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#TITLE:
Reproductive data for *Acanthurus guttatus, Acanthurus lineatus, Acanthurus nigricans, Ctenochatus striatus, Chlorurus japanensis and Scarus oviceps* collected near Tutuila, Territory of American Samoa, United States of America.

#ABSTRACT:
Natural-resource professionals from American Samoa's Department of Marine and Wildlife Resources performed novel research on the reproductive biology of exploited reef fishes. Participants worked as a group to produce new information for six species. These data can be used to describe: length-weight relationships (including sex-based differences), size-at-maturity for each sex (minimum size-at-maturity, and size-at-50%-maturity), reproductive mode (total vs batch spawning, gonochorism vs hermaphroditism), sex ratios (overall, operational, and size-specific), length-fecundity relationships, and reproductive periodicity (lunar, seasonal).

#PURPOSE:

The overall objective of the project was to train a cadre of natural-resource professionals from the Territory of American Samoa to use newly developed methods for rapid, low-cost, reproductive analysis of coral-reef fishes. These methods require minimal research infrastructure and are suitable for use on participants' home and neighboring islands.

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.

To address these issues, Longenecker *et al*. (2013) developed a method for rapid, low-cost, on-site, histology-based reproductive analysis that requires minimal research infrastructure. With this method, reproductive information can be generated quickly, and its low cost eliminates one of the arguments against broad-scale reproductive analysis surveys. Training a new crop of fish reproductive biologists who can independently use this method will increase the rate at which new reproductive information is generated, thus increasing the potential to effectively manage and conserve Pacific coral-reef fishes. The data presented here can be used to generate and disseminate novel reproductive information for commonly exploited Pacific reef fishes, including:

1) Length-weight relationships,
2) Size-at-maturity,
3) Reproductive mode,
4) Size-specific sex ratios,
5) Size-fecundity relationships, and
6) Reproductive periodicity.

#PROJECT:
American Samoa - Jungle Histology

#FUNDING:
2016 Saltonstall-Kennedy Competitive Research Program (opportunity number NOAA-NMFS-FHQ-2016-2004617, award number NA16NMF4270264)

#LOCATION EXTREMES:
SOUTHERNMOST LATITUDE: 14°23′
SOUTHERNMOST LATITUDE HEMISPHERE: S
NORTHERNMOST LATITUDE: 14°13′
NORTHERNMOST LATITUDE HEMISPHERE: S
WESTERNMOST LONGITUDE: 170°51′
WESTERNMOST LONGITUDE HEMISPHERE: W
EASTERNMOST LONGITUDE: 170°32′
EASTERNMOST LONGITUDE HEMISPHERE: W

#LOCATION KEYWORDS:
Tutuila, Aunu′u, American Samoa, South Pacific Ocean

#SAMPLING STATIONS:
Unknown, specimens were obtained from local fishers and markets near Pago Pago, American Samoa.

#BEGIN AND END DATES (YYYMMDD):
20161220 - 20171116

#SAMPLING PERIODS:
Unknown, specimens were obtained from local fishers and markets near Pago Pago, American Samoa.

#PARAMETERS:
Species (text, genus species)

Code (four-letter alpha code for location (AS) and species: ASAG = *Acanthurus guttatus*, ASAL = *Acanthurus lineatus*, ASAN = *Acanthurus nigricans*, ASCJ = *Chlorurus japanensis* ASCS = *Ctenochaetus striatus*, ASSO = *Scarus oviceps*)

Specimen (sequential Arabic numeral, in order processed)

Date (##-Aaa-####, day-month-year of specimen capture)

Length (cm, from front of head with mouth closed to end of middle caudal ray)

Weight (g, whole specimen)

Gross Sex (one- or two-letter alpha code for sex and reproductive status of specimen based on macroscopic examination of gonad upon excision: UD = undifferentiated, IF = immature female, IM = immature male, F = mature female, M = mature male)

Histo Sex (one- or two-letter alpha code for sex and reproductive status of specimen based on microscopic examination of gonad after histological processing: UD = undifferentiated, IF = immature female, IM = immature male, F = mature female, M = mature male, OT = ovotestis)

Egg Stage (Roman numeral [I – IV] indicating oocyte stage of females, possibly appended with "-A" or "-B" indicating sub-stage; OR the three-letter acronym "POF" for post-ovulatory follicle, based on the criteria of Wallace & Sellman 1981)

Whole gonad wt – (g, weight of whole gonad [W_s] upon excision from specimen and prior to fixation)

BF subsample wt – (g, weight of ovarian subsample for batch fecundity estimation [W_o])

Dilution – (ml, total volume [V] to which an ovarian subsample was diluted prior to sampling with a Stempel pipette)

Egg # 1 – (the number of the largest size class of oocytes in the first 1-ml subsample of the dilution volume [V] above)

Egg # 2 – (the number of the largest size class of oocytes in the second 1-ml subsample of the dilution volume [V] above)

Egg # 3 – (the number of the largest size class of oocytes in the third 1-ml subsample of the dilution volume [V] above)

Egg # 4 – (the number of the largest size class of oocytes in the fourth 1-ml subsample of the dilution volume [V] above)

Egg # 5 – (the number of the largest size class of oocytes in the fifth 1-ml subsample of the dilution volume [V] above)

Egg # 6 – (the number of the largest size class of oocytes in the sixth 1-ml subsample of the dilution volume [V] above)

Egg # 7 – (the number of the largest size class of oocytes in the seventh 1-ml subsample of the dilution volume [V] above)

Egg # 8 – (the number of the largest size class of oocytes in the eighth 1-ml subsample of the dilution volume [V] above)

Egg # 9 – (the number of the largest size class of oocytes in the ninth 1-ml subsample of the dilution volume [V] above)

Egg # 10 – (the number of the largest size class of oocytes in the tenth 1-ml subsample of the dilution volume [V] above)

Mean – (the mean number of the largest size class of oocytes in ten subsamples [N_o]}

Fecundity – (estimated batch fecundity [BF], the estimated number of oocytes that would have been released by the specimen during the next spawning event; $BF = (N_o \cdot V)(W_o \cdot W_s^{-1})$ 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

Notes – (text, observations or caveats entered by originators)

#METHODOLOGY:
Specimen Acquisition and Whole Specimen Processing: Specimens were purchased from local fishers or markets supplied by local fishers. 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 two hanging spring-scales (100 or 1000 g capacity, with 1 or 10 g increments, respectively). A mid-ventral incision was made from the vent through the pelvic girdle, sex and reproductive (based on gross examination) status were recorded, then gonads were excised and weighed to 0.001 g on a battery-powered jeweler'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, transferred to a skirted 50-ml centrifuge tube, and frozen 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 h.

Size-at-Maturity and Reproductive Mode: The Dietrich's-fixed gonad subsamples were dehydrated ethanol (30 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, 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 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 40X and testis sections at 100X 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).

Methods modified from Agger et al. (1974) were used to estimate batch fecundity. Ovarian samples of specimens that appeared mature upon visual examination were stored frozen for batch-fecundity analysis (above). We analyzed those that, based on the histological examination above, had reached at least the maturation stage (≥ stage 4a). A buffered collagenase solution (Marteinsdottir & Begg 2002) was be used to digest connective tissue (thus liberating oocytes); 6 ml of buffered enzyme solution (6 mg Collagenase Type 4, from Worthington Biochemical Corporation, dissolved in 6 ml 0.1 M Tris/5 mM CaCl2, pH 7.6) was added to the skirted centrifuge tubes containing ovary samples and digestion allowed to proceed at ambient temperature until oocytes were liberated (approximately 120 min). The samples were then fixed with approximately 2.5 ml of Dietrich's fixative (yielding an approximately 4% formaldehyde solution). The fixed oocyte samples were then 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.

#INSTRUMENT TYPES:
Hanging spring scales
Battery-operated jeweler's scale
MT1 Porter-Blum microtome
Stempel pipette
Dissecting microscope
Compound microscope

#REFERENCES:
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#SUBMITTING MEDIUM:
Email attachment

#DIRECTORY ORGANIZATION, FILE NAMES AND FORMATS:
File name: American Samoa fishes reproductive data.xlsx
Format: Microsoft Excel worksheet

#DATASET SIZE:
79.1 kb

#NUMBER OF DATA UNITS: 378

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The American Samoa Department of Commerce kindly provided space for laboratory analyses.

#MISCELLANEOUS:
Online teaching modules demonstrating the methods can be accessed at:
http://pbs.bishopmuseum.org/pacificfishes/modules.html