ACTA ICHTHYOLOGICA ET PISCATORIA (2013) 43 (1): 51–55 DOI: 10.3750/AIP2013.43.1.07

RAPID REPRODUCTIVE ANALYSIS AND LENGTH–WEIGHT RELATION FOR BLACKTAIL SNAPPER, *LUTJANUS FULVUS* (ACTINOPTERYGII: PERCIFORMES: LUTJANIDAE), FROM A REMOTE VILLAGE IN PAPUA NEW GUINEA

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Longenecker K., Langston R., Bolick H., Kondio U. 2013. Rapid reproductive analysis and length–weight relation of blacktail snapper, *Lutjanus fulvus* (Actinopterygii: Perciformes: Lutjanidae), from a remote village in Papua New Guinea. Acta Ichthyol. Piscat. 43 (1): 51–55.

Abstract. We present a length–weight relation and use rapid, low-cost histological methods to describe the reproductive biology of the blacktail snapper, *Lutjanus fulvus* (Forster, 1801), based on 124 specimens collected from a remote area in Papua New Guinea [$W = 0.0134(\mathrm{FL})^{3.100}$]. We estimate male L_{50} at 13.5 cm FL and female L_{50} at 18.8 cm FL. Sex ratio is not significantly different from 1 : 1. The species is a gonochore. Mature females from 17.8 to 21.7 mm FL produce a mean 35 305 ± 29 141 eggs per spawning event.

Keywords: blacktail snapper, Lutjanidae, reproduction, histology, Morobe Province

The blacktail snapper, *Lutjanus fulvus* (Forster, 1801), is widespread throughout the Indo-Pacific, ranging from the east coast of Africa to the Marquesas and from the northeast coast of Australia to southern Japan (Allen 1985). Individuals from the Society Islands were intentionally introduced to Hawaii in 1956 (Randall 1987). The species can grow to approximately 40 cm total length (TL), but most specimens commonly reach only 25 cm TL (Allen 1985). The blacktail snapper is an important component of artisanal fisheries throughout its natural range, ranking among the top five species at study sites in India (Lazarus et al. 1994), the Solomon Islands (Connell et al. 1998), and Kiribati (Beets 2000); and among the top ten species at study sites in Tonga (Kirch and Dye 1979) and Tuamotu (Caillart et al.1994). Despite its broad range and importance as a fishery species, little is known about the reproductive biology of *L. fulvus*.

Here we use a rapid, low-cost, histology-based reproductive analysis suitable for use in remote locations (i.e., it does not require electrical service) to describe the reproductive biology and length–weight relation of the blacktail snapper, *Lutjanus fulvus* (Fig. 1), at Kamiali Wildlife Management Area (KWMA), Papua New Guinea (7°18′S, 147°10′E). Approximately 600 villagers maintain traditional tenure over the wildlife management area and obtain the overwhelming majority of their dietary protein from fish caught in its 15 000 ha of marine habitat.

We followed the guidance of Froese et al. (2011) to determine a length–weight relation for fresh-caught specimens, and used methods modified from Longenecker et al. (2013) for size-at-maturity and sex-ratio analyses. Briefly, for each specimen we measured fork length (FL) to the nearest mm, and estimated whole body weight with a hanging spring-scale. We then removed and fixed gonads in a modified Dietrich's solution for at least 24 h. Whole gonads were weighed to 0.001 g on a battery-powered 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 Gilson's fluid for later batch fecundity analysis (below). For all gonads, we embedded an approximately 8-mm^3 section in plastic (JB4, Electron Microscopy Sciences). We mounted on a microscope slide at least five tissue sections from each specimen, stained them with Toluidine Blue, and examined them at 40× on a dissecting microscope for evidence of reproductive maturity. We classified ovaries according to Wallace and Sellman (1981) and testes according to Nagahama (1983). We considered females mature with the onset of vitellogenesis (appearance of yolk protein in the oocytes), and males mature when the testes contained visible spermatozoa. We report size at sexual maturity (L_{50}) as the size at which a regression (3-parameter, sigmoidal) of percent mature individuals in each 2-cm size class versus fork

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length (the average length of individuals within a size class) indicates 50% of individuals are mature.

To estimate batch fecundity, we used methods modified from Agger et al. (1974). Ovarian samples reserved for batch-fecundity analysis (above) were stored in Gilson's fluid for six weeks. We analyzed those that, based on the histological examination above, had reached at least late vitellogenesis (≥ stage 3b). Oocytes were liberated from the stroma by agitation in an ultrasonic cleaner. Samples were diluted with water to a total volume of 400 mL, stirred to distribute oocytes, and a Stempel pipette was used to obtain ten 1-mL subsamples. We counted the largest size-class of oocytes in each subsample (oocytes ≥ stage 3b were ≥ 400 µm in diameter, thus oocyte size was used as an indicator for oocyte maturity). 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. Regression analysis (2-parameter power function) was used to describe the relation between fork length and batch fecundity.

Total body weight (W) in g is a cubic function of fork length in cm:

$$W = 0.0134(FL)^{3.100}$$

$r^2 = 1$; $n = 124$; 95% CI of regression parameters a and b are 0.0134 ± 0.000 and 3.100 ± 0.000, respectively; length range = 10.2 to 23.3 cm; weight range = 17.9 to 232.3 g.

Individuals of a given length at the KWMA tend to weigh slightly less than individuals at New Caledonia and markedly (~ 40%–50%) less than individuals from Tuamotu (Fig. 2). Given the latter observation and the phylogenetic distinctiveness of *Lutjanus fulvus* in French Polynesia (Gaither et al. 2010), the reproductive parameters we present below may not apply to eastern populations (or to the introduced Hawaiian population that originated from an eastern population).

We histologically examined gonads of 2 undifferentiated, 47 male, and 65 female *Lutjanus fulvus*. Figure 3 shows examples of immature and mature testes and ovaries. Sexual differentiation occurs around 11–12 cm FL. The smallest male with spermiated testes was 12.8 cm FL. Male L_{50} is 13.5 cm FL (Fig. 4). With the exception of a single 21.4 cm FL specimen, all males ≥ 17.2 cm were mature. Ovaries contained vitellogenic oocytes in females as small as 17.6 mm FL. We estimate female L_{50} at 18.8 cm FL (Fig. 4). This estimate ignores a single, immature, 23.3-cm female which was the only female in the largest size class. Our minimum-size-at-maturity (L_m) and L_{50} values for both sexes are substantially smaller than an earlier estimate that the species matures at about 20–30 cm (Allen 1985). Likewise, the L_m and L_{50} values presented here are smaller than L_m estimates (21.5 cm for males, 25.6 cm for females) derived from the empirical equations of Froese and Binohlan (2000). For instance, considering the more-conservative of the size-at-maturity values we present, L_{50} is only 63% (for males) or 71% (for females) of the empirically-derived L_m values.

Because macroscopic gonad classification systems are known to introduce excessive error when describing reproductive parameters (Vitale et al. 2006, Longenecker et al. 2013), we evaluated our accuracy in determining sex and reproductive status by macroscopic examination. We misclassified 43% of specimens examined (could not determine sex, 8.8%; incorrect sex, 4.4%; incorrect status, 20.2%; incorrect sex and status, 9.6%). Interestingly, in all incorrect sex classifications we called immature females immature males; in half of incorrect status classifications we thought mature males were immature; in the other half

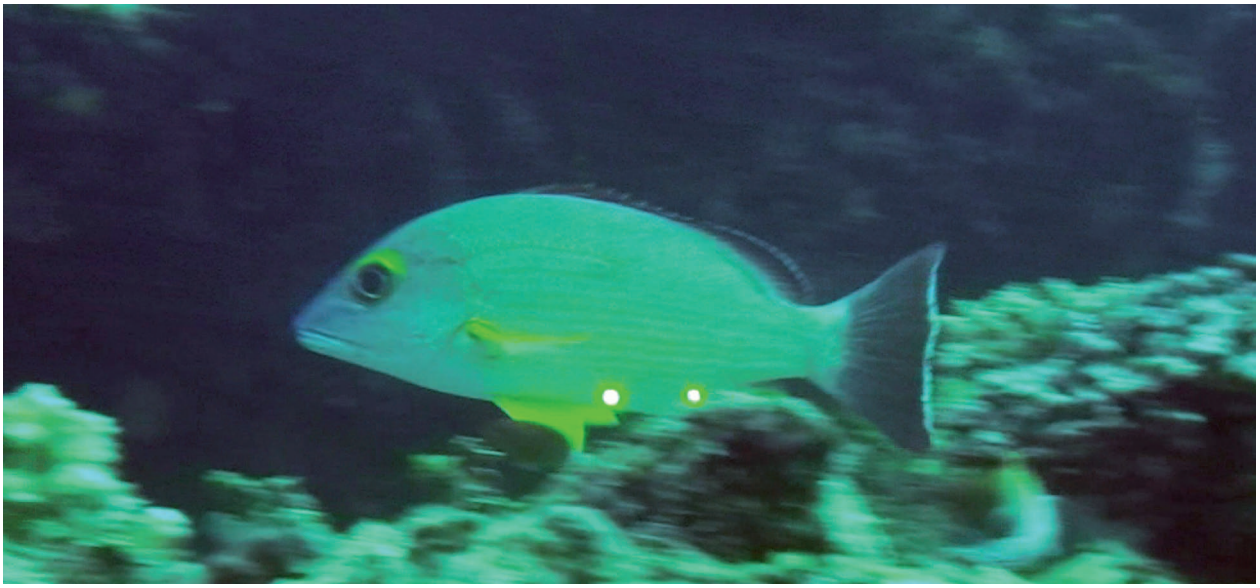

Fig. 1. *Lutjanus fulvus* „captured" during a laser videogrammetry study at Kamiali Wildlife Management Area, Papua New Guinea; Laser dots are separated by 31 mm; Image: Ross Langston and Ken Longenecker

of incorrect status classifications we thought immature females were mature; in all incorrect sex and status classifications we called immature females mature males. Importantly, macroscopic examination overestimated the number of mature females and underestimated the number of mature males. Further, we grossly underestimated the number of immature females.

Sex ratios can have profound impacts when predicting population-level reproductive output. Sex-ratio patterns are variable within the lutjanids; some species occur in a 1 : 1 ratio independent of size (Loubens 1980, Kritzer 2004, Russell and McDougall 2008, Longenecker et al. 2013), whereas the sex ratio of other species varies predictably with size (Loubens 1980, authors' interpretation of Davis and West 1992; authors' interpretation of M. Heupel, L. Currey, A. Williams, C. Simpendorfer, A. Ballagh and A. Penny unpublished data). Sex ratio in this *Lutjanus fulvus* population, from the size class at male maturity (12 to < 14 cm) through maximum observed size (23.3 cm), is 1 : 1.38 (♂ : ♀). However, a χ^2 analysis indicates the observed ratio

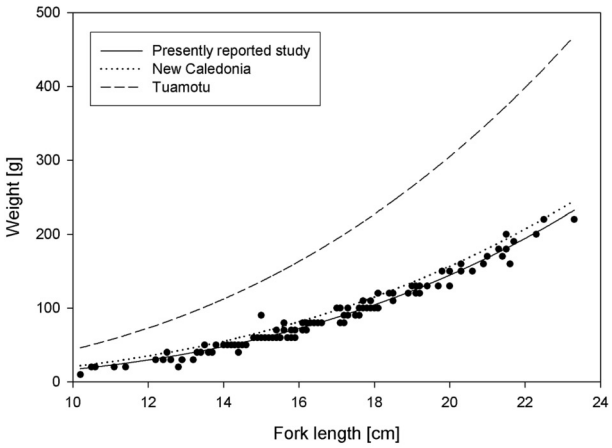

Fig. 2. Length–weight relations for *Lutjanus fulvus* from the Kamiali Wildlife Management Area (KWMA), Papua New Guinea; from New Caledonia (Letourneur et al. 1998); and from Tuamotu (Caillart et al. 1994); Circles represent specimens from KWMA

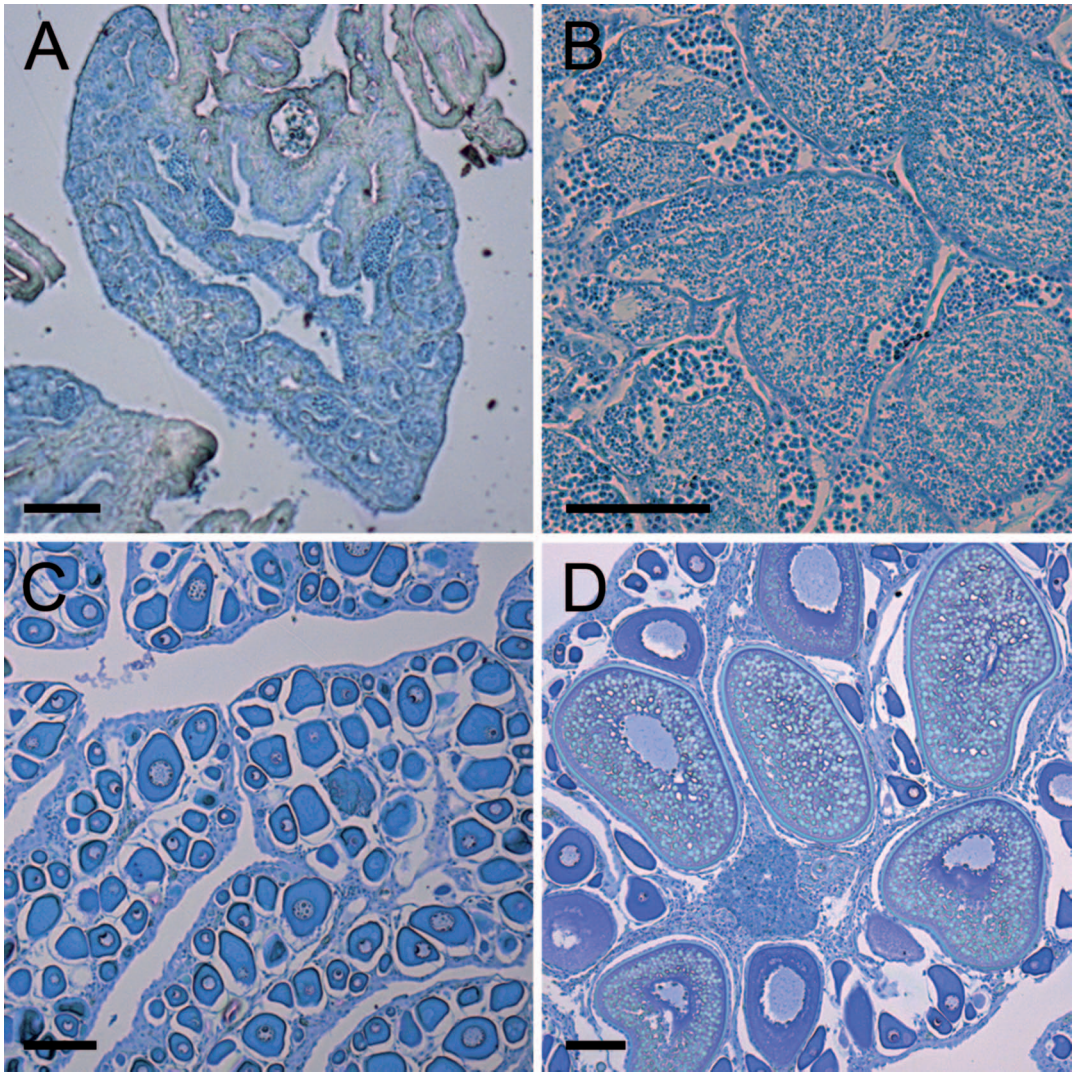

Fig. 3. Histological preparations of gonads of *Lutjanus fulvus* from the Kamiali Wildlife Management Area, Papua New Guinea; (**A**) immature male with lumen, 14.4 cm fork length FL, (**B**) mature male, 17.2 cm FL, (**C**) immature female, 16.6 cm FL, (**D**) mature female, 21.7 cm FL; Scale bars = 100 μm

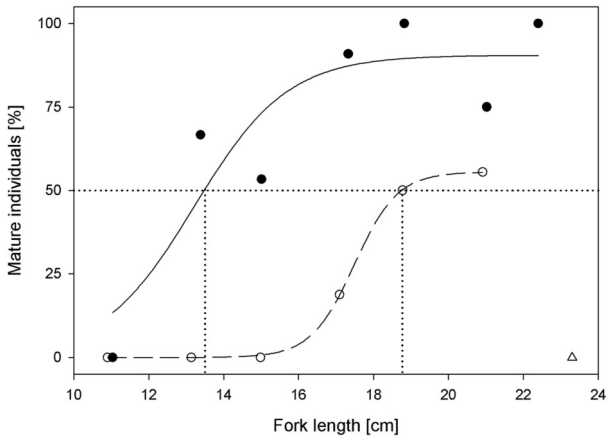

Fig. 4. L_{50} for *Lutjanus fulvus* from the Kamiali Wildlife Management Area, Papua New Guinea; 50% of males (closed circles, solid line) are mature at 13.5 cm fork length, 50% of females (open circles, dashed line) are mature at approximately 18.8 cm; The latter estimate ignores a single, immature 23.3 cm female (open triangle) in the largest size class

is not significantly different from 1 : 1 (df = 1, $P = 0.0890$). Further, sex ratio does not vary predictably with size.

Testes of two immature males contained a central membrane-lined lumen (see Fig. 3A). Testicular lumina are frequently found in secondary males of protogynous species (Sadovy and Shapiro 1987), however we do not think they indicate sex change in *Lutjanus fulvus*. They were rare in *L. fulvus*, but tend to be common (> 40%) in protogynous species (Sadovy and Shapiro 1987). Further, testicular lumina have been observed in gonochoristic species (Takahashi 1977), where they may be an artifact of phylogeny or result from environmental influences. There was no other evidence of sex change in *L. fulvus*. A t-test for a sex-based bimodal size distribution was not significant, nor did ovaries contain spermatogenic tissue. In agreement with general expectations for lutjanids (Allen 1985), we classify *L. fulvus* as a gonochore.

We did not find a statistically significant relation between fork length and our estimates of batch fecundity. Conceding that a descriptive relation may not exist, we offer three non-mutually-exclusive reasons for our failure to find a length-fecundity relation should one in fact exist. First, our fecundity estimates can be profoundly altered by errors in weighing gonads and gonad samples, by errors in measuring dilution volume or subsample volume, and by unequal dispersion of oocytes when obtaining subsamples. Second, we only had 11 samples for fecundity analysis, and these were from a relatively small size-range (3.9 cm) of specimens. Third, although *Lutjanus fulvus* appears to spawn year-round (Allen 1985, Caillart et al 1994), Randall and Brock (1960) report peak spawning occurs around the full moon in the Society Islands. Only two of our fecundity calculations were from females collected around the full moon. These small- to average-sized individuals, contrary to expectations, had the highest fecundity estimates. Perhaps confining fecundity analysis to specimens collected near peak-spawning time would lead to more consistent estimates. Given the above concerns, we report that on average, females from 17.8 to 21.7 mm FL (mean = 19.4) produce 35 305 ± 29 141 (mean ± standard deviation) eggs per spawning event. Our batch fecundity estimates ranged from 10 144 for an 18.1 cm female to 94 501 for a 17.9 cm female.

ACKNOWLEDGEMENTS

A private foundation, wishing to remain anonymous, provided financial support. Hawaiian Airlines provided in-kind travel support. We thank the fishers of Kamiali Wildlife Management Area for collecting specimens (national-level approval included in research visas for KL, RL, and HB; local-level approval by the Kamiali Wildlife Management Committee). Nine-year-old Kamiali resident, John Wagner Geamsa, demonstrated remarkable skill on the microtome. We thank all the residents of Kamiali for their hospitality, openness, and willingness to have their environment and fishing practices examined. Bulisa Iova provided invaluable help obtaining an export permit. This is contribution 2012-020 to the Pacific Biological Survey.

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Received: 30 July 2012
Accepted: 3 January 2013
Published electronically: 31 March 2013