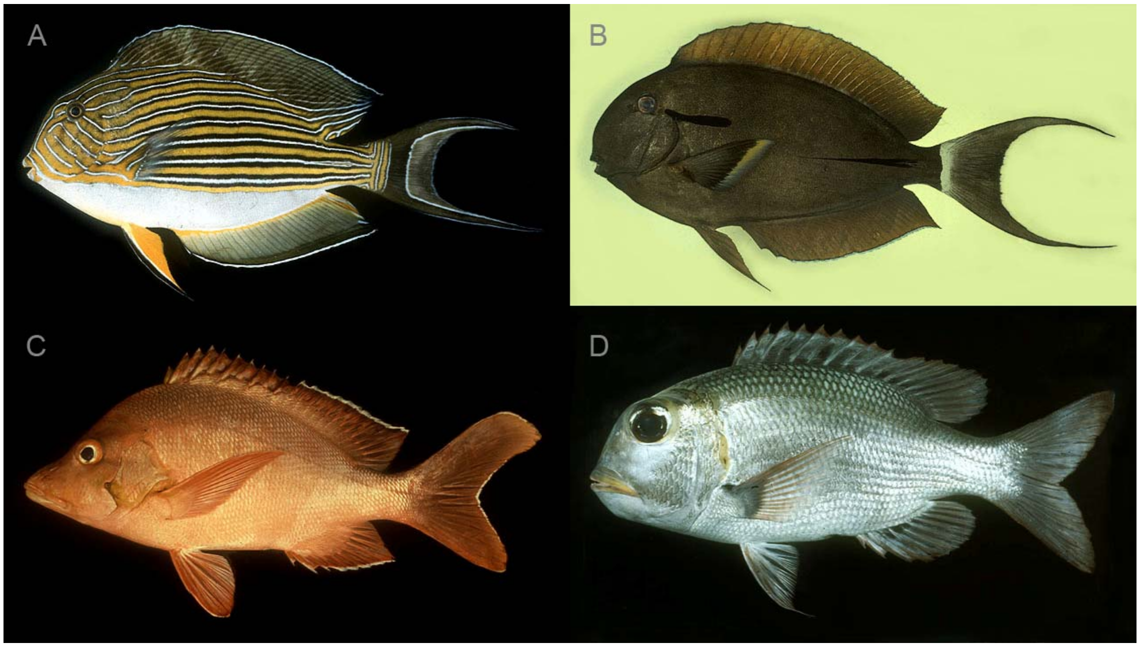

Figure 2. Study species. (A) *Acanthurus lineatus*, (B) *Acanthurus nigricauda*, (C) *Lutjanus gibbus*, (D) *Monotaxis grandoculis*. All images courtesy of J. Randall.

Specimen Acquisition and Whole Specimen Processing: All but seven specimens were purchased from fish markets on the island of Pohnpei, Federated States of Micronesia (Fig. 3). The remaining specimens were speared: one *Acanthurus lineatus* at Pakin Atoll; 3 *A. lineatus* near Palikir Pass, Pohnpei; and 3 *Monotaxis grandoculis* at Ant Atoll. Length, from the front of the head with mouth closed to the end of the middle caudal ray, was measured to 0.1 cm. Whole body weight was measured with the smallest-possible of three hanging spring-scales (100, 1000, or 2500 g capacity, with 1, 10, or 20 g increments, respectively). A mid-ventral incision was made from the vent through the pelvic girdle, sex and reproductive status (based on gross examination) were recorded, and then gonads were excised and weighed to 0.001 g on a portable jeweller's scale. For each ovary that appeared to be at or nearing maturity, 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 x 3 mm x 3 mm subsample was excised, placed in one well of a tissue culture plate, and fixed in Dietrich's solution for at least 24 hours.

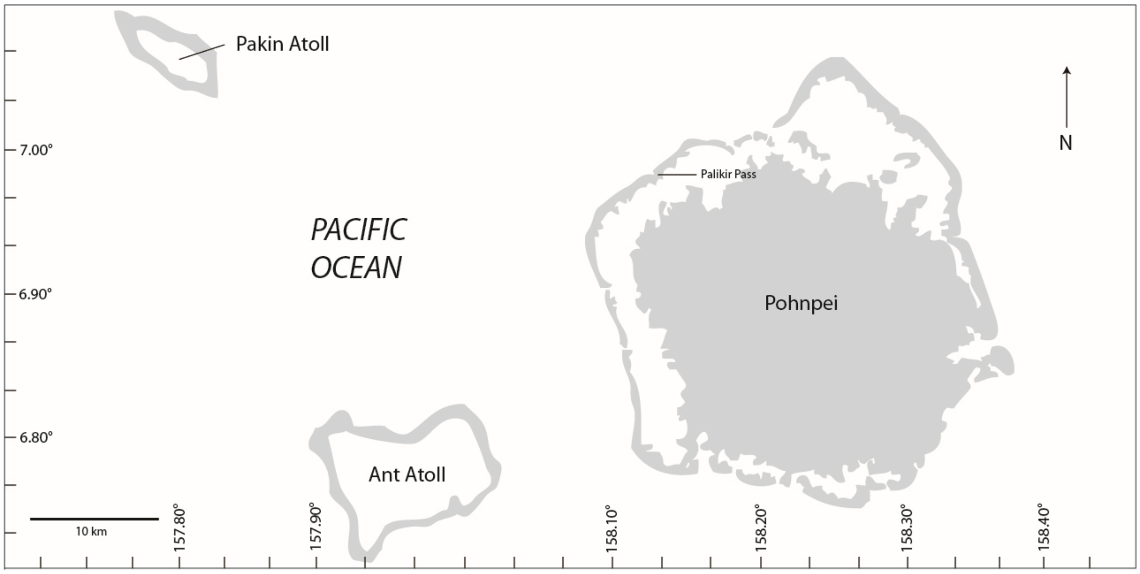

Figure 3. The study area in Pohnpei State, Federated States of Micronesia.

Size-at-Maturity, Sexual Pattern, and Sex Ratios: The Dietrich's-fixed gonad subsamples were trimmed to a volume of about 8 mm^3, then dehydrated in ethanol (30-60 min in each of 50%, and two changes of 95% ethanol). Using plastic embedding medium (JB4, Electron Microscopy Sciences) and following kit instructions, gonad sections were infiltrated in two changes of infiltration solution (1 hr and > 8 hrs, respectively), transferred into embedding capsules (BEEM®, size 00), and embedded. Because high humidity in tropical locations often prevents tissue blocks from hardening completely, tissue blocks were dehydrated for 12 hours in a "desiccating chamber" (a diver's dry box containing silica gel packets). 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 metal baking sheet warmed on an electric stove). Tissue sections (now affixed to the slides) were stained with either toluidine blue or hematoxylin and eosin (H & E). For the former, slides were immersed 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". For H & E staining, slides were immersed in Shandon^{TM} Instant Hematoxylin for 5 minutes, rinsed under running tap water for 2 minutes, destained with acid water (distilled vinegar diluted to 0.5 – 1% acetic acid content) for 20 seconds, rinsed under running tap water for 1 minute, immersed in Scott's tap-water substitute (3.5 g sodium bicarbonate and 20 g magnesium sulfate dissolved in 1 L distilled water) for 45 seconds, rinsed under running tap water for 3 minutes, immersed in Shandon^{TM} Instant Eosin-Y Aqueous for 3 minutes, rinsed under running tap water for 5 minutes, and dried on the "warmer". Ovary sections were examined at 100X and testis sections at 400X 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).

Batch Fecundity: Methods modified from Agger *et al.* (1974) were used to estimate batch fecundity. Ovarian samples reserved for batch-fecundity analysis (above) were preserved in Gilson's Fluid and 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, W_s is the sample weight.

Gonadosomatic Index: We used a gonadosomatic index (GSI) as a proxy for spawning state.

$$GSI = 100GS^{-1}$$

where: *G* is total gonad weight and *S* is somatic body weight (total body weight minus total gonad weight). We only produced GSI values for histologically confirmed mature individuals. We assumed that higher GSI values indicated spawning readiness.

Data Analysis: We constructed length–weight relationships (LWR) using log-transformed data and following the guidance of Froese *et al.* (2011). We considered any data point with a residual > 0.1 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 average length of individuals within a size class) indicates 50% of individuals are mature. We used 1-cm size classes for *Acanthurus lineatus*, *A. nigricauda*, and *Lutjanus gibbus*. We used 5-cm size classes for *Monotaxis grandoculis*. We assigned one-half of any 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 average 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 relationships between length and batch fecundity using linear regression analysis of log-transformed data. We plotted mean (± standard deviation) GSI values versus date to search for patterns of reproductive periodicity.

RESULTS

A summary of length-weight relationships and reproductive information for all four study species is presented in Table 1. More, detailed information is presented in separate species accounts, below.

Acanthurus lineatus

Analysis of covariance (ANCOVA) did not detect a significant sex-based difference in the length-weight relationship for this population (F = 0.06, DF = 1, P = 0.813). Total body weight (W) in g is an approximately cubic function of fork length (FL) in cm (Table 1, Figure 4). The 95% CI of regression parameters a and b are 0.0256–0.0603 and 2.74–3.05, respectively (r^2 = 0.910, n = 142, FL range: 14.0–20.5, W range: 84–240).

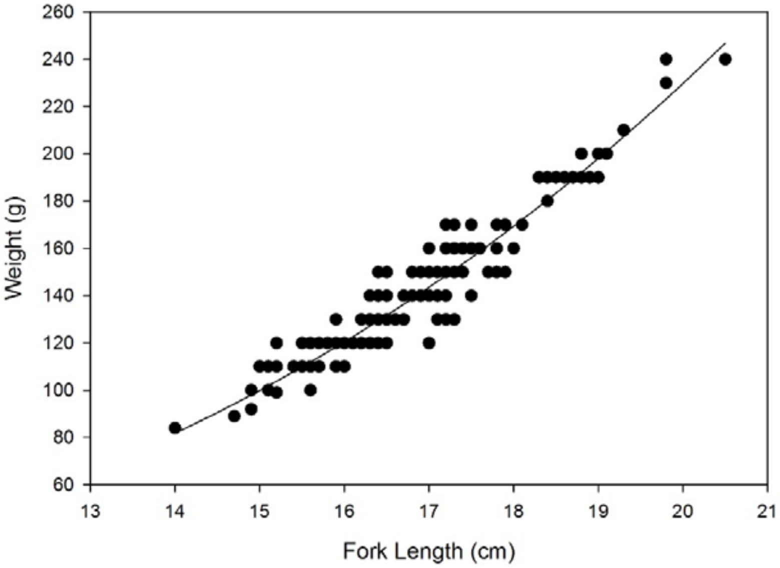

Figure 4. Relationship between length and weight for *Acanthurus lineatus* from Pohnpei State, Federated States of Micronesia.

We histologically examined gonads of 68 male and 59 female *Acanthurus lineatus*. Figure 5 shows examples of immature ovaries 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 classify *A. lineatus* as a batch spawner. Ovaries contained vitellogenic oocytes in females as small as 15.2 cm FL. Inactive and immature females (range 14.0-19.8) were smaller than, or scattered throughout the size range of, mature females (range 15.2-20.5). We estimate female L_{50} at 16.8 cm FL (Fig. 6). The smallest male with spermiated testes was 15.1 cm FL. We observed only two inactive or immature males (15.9 and 16.8 cm FL); both were within the size range of mature males (range 15.1-19.8). 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 averaged 97.1% (Fig. 6).

We did not see evidence of a sex-based bimodal size distribution in *Acanthurus lineatus*. Mean lengths of males and females were 169.7 and 168.9 cm, respectively. Our data did not meet the underlying assumptions for parametric statistical tests. Further, length distributions appeared to differ between sexes. Therefore, we tested for median differences using Mood's Median Test (χ^2 = 1.05, DF = 1, P = 0.306). Nor was there 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 classify *A. lineatus* as a gonochore.

Table 1. Reproductive parameters for four exploited reef fishes from Pohnpei State, Federated States of Micronesia. LWR = length-weight relationship, *L_m* = minimum mature length (the smallest mature individual observed during this study), *L_{50}* = length at 50% maturity (50% of all individuals at this length are expected to be mature).

	Acanthurus lineatus	*Acanthurus nigricauda*	*Lutjanus gibbus*	*Monotaxis grandoculis*
LWR (overall)	$W = 0.0393(\mathrm{FL})^{2.90}$	$W = 0.0346(\mathrm{FL})^{2.92}$	$W = 0.0286(\mathrm{FL})^{2.90}$	$W = 0.0394(\mathrm{FL})^{2.86}$
LWR (♂)	-----	-----	-----	$W = 0.0440(\mathrm{FL})^{2.84}$
LWR (♀)	-----	-----	-----	$W = 0.0534(\mathrm{FL})^{2.77}$
♂L_m	15.1	16.3	20.8	19.5
♀L_m	15.2	15.5	20.5	18.1
♂L_{50}	< 15.6	< 16.6	< 20.9	< 19.5
♀L_{50}	16.8	18.4	21.5	27.5
Sexual pattern	gonochore	gonochore	gonochore	gonochore
Oocyte development	group synchronous	group synchronous	group synchronous	group synchronous
Sex ratio (overall) ♂ : ♀	1 : 0.88	1 : 0.74	1 : 2.03	1 : 0.88
Sex ratio (mature) ♂ : ♀	1 : 0.44	1 : 0.28	1 : 1.68	1 : 0.62
Size-specific sex ratio	$\% ♀ = \frac{47890522.77}{1+e^{-\left(\frac{\mathrm{FL}-56.19}{2.72}\right)}}$	$\% ♀ = 50.00e^{-19.52(\mathrm{FL})} + 13281854.01e^{-0.76(\mathrm{FL})}$	$\% ♀ = 94.07e^{\left(-0.5\left(\frac{\mathrm{FL}-24.57}{2.40}\right)^2\right)}$	$\% ♀ = 60.41e^{\left(-0.5\left(\frac{\mathrm{FL}-24.97}{9.24}\right)^2\right)}$

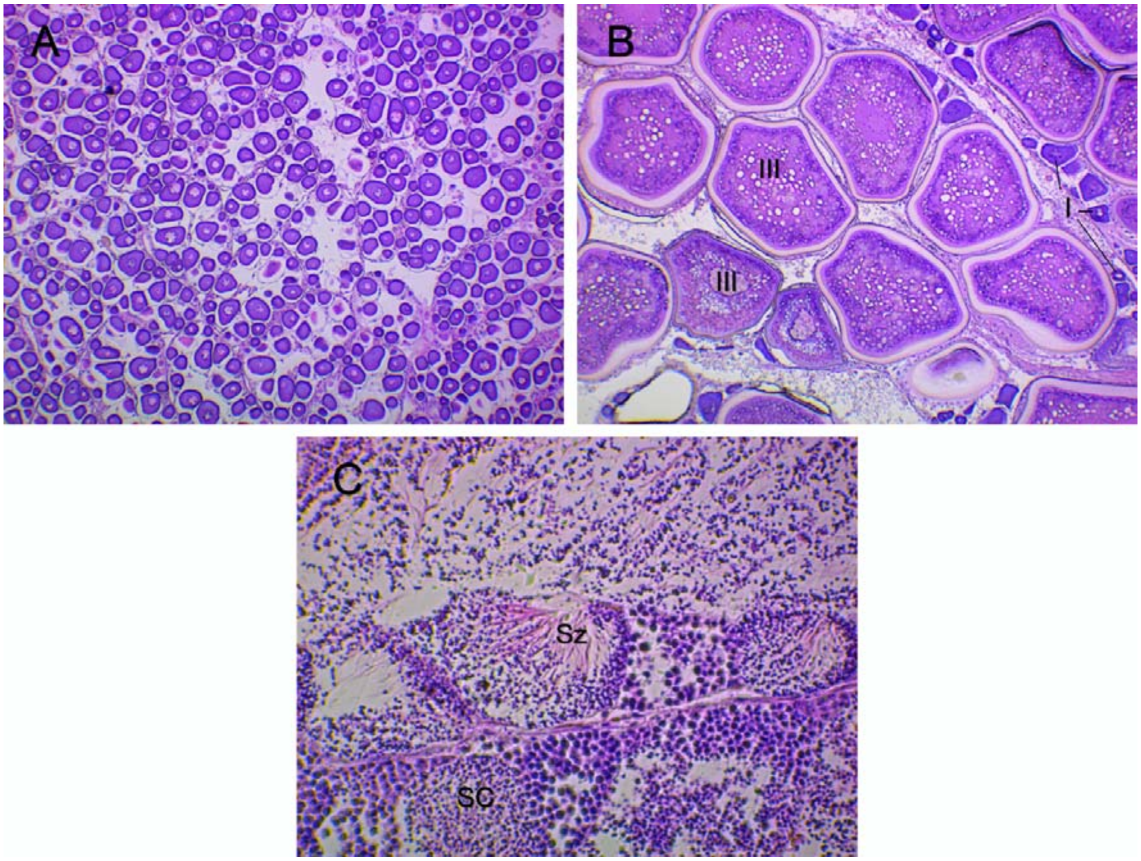

Figure 5. Photomicrographs of *Acanthurus lineatus* gonads. (A) 16.9 cm FL immature female containing only primary-growth oocytes, 100X; (B) 18.3 cm FL mature female with primary-growth (I) and vitellogenic (III) oocytes, 100X; (C) 17.5 cm FL mature male containing spermatogenic cysts (SC) and tailed spermatozoa (Sz), 400X. All slides stained with hematoxylin and eosin.

Overall sex ratio in this *Acanthurus lineatus* population is not significantly different from 1 male : 1 female (Table 1, $\chi^2 = 0.500$, DF = 1, $P = 0.480$). Considering only mature individuals, the observed sex ratio is male-biased (Table 1, $\chi^2 = 14.411$, DF = 1, $P = 0.0001$). However, we saw size-specific sex ratios in the mature size classes; the sex ratio of mature individuals varied predictably with length. Smaller size classes are male biased, and the largest size classes are female biased (Fig. 7). An equation describing the percent of mature females (% ♀), throughout the size range of mature specimens (Table 1, $r^2 = 0.947$), predicts that the population is female biased at lengths ≥ 18.7 cm FL.

We had too few female *Acanthurus lineatus* with stage IV oocytes to construct a length-batch fecundity relationship. However, batch fecundity averaged 48,845 eggs in two females with a mean length of 18.7 cm FL.

We generated GSI values for 62 male and 27 female *Acanthurus lineatus*. Specimens were collected from one day after the full moon to three days after the new moon. Figure 8 shows that GSI values for both sexes were lowest just after the quarter moon, and that values were generally highest around the full and new moons.

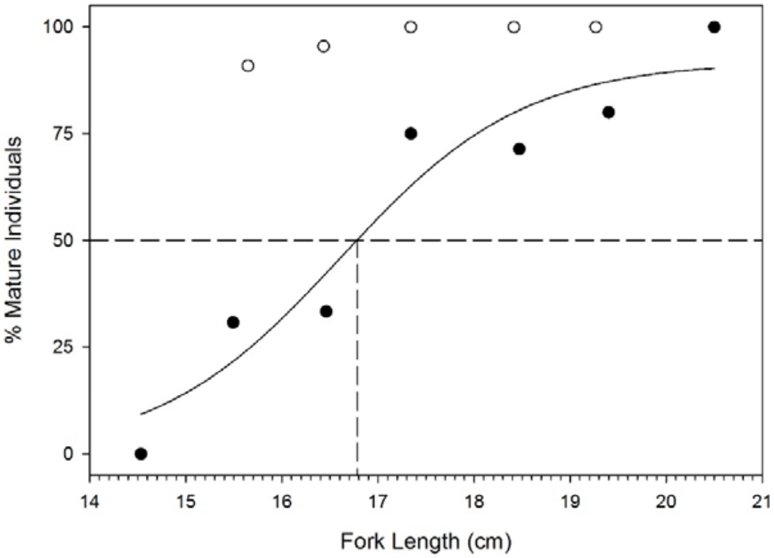

Figure 6. Size-at-maturity (*L_{50}*) for *Acanthurus lineatus* from Pohnpei State, Federated States of Micronesia. Females are represented by closed circles and the solid line, males are represented by open circles.

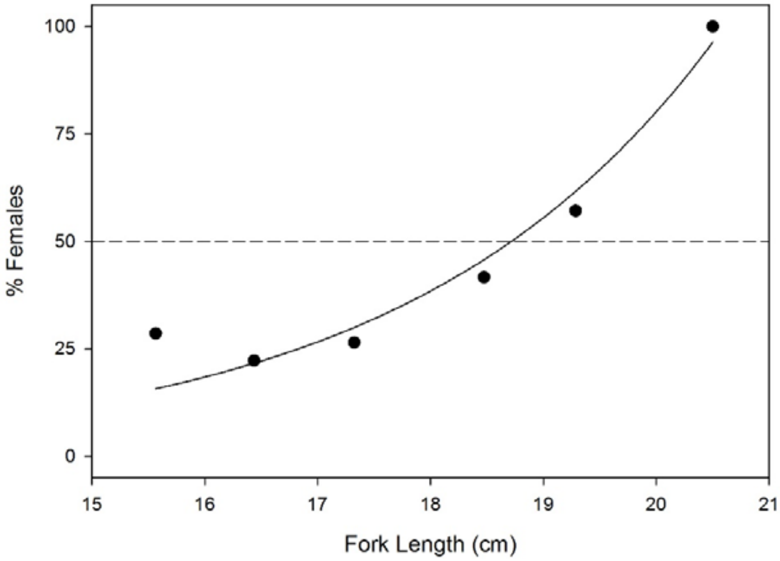

Figure 7. Percentage of mature females, relative to all mature individuals, versus length for *Acanthurus lineatus* from Pohnpei State, Federated States of Micronesia.

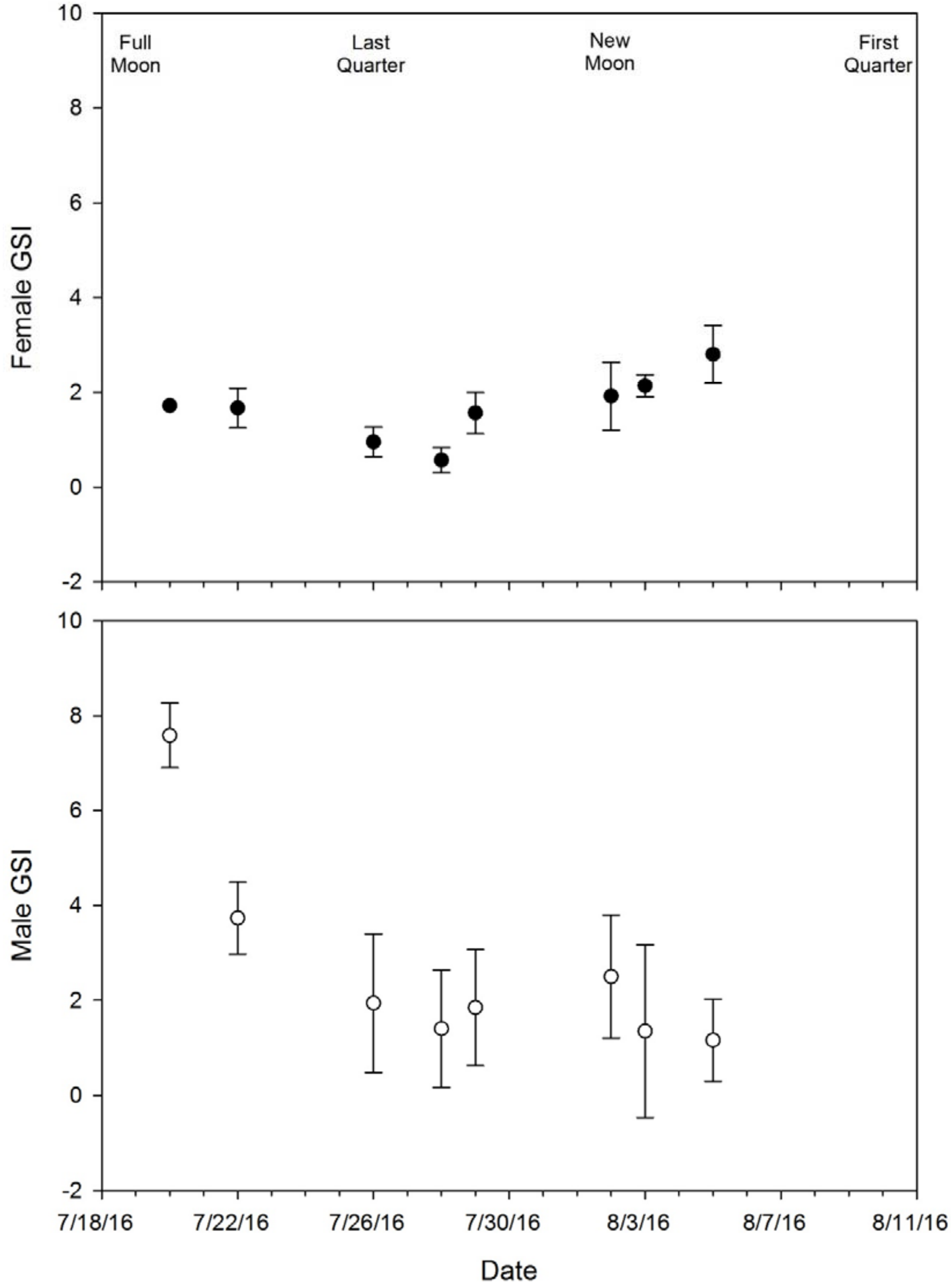

Figure 8. Mean (± standard deviation) gonadosomatic index values by date for *Acanthurus lineatus* from Pohnpei State, Federated States of Micronesia. Moon phases are indicated in the top (female) panel.

Acanthurus nigricauda

ANCOVA did not detect a significant sex-based difference in the length-weight relationship for this population ($F = 0.02$, DF = 1, $P = 0.879$). Total body weight (W) in g is an approximately cubic function of fork length (FL) in cm (Table 1, Figure 9). The 95% CI of regression parameters a and b are 0.0178–0.0673 and 2.69–3.15, respectively ($r^2 = 0.920$, $n = 60$, FL range: 15.5–22.6, W range: 100–320).

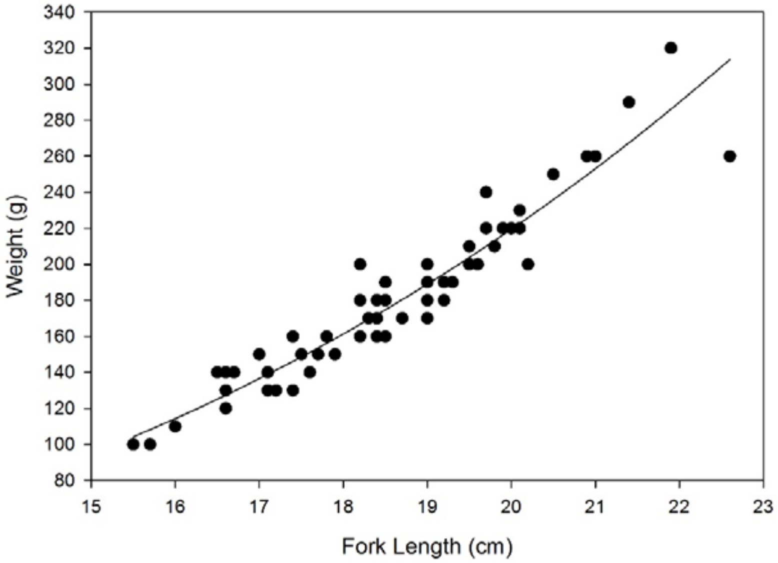

Figure 9. Relationship between length and weight for *Acanthurus nigricauda* from Pohnpei State, Federated States of Micronesia.

We histologically examined gonads of 19 male and 14 female *Acanthurus nigricauda*. Figure 10 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 classify *A. lineatus* as a batch spawner. Ovaries contained vitellogenic oocytes in females as small as 15.5 cm FL. Inactive and immature females (range 16.1-18.4) were scattered throughout the size range of mature females (range 15.5-19.0). We had too few ovaries to confidently determine female L_{50}, however we tentatively estimate it at 18.4 cm FL (Fig. 11). This estimate ignores the single (mature) female in the smallest size class. The smallest male with spermiated testes was 16.3 cm FL. We observed only one inactive or immature male (19.8 cm FL); it was in the middle of the size range of mature males (range 16.3-21.9). 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 averaged 94.7% (Fig. 11).

We saw a sex-based bimodal size distribution in *Acanthurus nigricauda*. A t-test indicated mean length of males (189.9) is significantly greater than that of females (172.2 cm) ($t = –3.62$, DF = 31, $P = 0.001$). 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 classify *A. nigricauda* as a gonochore.

Overall sex ratio in this *Acanthurus nigricauda* population is not significantly different from 1 male : 1 female (Table 1, $\chi^2 = 0.758$, DF = 1, $P = 0.384$). Considering only mature individuals, the observed

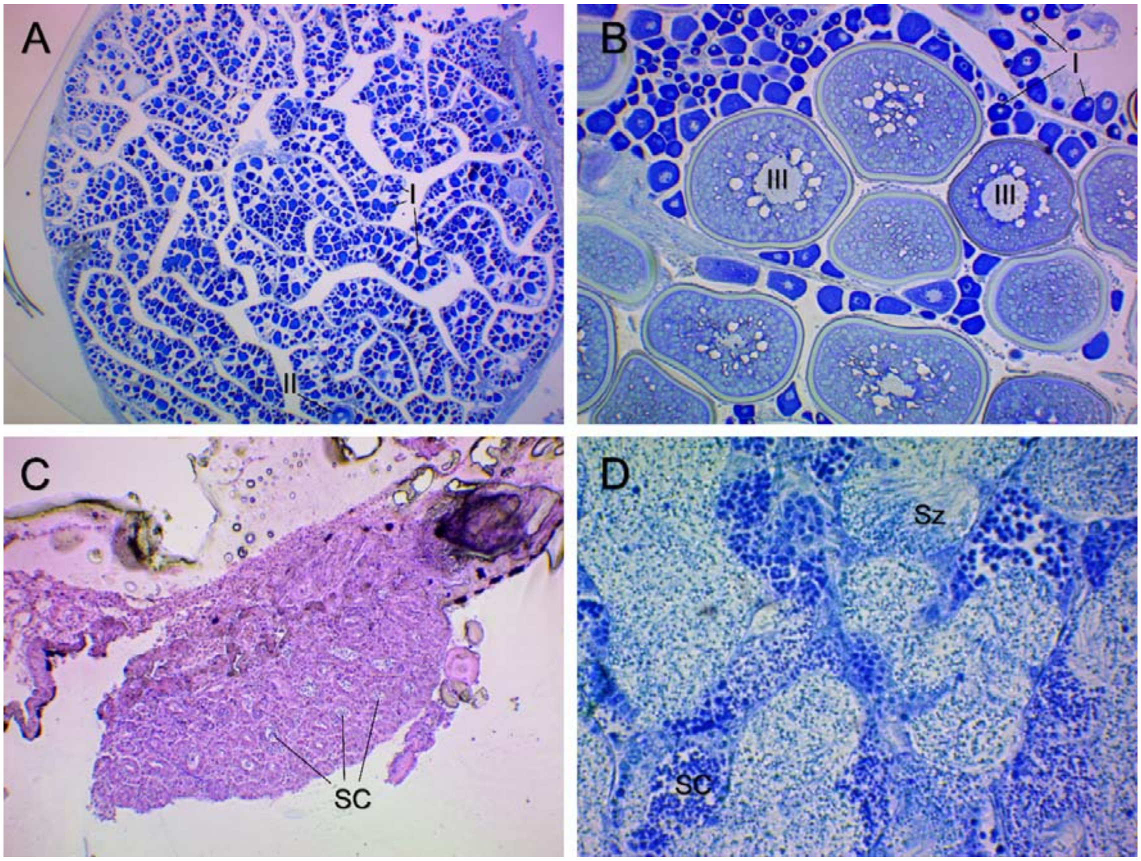

Figure 10. Photomicrographs of *Acanthurus nigricauda* gonads. (A) 16.6 cm FL immature female containing primary-growth (I) and cortical vesicle (II) oocytes, 40X; (B) 19.0 cm FL mature female with primary-growth (I) and vitellogenic (III) oocytes, 100X; (C) 19.8 cm FL immature male containing spermatogenic cysts (SC), 100X; (D) 18.2 cm FL mature male containing spermatogenic cysts (SC) and tailed spermatozoa (Sz), 400X. Slides A, B, and D stained with toluidine blue; slide C stained with hematoxylin and eosin.

sex ratio is male-biased (Table 1, $\chi^2 = 7.348$, DF = 1, $P = 0.007$). Further, although our sample size is very small (23 individuals) we saw size-specific sex ratios in the mature size classes; the sex ratio of mature individuals varied predictably with length. The smallest size classes are female biased, and larger size classes are male biased (Fig. 12). An equation describing the percent of mature females (% ♀), throughout the size range of mature specimens (Table 1, $r^2 = 0.913$), predicts that the population is male biased at lengths $\geq$ 16.3 cm FL, that less than 25% of individuals are female at lengths $\geq$ 17.3 cm FL, and that less than 1 % of individuals are female at lengths $\geq$ 20.9 cm FL.

We had too few female *Acanthurus nigricauda* with stage IV oocytes to construct a length-batch fecundity relationship. However, batch fecundity averaged 20,908 eggs in two females with a mean length of 17.0 cm FL.

We generated GSI values for 10 male and 4 female *Acanthurus nigricauda*. Specimens were collected from two days after the full moon to four days after the new moon. Although our sample size is very small, Figure 13 suggests that GSI values for both sexes were lowest just after the quarter moon.

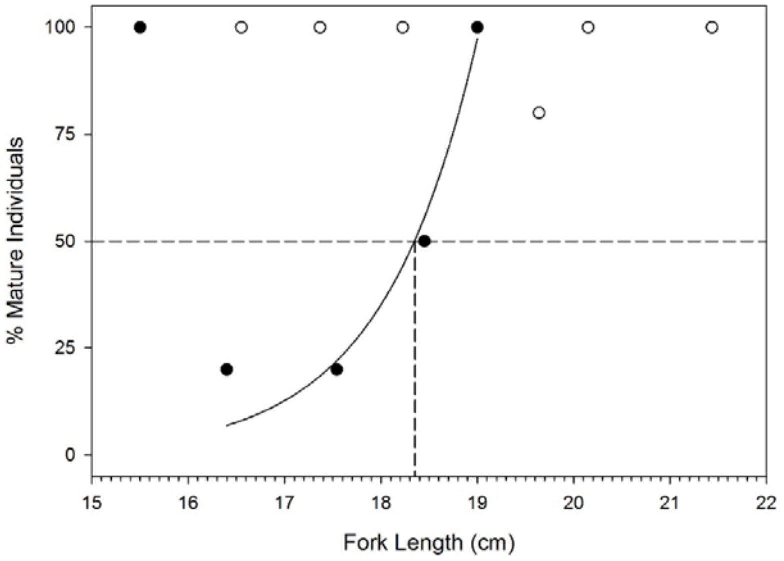

Figure 11. Size-at-maturity (*L_{50}*) for *Acanthurus nigricauda* from Pohnpei State, Federated States of Micronesia. Females are represented by closed circles and the solid line, males are represented by open circles.

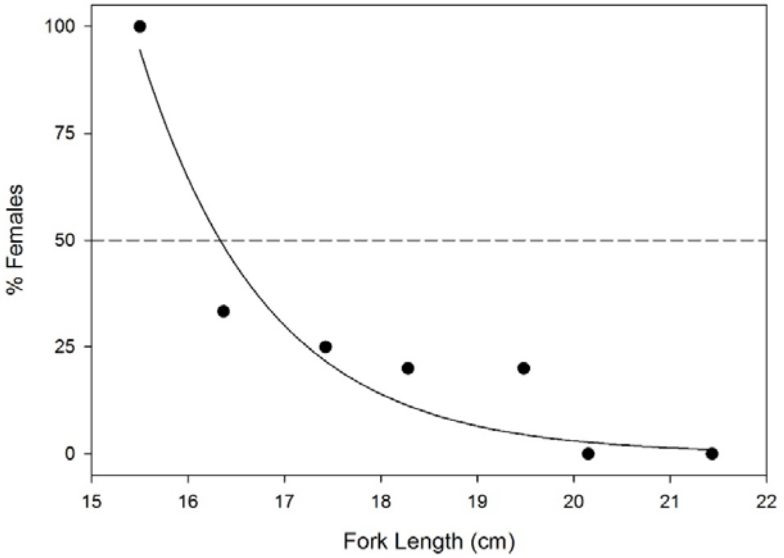

Figure 12. Percentage of mature females, relative to all mature individuals, versus length for *Acanthurus nigricauda* from Pohnpei State, Federated States of Micronesia.

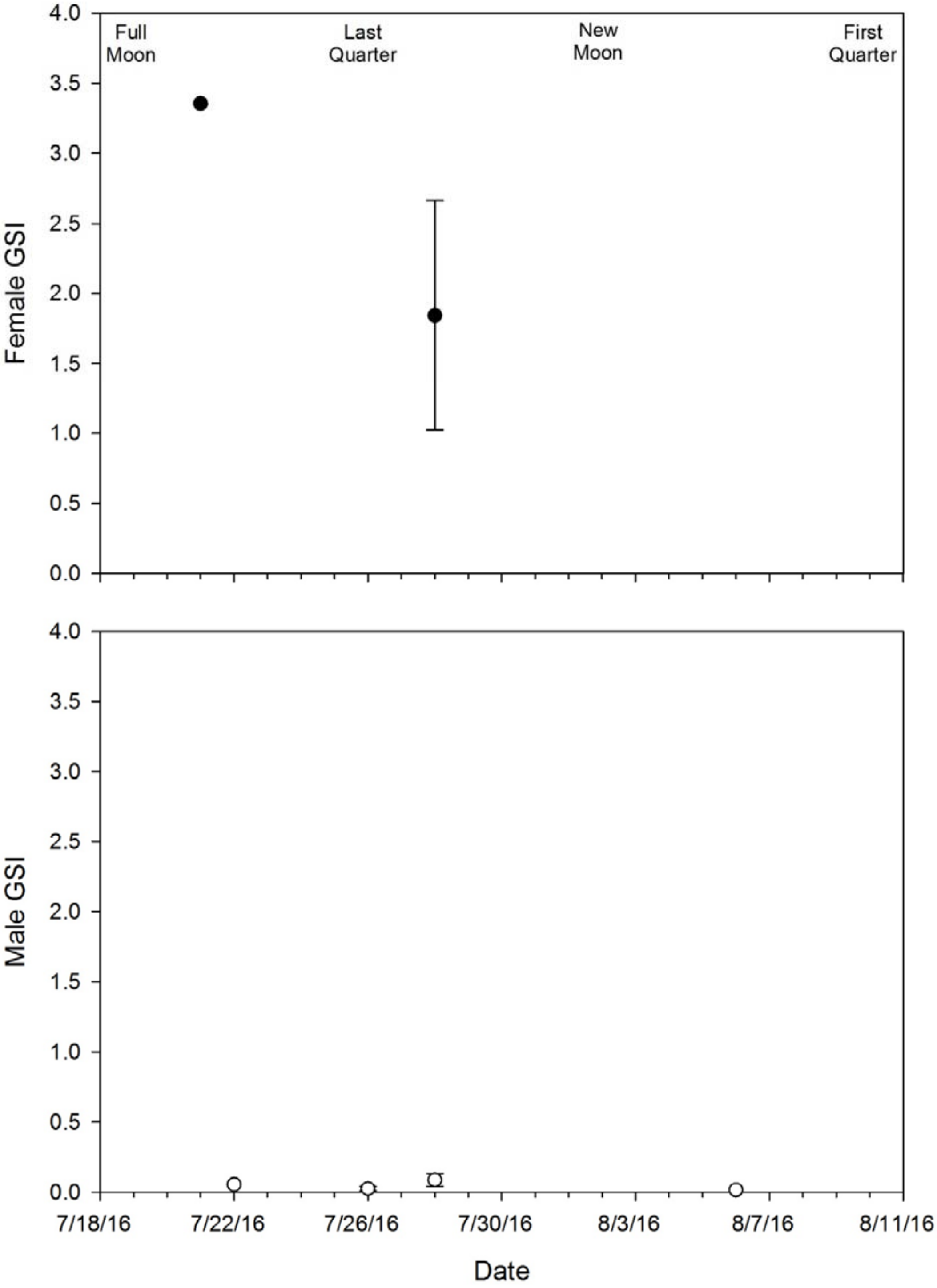

Figure 13. Mean (± standard deviation) gonadosomatic index values by date for *Acanthurus nigricauda* from Pohnpei State, Federated States of Micronesia. Moon phases are indicated in the top (female) panel.

Lutjanus gibbus

ANCOVA did not detect a significant sex-based difference in the length-weight relationship for this population (F = 1.31, DF = 1, P = 0.255). Total body weight (W) in g is an approximately cubic function of fork length (FL) in cm (Table 1, Figure 14). The 95% CI of regression parameters a and b are 0.0208–0.0394 and 2.80–3.00, respectively (r^2 = 0.969, n = 109, FL range: 19.8–33.5, W range: 150–760).

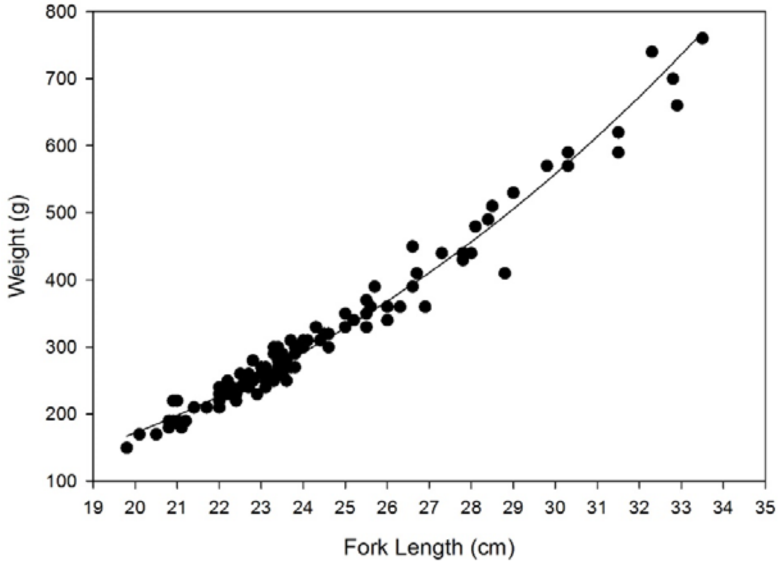

Figure 14. Relationship between length and weight for *Lutjanus gibbus* from Pohnpei State, Federated States of Micronesia.

We histologically examined gonads of 31 male and 63 female *Lutjanus gibbus*. All specimens were ≥ 19.8 cm FL. 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). We therefore classify *A. lineatus* as a batch spawner. Ovaries contained vitellogenic oocytes in females as small as 20.5 cm FL. Inactive and immature females (range 19.8-28.0) were scattered throughout, and slightly exceeded, the size range of mature females (range 20.5-27.3). We estimate female L_{50} at 21.5 cm FL (Fig. 16). This estimate ignores the single (immature) female in the largest size class. The smallest male with spermiated testes was 20.8 cm FL. We observed only three inactive or immature males (23.4-25.0 cm FL) all within the size range of mature males (range 20.8-33.5). We could not generate a satisfactory L_{50} curve for males. The percent of mature individuals was ≥ 50% for all size classes sampled and averaged 90.3% (Fig. 16).

Although the mean length of males (259.5 cm) and females (237.4 cm) differed by more than 20 cm, we did not see evidence of a sex-based bimodal size distribution in *Lutjanus gibbus*. Our data did not meet the underlying assumptions for parametric statistical tests. Further, length distributions appeared to differ between sexes. Therefore, we tested for median differences using Mood's Median Test (χ^2 = 0.43, DF = 1, P = 0.510). Nor was there 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 classify *L. gibbus* as a gonochore.

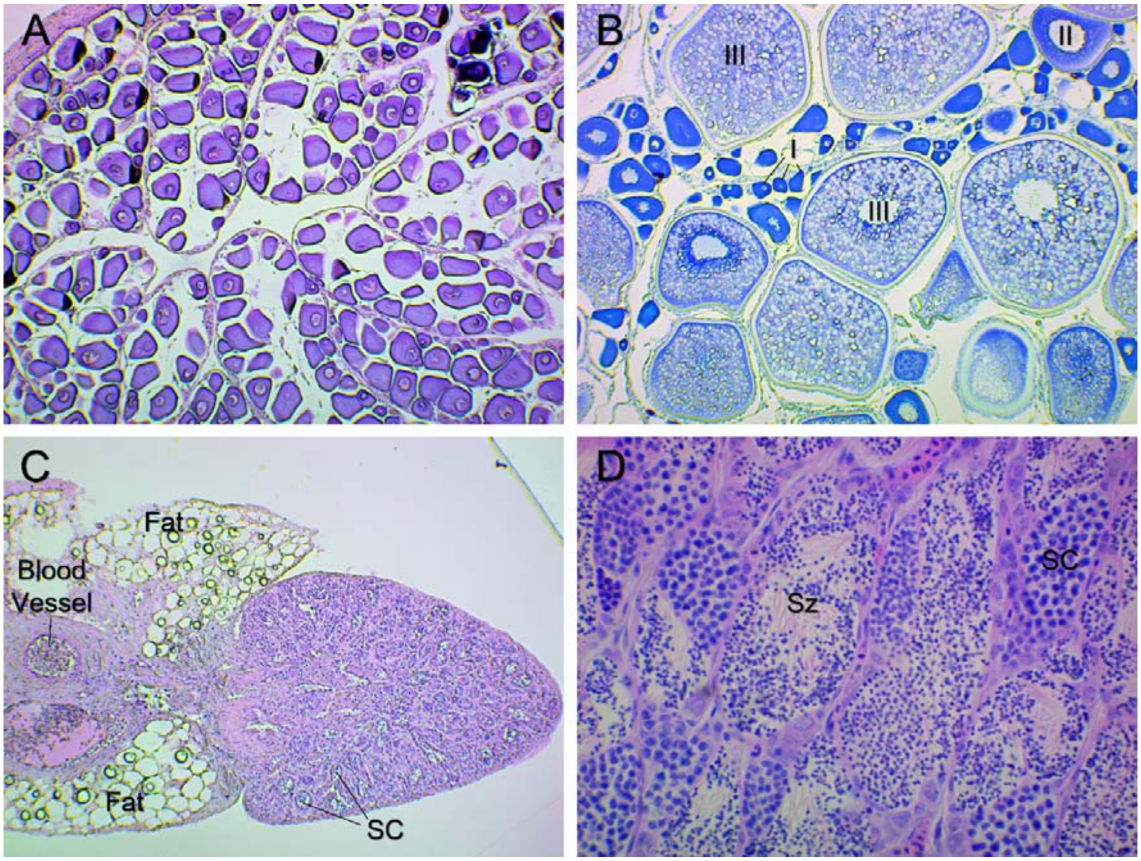

Figure 15. Photomicrographs of *Lutjanus gibbus* gonads. (A) 22.4 cm FL immature female containing only primary-growth oocytes, 100X; (B) 22.5 cm FL mature female with primary-growth (I), cortical vesicle (II), and vitellogenic (III) oocytes, 100X; (C) 23.6 cm FL immature male containing spermatogenic cysts (SC), 100X; (D) 22.4 cm FL mature male containing spermatogenic cysts (SC) and tailed spermatozoa (Sz), 400X. Slides A, C, and D stained with hematoxylin and eosin; slide B stained with toluidine blue.

Overall sex ratio in this *Lutjanus gibbus* population is female biased (Table 1, $\chi^2 = 10.894$, DF = 1, $P = 0.001$). Considering only mature individuals, the observed sex ratio is also female-biased (Table 1, $\chi^2 = 4.813$, DF = 1, $P = 0.028$). However, the sex ratio of mature individuals varied predictably with length. Smaller size classes are male biased, switch to a female-biased state as length increases, and the largest size classes are male biased (Fig. 17). An equation describing the percent of mature females (% ♀), throughout the size range of mature specimens (Table 1, $r^2 = 0.879$), predicts that the population is female biased between 21.9 and 27.2 cm FL, and that the percentage of females is effectively zero (< 1 %) at fork lengths ≥ 31.8 cm.

We had too few female *Lutjanus gibbus* with stage IV oocytes to construct a length-batch fecundity relationship. However, batch fecundity averaged 16,023 eggs in two females with a mean length of 24.1 cm FL.

We generated GSI values for 24 male and 45 female *Lutjanus gibbus*. Specimens were collected from one day after the full moon to three days after the new moon. Figure 18 shows that GSI values for both sexes are highest around the new moon.

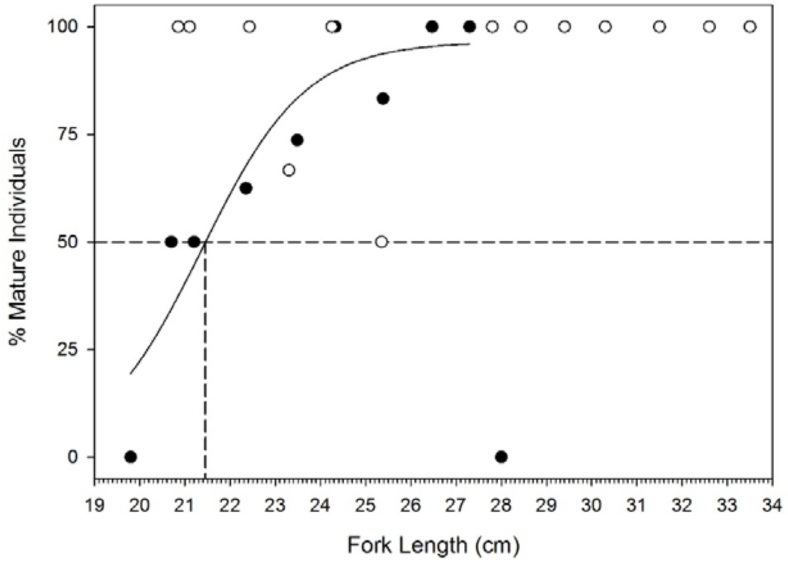

Figure 16. Size-at-maturity (L_{50}) for *Lutjanus gibbus* from Pohnpei State, Federated States of Micronesia. Females are represented by closed circles and the solid line, males are represented by open circles.

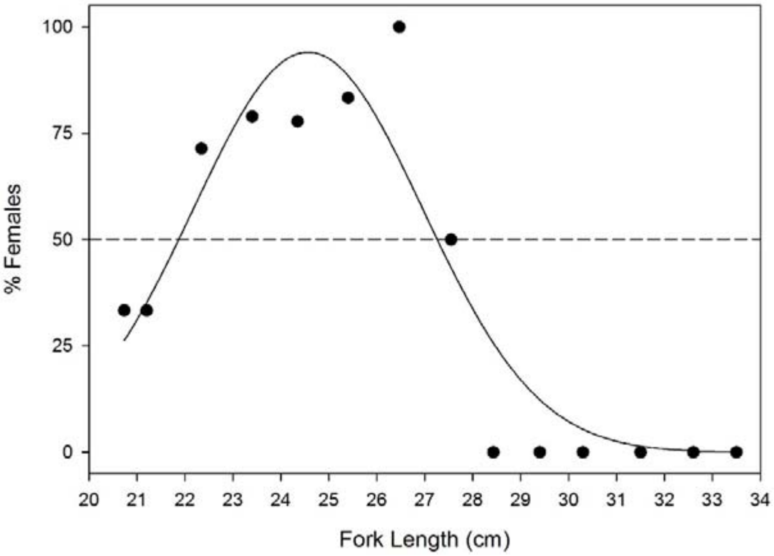

Figure 17. Percentage of mature females, relative to all mature individuals, versus length for *Lutjanus gibbus* from Pohnpei State, Federated States of Micronesia.

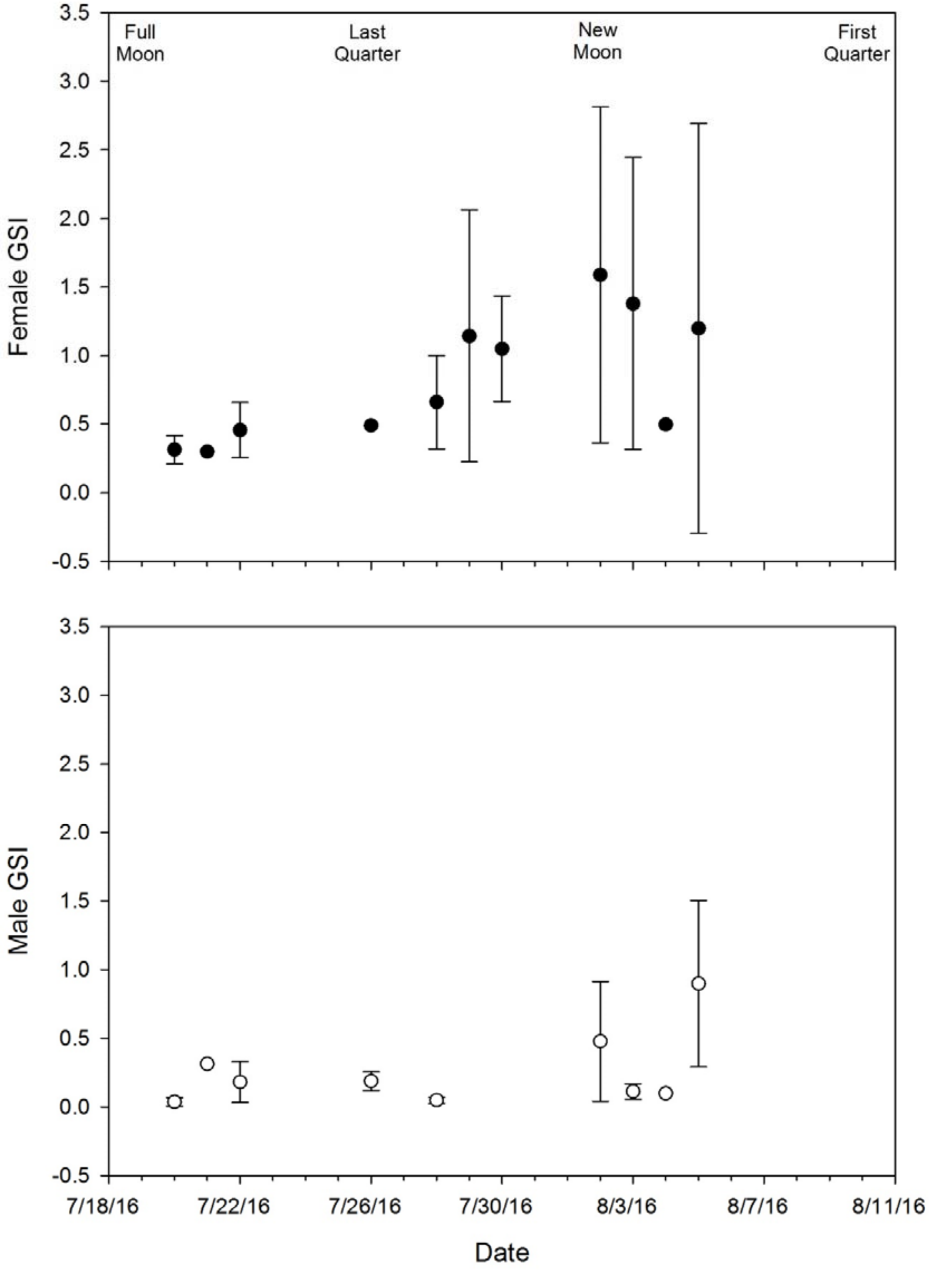

Figure 18. Mean (± standard deviation) gonadosomatic index values by date for *Lutjanus gibbus* from Pohnpei State, Federated States of Micronesia. Moon phases are indicated in the top (female) panel.

Monotaxis grandoculis

ANCOVA detected a significant sex-based difference in length-weight relationships (F = 6.12, DF = 1, P = 0.020). Overall and sex-based LWRs are presented in Table 1 and plotted in Figure 19. Overall, total body weight (W) in g is an approximately cubic function of TL in cm. 95% CI of regression parameters a and b are 0.0308–0.05047 and 2.79–2.94, respectively (r^2 = 0.994, n = 38, FL range: 18.1–44.5, W range: 160–2100). Males tend to weigh more per unit length than females. For females, the 95% CI of regression parameters a and b are 0.0344–0.0829 and 2.63–2.90, respectively (r^2 = 0.994, n = 14, FL range: 18.1–33.0, W range: 160–850). For males, the 95% CI of regression parameters a and b are 0.0331–0.0586 and 2.75–2.92, respectively (r^2 = 0.997, n = 16, FL range: 19.5–44.5, W range: 190–2100).

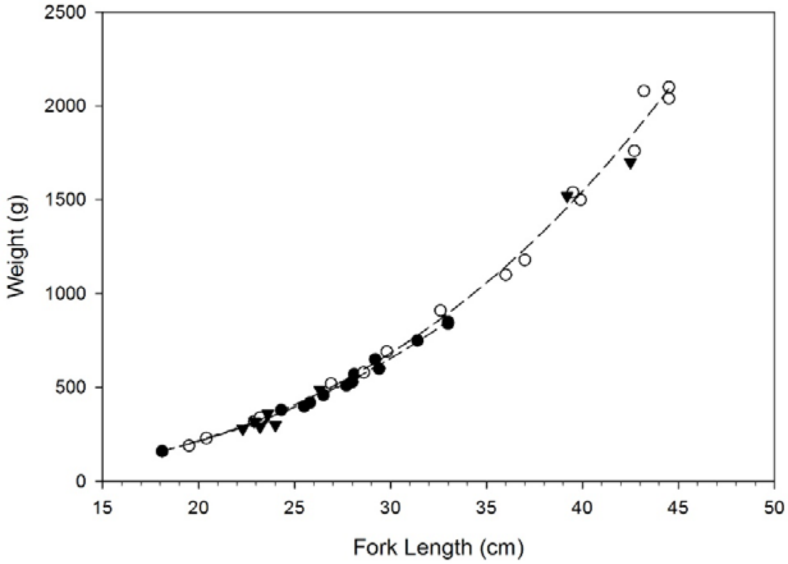

Figure 19. Sex-specific relationships between length and weight for *Monotaxis grandoculis* from Pohnpei State, Federated States of Micronesia. Females are represented by filled circles and the solid line, males are represented by open circles and the dashed line, individuals without histology-based sex determinations are represented by triangles (these were included in the overall length-weight relationship, Table 1, but not in either of the sex-specific relationships).

We histologically examined gonads of 16 male and 14 female *Monotaxis grandoculis*. All specimens were ≥ 18.1 cm FL. Figure 20 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 classify *M. grandoculis* as a batch spawner. Ovaries contained vitellogenic oocytes in females as small as 18.1 cm FL. Inactive and immature females (range 23.4-28.1) were scattered throughout the lower half of the size range of mature females (range 18.1-33.0). We had too few ovaries to confidently determine female L_{50}, however we tentatively estimate it at 27.5 cm FL (Fig. 21). This estimate ignores the single (mature) female in the smallest size class. The smallest male with spermiated testes was 19.5 cm FL. We observed only three inactive or immature males (26.9-29.8 cm FL), all in a single size class within the size range of mature males (range 19.5-44.5). We could not generate a satisfactory L_{50} curve for males; the percent of mature individuals averaged 81.3% (Fig. 21).

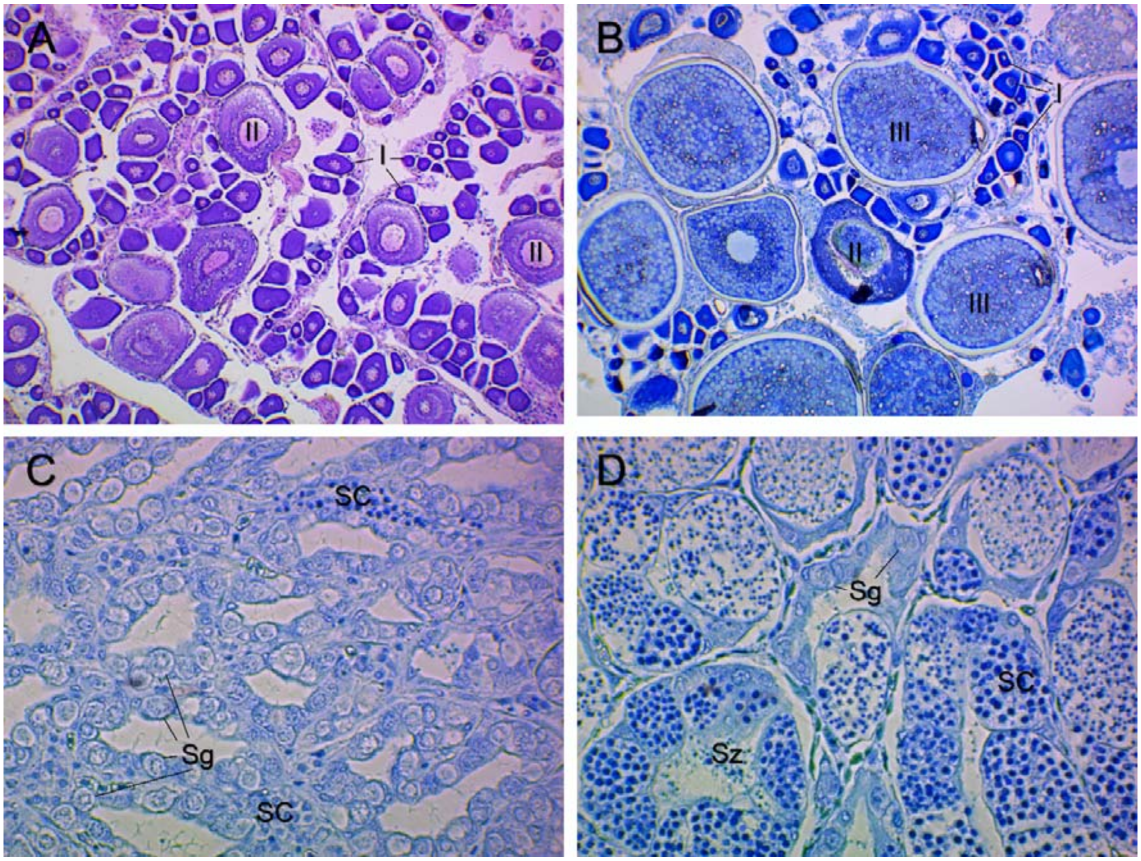

Figure 20. Photomicrographs of *Monotaxis grandoculis* gonads. (A) 23.4 cm FL immature female containing primary-growth (I) and cortical vesicle (II) oocytes, 100X; (B) 18.1 cm FL mature female with primary-growth (I), cortical vesicle (II), and vitellogenic (III) oocytes, 100X; (C) 28.6 cm FL immature male containing spermatogenic cysts (SC) and spermatogonia (Sg), 400X; (D) 20.4 cm FL mature male containing spermatogenic cysts (SC), spermatogonia (Sg), and tailed spermatozoa (Sz), 400X. Slide A stained with hematoxylin and eosin; slides B, C, and D stained with toluidine blue.

We saw a sex-based bimodal size distribution in *Monotaxis grandoculis*. A *t*-test, assuming unequal variances, indicated mean length of males (332.0) is significantly greater than that of females (273.9 cm) ($t = -2.36$, DF = 21, $P = 0.028$). 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 classify *M. grandoculis* as a gonochore.

Overall sex ratio in this *Monotaxis grandoculis* population is not significantly different from 1 male : 1 female (Table 1, $\chi^2 = 0.133$, DF = 1, $P = 0.715$). Nor does the sex ratio of mature individuals significantly differ from 1 : 1 (Table 1, $\chi^2 = 1.190$, DF = 1, $P = 0.275$). However, although our sample size is very small (21 individuals) we saw size-specific sex ratios in the mature size classes; the sex ratio of mature individuals varied predictably with length. The smallest size classes are female biased, and larger size classes are male biased (Fig. 22). An equation describing the percent of mature females (% ♀), throughout the size range of mature specimens (Table 1, $r^2 = 0.913$), predicts that the population is male biased at lengths ≥ 30.6 cm FL, that less than 25% of individuals are female at lengths ≥ 37.3 cm FL, and that less than 10 % of individuals are female at lengths ≥ 42.0 cm FL.

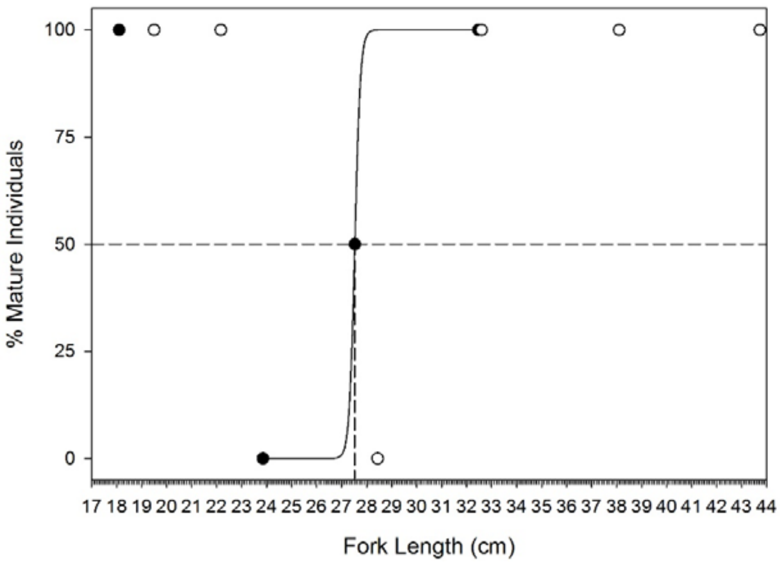

Figure 21. Size-at-maturity (L_{50}) for *Monotaxis grandoculis* from Pohnpei State, Federated States of Micronesia. Females are represented by closed circles and the solid line, males are represented by open circles.

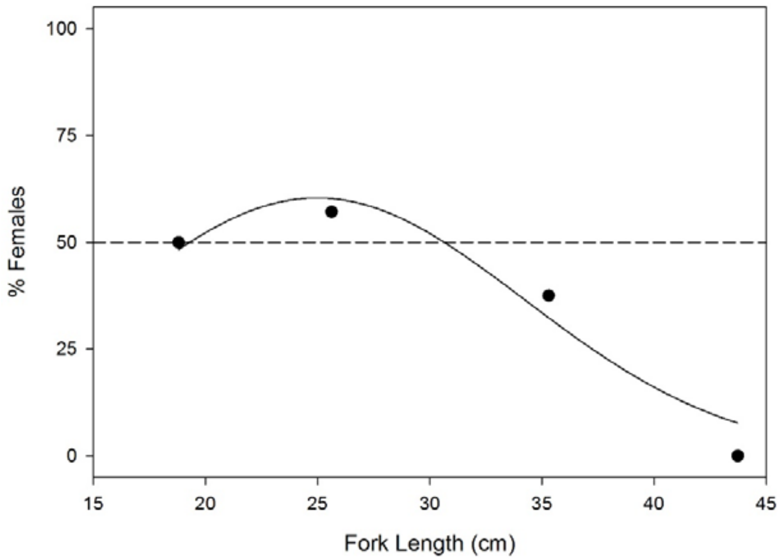

Figure 22. Percentage of mature females, relative to all mature individuals, versus length for *Monotaxis grandoculis* from Pohnpei State, Federated States of Micronesia.

We had too few female *Monotaxis grandoculis* with stage IV oocytes to construct a length-batch fecundity relationship. However, batch fecundity averaged 80,875 eggs in four females with a mean length of 30.4 cm FL.

We generated GSI values for 11 male and 19 female *Monotaxis grandoculis*. Specimens were collected from two days after the full moon to four days after the new moon. Figure 23 shows no detectable pattern for male GSI, but that female GSI values for females were highest around the full moon.

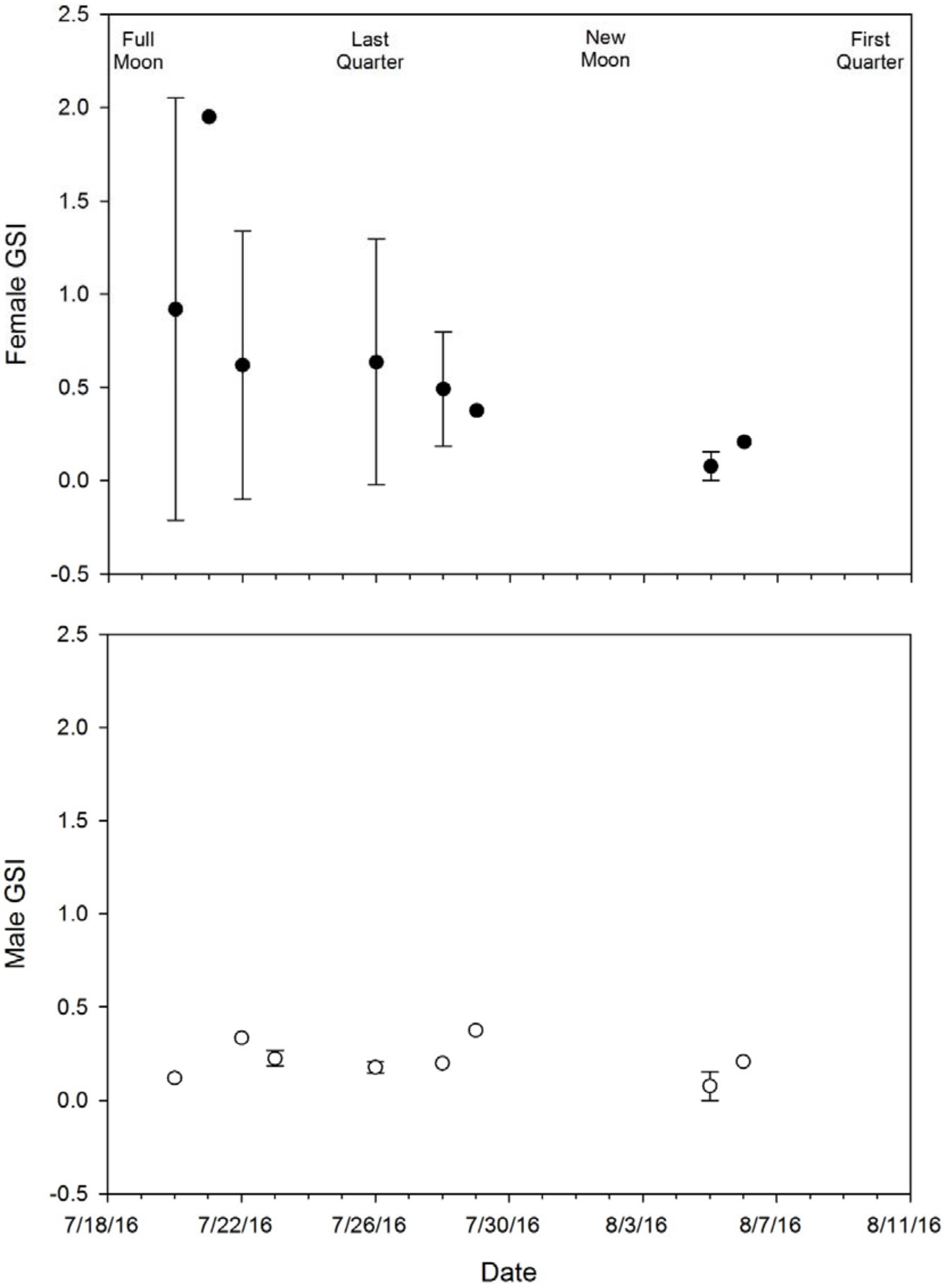

Figure 23. Mean (± standard deviation) gonadosomatic index values by date for *Monotaxis grandoculis* from Pohnpei State, Federated States of Micronesia. Moon phases are indicated in the top (female) panel.

DISCUSSION

Limitations and Applications

We generated histology-based reproductive information about four heavily exploited reef-fish species from Pohnpei State, Federated States of Micronesia, for which reproductive information was scant or lacking. However, in the interest of rapidly increasing the amount of available reproductive information, our Jungle Histology approach is to analyze only one gonad subsample from the minimum number of specimens necessary to generate estimates of reproductive parameters. It is possible that different results would be obtained by a more-conventional histological study that would, at least initially, examine whole cross-sections from multiple regions of a gonad (rather than a single ~8 mm^3 subsample) from a larger number of specimens (rather than our small sample-size target of 125 specimens). Therefore, Jungle Histology results should be viewed as preliminary. However, we think the results are a useful contribution to the currently insufficient body of knowledge about reproduction in Pacific coral-reef fishes.

Because all four species are widespread in the Indo-Pacific region, we suggest the information presented in this report will be useful outside Pohnpei State. For instance, creel surveys in American Samoa indicate that *Acanthurus lineatus* is the most common species landed by commercial spearfishing, representing 66% by number of 14 commonly speared species (Domingo Ochavillo, personal communication). The same creel survey indicates that *Lutjanus gibbus* is the 14th most common commercially caught reef-fish species. Thus, our results may be useful for length-based management of American Samoa's commercial reef fishery.

Jungle Histology versus Data-Poor and Gross Methods

The data-poor F&B (Froese & Binohlan 2000) method, shown in Figure 1 to systematically overestimate female size-at-maturity for exploited reef fishes in Papua New Guinea, also overestimates female size-at-maturity for the four reef fishes we studied in Pohnpei State, Federated States of Micronesia (Figure 24). For the species we examined in this report, the data-poor method overestimates female size-at-maturity by an average 21.0% (range: 14.2-33.0%).

This error can have a profound impact when evaluating the sustainability of a fishery. For instance, 99.3% of the specimens we obtained from Pohnpei fish markets were shorter than the F&B estimate of 20.3 cm FL for the size-at-maturity of female *Acanthurus lineatus*. By most standards, this would be considered an unsustainable fishery because so few fish would have had the chance to reproduce before being harvested. However, only 51.8% of the catch was shorter than our histologically derived female size-at-maturity estimate of 16.8 cm FL (and when the length of histologically sexed specimens was compared to histologically derived, sex-specific, size-at-maturity values, below, the percentage of immature individuals is even lower). Thus, our histology-based results raise the possibility that the Pohnpei fishery for *Acanthurus lineatus* is sustainable.

Similar differences were seen for the other three species we studied. For *Acanthurus nigricauda*, 96.2% of specimens we obtained from Pohnpei fish markets were shorter than the F&B female size-at-maturity estimate of 21.4 cm FL, but only 51.9% were shorter than the histologically derived value. For *Lutjanus gibbus*, 89.9% of specimens were shorter than the F&B estimate of 28.6 cm FL, but only 11.0% were shorter than the histologically derived value. For *Monotaxis grandoculis*, 60.0% of specimens were shorter than the F&B estimate of 31.4 cm FL, but only 40.0% were shorter than the histologically derived value.

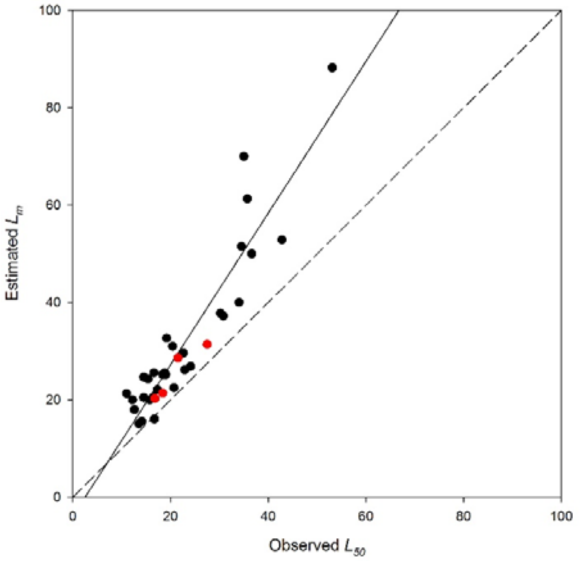

Figure 24. The data-poor method of Froese & Binohlan (2000), solid line, systematically overestimates female size-at-maturity of exploited reef fishes from Pohnpei State, Federated States of Micronesia (red circles). The dashed line is a 1:1 reference. Sources and species for black circles as in Figure 1.

Gross (macroscopic) reproductive analysis can introduce similar errors. Our gross evaluations, done when removing gonads for histological processing, were incorrect for 12.5% of *Acanthurus lineatus* specimens, 62.2% of *Acanthurus nigricauda* specimens, 51.6% of *Lutjanus gibbus* specimens, and 35.5% of *Monotaxis grandoculis* specimens. For most errors, we assigned the wrong sex or maturity status. However, for 4.8% of specimens, we were wrong about both sex and maturity status. Even more disconcerting is that we sometimes used non-gonadal structure to assign sex and maturity status; we sometimes mistook fat bodies for testes, or thought that gut was the gonad of an immature individual.

The relatively low error of our gross reproductive analysis of *Acanthurus lineatus* had little impact on our final interpretation of the reproductive status of the Pohnpei catch of the species. Our macroscopically derived size-at-maturity estimates were only 0.2 cm shorter for females and 0.1 cm shorter for males. When we applied the appropriate sex-specific size-at-maturity estimate to each specimen (unsexed individuals were assumed to be larger-maturing females) to evaluate the reproductive status of our sample, our gross-level results suggest 67.4% of specimens were mature whereas histological results indicate that at least 65.2% were mature.

Gross-level reproductive analysis differed greatly from histology-based results for the other three species. In general, the gross-level data would not permit an estimate of female L_{50} for any species (or a male L_{50} for *Acanthurus nigricauda*). Also, as we have reported for other species (Longenecker *et al.* 2013a, 2013b), gross-level reproductive analysis overestimated male size-at-maturity for *Lutjanus gibbus* and *Monotaxis grandoculis*. The error was ≥ 12.4% for *L. gibbus* and ≥ 47.7% for *M. grandoculis*.

Combined, these methods-based differences can have profound impacts when evaluating the reproductive status of a sample. Our gross assessment of *Acanthurus nigricauda* gonads indicated that only 26.7% of females and 20.0% of males were mature. However, our histology-based results suggest that least 59.5% of specimens, overall, were mature. For *Lutjanus gibbus*, our gross-level assessment indicated that only 36.5% of females and 47.2% of males were mature, whereas our histology-based results suggest that least 91.7% of specimens were mature. Finally, gross-level reproductive analysis of *Monotaxis grandoculis* indicated that 20.8% of females and 76.9% of males were mature, whereas histological analysis suggests that, overall, at least 68.6% of the specimens we obtained from Pohnpei fish markets were mature. For the three species, combined, gross-level reproductive analysis indicated that only about 38% of the specimens we examined were mature. This low value could lead to the conclusion that these species are being unsustainably harvested. However, a more-detailed histological analysis of the same set of gonads indicated that at least 73%, nearly twice the number of specimens, were mature. In other words, most of the individuals in Pohnpei fish markets had the chance to reproduce before being harvested.

Overall, we generated eight data-poor or gross-level estimates of reproductive parameters. Only one of those estimates was close to the parameters resulting from our inherently more-detailed and -informative histological analyses. The data-poor and gross-level approaches could lead fishery managers to impose fishing restrictions in an unnecessary attempt to change fishing patterns on the four species we examined. Thus, our Jungle Histology approach can help avoid the expense of unnecessary management actions that would make it more difficult for subsistence fishers to obtain food.

A Comparison with Results from Other Locations

Craig *et al.* (1997) published a length-weight relationship and gross-level reproductive analysis of *Acanthurus lineatus* from American Samoa. Similar to our results, there was no sex-based difference in the length-weight relationship. However, individuals of the same length are about 9 g heavier in Pohnpei State, FSM (Figure 25).

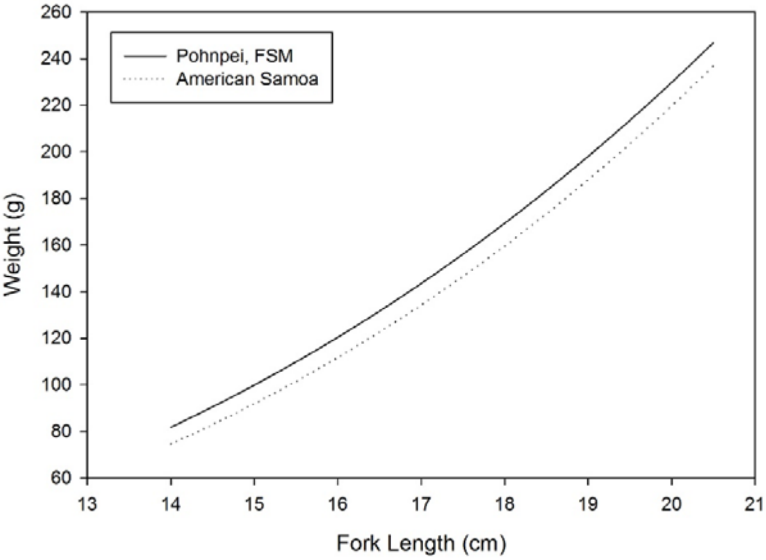

Figure 25. Geographic difference in the length-weight relationship for *Acanthurus lineatus*. American Samoa relationship from Craig *et al.* 1997.

Also similar to our results, Craig *et al.* (1997) did not find a significant sex-based bimodal size distribution, nor did the overall sex ratio significantly differ from 1 : 1. However, the American Samoa population had more females than males (1 male : 1.1 females), whereas the Pohnpei population had more males than females (Table 1). Craig *et al.* (1997) report that L_{50} is ~18.0 and ~17.4 cm FL for females and males, respectively. Our gross-level estimates for the Pohnpei population were shorter: 16.6 cm FL for females and <15.5 cm FL for males. The above differences warrant a histology-based study in American Samoa to evaluate whether the differences between the two populations are geography- or method-based.

Anand & Pillai (2002) studied *Lutjanus gibbus* in India. They reported minimum size-at-maturity (L_m) based on a combination of gross and histological examination of individuals in variable size classes. Using the mean of their minimum and maximum size-class limits suggests L_m is about 18 cm FL for females and about 14 cm FL for males. Our histology-based L_m estimates were longer (20.5 and 20.8 cm FL, respectively). We cannot determine whether the differences were methods- or geography-based (or both). Our L_{50} estimate of 21.5 cm FL for female *L. gibbus* is consistent with Huepel *et al.* (2009), who could not construct an L_{50} curve, but reported that all females > 23 cm FL were mature. However, 78% of our immature or inactive female *L. gibbus* specimens were larger than 23 cm. Our largest immature/inactive female was 28.0 cm FL.

Emergent Patterns

One of the benefits of our Jungle Histology approach to reproductive analyses is that rapid production of reproductive information helps to identify emergent patterns. For instance, females of three of the four species in this study become less abundant as length increases (*Acanthurus nigricauda*, *Lutjanus gibbus*, *and Monotaxis grandoculis*). Similar patterns have been reported for confamilial species (Langston *et al.* 2009, Pyle *et al.* 2016, Longenecker *et al.* 2014b, in preparation) and members of other families (Longenecker & Langston 2008, Longenecker *et al.* 2016) subject to our rapid reproductive analyses.

Although similar patterns have been reported elsewhere (Young & Martin 1985, Russell 1990, Lau & Sadovy 2001, Mant *et al.* 2006, Boaden & Kingsford 2013), the relevance of these size-specific sex ratios is currently under-recognized (but see Longenecker *et al.* 2014b, 2016, in preparation). It is a common assumption that large fish are disproportionately responsible for population-level reproductive output because large females typically produce an exponentially greater number of eggs than small females (see Roberts & Polunin 1993, Allison *et al.* 1998, Halpern 2003, Froese 2004, Birkeland & 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. The consequence of this observation cannot be overstated. 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, harvesting fish at or near their maximum size 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.

To date, we have analysed size-specific sex ratios for 26 Pacific reef-fish species. For almost all species, the proportion of females decreased with increasing length or there was no detectable length-related pattern. *Acanthurus lineatus* is the first species in which we have seen the proportion of females increase with increasing length (Figure 7). For those species with decreasing proportions of females as length increases, we have assumed (without any hard evidence) that egg production is energetically costly and that, as females mature, they shunt a higher proportion of energy to gonads than do males. This would result in female somatic growth slowing as males continued to grow, and thus changing sex ratios as length increases. Interestingly, mean male *Acanthurus lineatus* GSI values were equal to or, in the extreme case, four times higher than female GSI values (Figure 8). Perhaps males of this species invest more heavily in reproductive output than females. Such differential energy allocation would allow females to grow larger and to dominate the largest size classes.

Fishery Recommendations

Acanthurus lineatus

Sixty-five percent of the specimens we obtained from Pohnpei fish markets were mature. If fishery managers are concerned that *Acanthurus lineatus* is overfished, the local population may benefit from a minimum-size restriction of 17 cm FL. This would insure that most females and virtually all males had the chance to reproduce.

Our data did not allow us to describe a relationship between length and batch fecundity. However if, as generally assumed, fecundity of *Acanthurus lineatus* is an exponential function of length, a maximum-size limit may also be helpful. Because the population is female biased at lengths ≥ 18.7 cm FL (Figure 7), a slot limit may help protect the individuals responsible for the majority of population-level egg production. We emphasize that for the three other species we studied, because females are rare or absent in the largest size classes, maximum-size limits are unlikely to increase population-level egg production.

Acanthurus nigricauda

Sixty percent of the specimens we obtained from Pohnpei fish markets were mature. If fishery managers are concerned that *Acanthurus nigricauda* is overfished, the local population may benefit from a minimum-size restriction of 19 cm FL. This would insure that most females and virtually all males had the chance to reproduce. This size limit would disproportionately protect reproductive females because < 6.5% of individuals ≥ 19 cm FL are females (Figure 12).

Lutjanus gibbus

Ninety-two percent of the specimens we obtained from Pohnpei fish markets were mature. Interestingly, in Pohnpei State, *Lutjanus gibbus* is subject to a relatively new minimum-size limit of 10 inches (total length). This corresponds to approximately 21.5 cm FL, our histology-based estimate of female L_{50}, and insures that most females and virtually all males had the chance to reproduce.

Females were absent at lengths ≥ 27.8 cm FL (Figure 17). If Pohnpei fishery managers perceive the need for additional regulation of the *Lutjanus gibbus* fishery, we suggest that a minimum-size limit of 28 cm FL would protect virtually all reproductive females.

Monotaxis grandoculis

Sixty-nine percent of the specimens we obtained from Pohnpei fish markets were mature. If fishery managers are concerned that *Monotaxis grandoculis* is overfished, the local population may benefit from a minimum-size restriction of 28 cm FL. This would insure that most females and virtually all males had the chance to reproduce. The population is male biased at lengths ≥ 31 cm FL (Figure 22), thus a slightly larger minimum-size limit would protect a larger percentage of reproductive females.

ACKNOWLEDGMENTS

Financial support for this research was provided by a private foundation wishing to remain anonymous. Wilbur Walter provided in-kind support via a significant discount on lodging at Nihco Marine Park and Resort. We thank our colleagues from the Association for Marine Exploration and the California Academy of Sciences for cost-sharing on lodging and ground-transportation expenses. The following organizations and their staff were instrumental to the success of this project: Conservation Society of Pohnpei, FSM Department of Land and Natural Resources, FSM Office of Fisheries and Aquaculture, Nihco Marine Park and Resort, Saimon's Market, and The Nature Conservancy. We thank the communities of Ant and Pakin Atolls for their hospitality and for permission to work in their water. Our sincere thanks to the following individuals for their outstanding contributions to this project: Holly Bolick for support during logistical planning; Leah Briones for helping to navigate the permitting process, Scotty Malakai for introductions to Pohnpei fishers; Saimon Mix for priceless insight into Pohnpei's fishing practices and rhythms; and Kevin Rhodes for advice on Pohnpei fisheries, making key introductions, and consulting on our choice of study species.

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