

**Growth, mortality and reproduction of manini,
*Acanthurus triostegus sandvicensis***

Final Report

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EXECUTIVE SUMMARY

Attempts to manage exploited fish populations ideally incorporate information on the life history and demographic structure of the target species. In Hawaii, a complete set of the necessary information does not seem to exist for any one reef-fish species, making it nearly impossible to predict the outcome of new management strategies or evaluate the effectiveness of existing ones. We generated information on morphometric relationships, growth, sex-specific size-at-maturity, sex ratios, size-fecundity relationships, age structure, and forces of mortality for manini, *Acanthurus triostegus sandvicensis*, an exploited reef fish.

We measured the lengths and weights of specimens collected on Oahu, Maui, and Hawaii to describe morphometric relationships. Most important for fishery modeling is the length-weight relationship, which for manini is: $W = 0.00001214(FL)^{3.1557}$. We examined histological preparations of gonadal tissue to describe sex-ratio and size at maturity (L_{50}). The populations we studied had a physical sex-ratio of 43:57 (M:F), although operational sex ratio (counting only reproductively mature males and females) was affected by the size-structure of the population surveyed. Seventy percent of males were mature at 90 mm (the smallest size class of males obtained), indicating that L_{50} for males occurs below this size. Females mature at approximately 164 mm. We sub-sampled ovaries of gravid females to estimate batch fecundity. A linear regression best described variation in fecundity with size: $BF = -648,588.17 + FL(4226.06)$. Age was estimated from otolith microstructure. The relationship between length and age, assuming otolith increments are formed daily, can be described by a vonBertalanffy growth equation: $l_t = 202.798(1 - e^{-0.0039276(t-18.6533)})$. Males mature by 168 days, and females mature around 440 days. Growth does not appear to vary between sexes or among locations. We did not determine maximum life span; however, results suggest that manini live for more than four years. We compared mortality estimates obtained from a series of marine reserves with those from comparable fished sites to determine forces of natural (M) and fishing (F) mortality. Quarterly natural mortality up to 2.5 years is 0.53549 and, although data were too limited to be certain, mortality appears to decrease after 2.5 years. Quarterly fishing mortality through two years of age is 0.14621, or about 20% of total mortality in exploited populations.

From a fishery-management standpoint, the estimate deserving the most immediate attention may be size at maturity for females. Hawaii's current legal size limit for manini is 127 mm. With an L_{50} of 164 mm, most, if not all, females enter the fishery before maturation.

INTRODUCTION

Acanthurus triostegus sandvicensis (or manini) is the most abundant and, commercially, the most important surgeonfish in Hawaii (Randall 1961). It is important in recreational/subsistence fishing, and ranked second among speared fish in Waikiki creel surveys (Meyer 2003). Some fragmentary life-history information is reported for *A. triostegus*. However, the biology of manini in Hawaii is known to differ substantially from populations in equatorial regions (Randall 1961), making us suspect the relevance of studies from other geographic regions to the Hawaiian subspecies. Here we estimate the following life-history descriptors: length-weight relationship, sex ratio, size at 50% maturity, and size-fecundity relationship, growth rate, age structure and mortality. With the exception of growth for tank-reared specimens and (problematic) mark-recapture studies, and sex ratio (Randall 1961), there does not appear to be published information on any of the above life-history parameters for this abundant and economically important species. All of them are needed to estimate biomass production and reproductive output. The latter estimates should provide the least ambiguous means of evaluating various management strategies.

METHODS

1. Study Sites

Morphometric, reproductive, and growth analysis was performed on specimens collected opportunistically from various locations on the islands of Oahu, Maui, and Hawaii. Natural and fishing mortality was estimated by comparing the total mortality estimate generated in a combination of four marine reserves with that from four fished areas that contain, at least superficially, similar habitat types and depth distributions (Figure 1). On Oahu, Hanauma Bay Marine Life Conservation District (N 21° 16'10", W 157° 41'38") encloses approximately 380,589 m² and fishing has been prohibited for 40 years. Our comparison fished site was 3.2 km west in Maunalua Bay, adjacent to Portlock (N 21° 15'41", W 157° 42'41"), and occupied an equivalent area. On Maui, Honolua-Mokuleia Marine Life Conservation District (N 21° 00'51", W 156° 38'30") encloses approximately 182,109 m² and fishing has been prohibited for 30 years. Our comparison fished site was approximately 3 km to the southwest in Kapalua Bay (N 21° 00'07", W 156° 40'05"), and enclosed approximately 104,166 m². We surveyed two pairs of sites on Hawaii. Subzone A of Lapakahi Marine Life Conservation District (N 20° 10'36", W 155° 54'04") encloses approximately 83,479 m², and fishing has been prohibited for 29 years. Our comparison fished site was Mahukona (N 20° 11'03", W 155° 54'06"), 0.25 km to the north. The area surveyed enclosed approximately 92,600 m². The other Big Island reserve surveyed was Waialea Bay Marine Life Conservation District (N 19° 58'54", W 155° 49'48"), enclosing approximately 141,640 m². Although hook and line fishing is allowed throughout the reserve and the use of nets is permitted over sandy areas, neither fishing method targets manini, so this area has effectively fully protected manini for the past 23 years. Our comparison fished site was 10 km to the southwest in the southern end of Anaehoomaluhia Bay (N 19° 54'47", W 155° 53'25"), and enclosed approximately 77,471 m². Datum for the above coordinates is WGS84.



Figure 1 Survey sites on 3 main Hawaiian Islands. Yellow symbols are marine reserves, red symbols are areas open to fishing. Image from Google Earth.

2. Life History Analysis

We collected most specimens using nets or spears. New recruits were collected from tidepools with hand-nets or by pumping the water out of the pool with a battery-operated bilge pump. Specimens were stored on ice until processing. We measured, to the nearest 0.5 mm, standard length, total length, the distance between the origins of the dorsal and pelvic fins, and the length from the anterior-most part of the head to the end of the middle caudal rays. The latter measurement is referred to as fork length throughout this report. We then measured total body mass (to 0.1 g), removed saccular otoliths (sagittae), and fixed gonads in Dietrich's fixative (60% distilled water, 28% absolute ethanol, 10% formaldehyde, and 2% glacial acetic acid). Morphometric relationships were described using linear regression for lengths and a 2-parameter power function for length vs. weight. Growth and reproduction were described following the methods in Longenecker and Langston (2006), summarized below.

Growth

We prepared a single, transverse section of each saggita by mounting the otolith, lateral side down, on a glass microscope slide in thermoplastic glue (Crystal Bond #509 from Electron Microscopy Sciences, Hatfield, PA) then removing a section containing the core using an Isomet 11-1180 low-speed saw (Beuhler, Lake Bluff, IL). We affixed this section to a glass microscope slide, a cut side down, with thermoplastic glue; ground close to the core using a series of 600 and 1500 grit sandpaper; then polished the section with 0.3 and 0.05 μm alumina slurry on felt. We etched the polished surface with a 2.5% solution of unbuffered EDTA for 4-7 min, then rinsed

with deionized water. We dissolved the thermoplastic glue with acetone and mounted prepared otolith sections on aluminum stubs. We coated these sections with a gold-palladium film in a Hummer II sputtercoater (Technics, Alexandria, VA), and viewed them on a Hitachi S-800 field emission scanning electron microscope at 700X (Hitachi High Technologies America, Schaumburg, IL).

We used Photoshop 7.0 (Adobe Systems, San Jose, CA) to examine digital images of the otolith sections (see Figure 2). Otolith preparations rarely included the primordium, so total number of rings was estimated by counting the number of increments past an easily identifiable settlement mark, and adding an assumed number of days for the region inside the mark. Basch (unpublished data) showed that the presettlement phase for *A. triostegus sandvicensis* lasts an average of 54 d. We assumed that each otolith increment represented one day, and constructed a vonBertalanffy growth curve using Simply Growth version 2.1.0.48 (Pisces Conservation, Lymington, Hampshire, UK).

We attempted to validate daily ring formation by chemically marking otoliths of captive fish. Ten juvenile manini were collected from tidepools and immersed in a solution of Calcein and seawater (125 mg/l) for 48 hrs. The fish were then transferred to flow-through seawater system at the Hawaii Institute of Marine Biology and fed algae and brine shrimp daily. We killed one to two individuals at 1, 2, and 14 days post-mark. We removed the otoliths of these individuals, sectioned and polished as described above and examined them under UV microscopy for the presence of a florescent chemical mark.

Reproduction

We removed a small tissue biopsy from each of the Dietrichs'-preserved gonads, dehydrated in a graded ethanol series and embedded them in glycol methacrylate (JB-4 Embedding Kit from Electron Microscopy Sciences, Hatfield, PA). Embedded gonads were then sectioned at 2 - 5 μm on a rotary microtome (Sorvall Products, Newtown, CT) fitted with a glass knife. We affixed these sections to glass microscope slides, stained them in toluidine blue or hematoxylin and eosin and examined them for evidence of reproductive maturity. We classified ovaries according to Wallace & Sellman (1981) and testes according to Nagahama (1983). We considered female fish mature with the onset of vitellogenesis, and males mature when the testes contained visible spermatozoa. We report size at sexual maturity as the size at which a regression equation (3-parameter, sigmoidal) of percent mature individuals in each 12 mm size class versus standard length indicates 50% of individuals are mature.

Ovaries selected for batch fecundity were weighed to the nearest 0.001 g on a digital microbalance. We collected 3 subsamples (chosen randomly from right or left lobe of ovary) of tissue (8-15 mg each) from the anterior, middle, and posterior of the gonad and weighed these to the nearest 0.01 mg on a CAHN 28 electrobalance. We estimated batch fecundity from the subsamples using the oocyte size-frequency method (Hunter et al. 1985; MacGregor 1957), assuming the largest mode of yolked oocytes formed the next spawning batch. We used a linear regression to describe the relationship between batch fecundity and standard length.

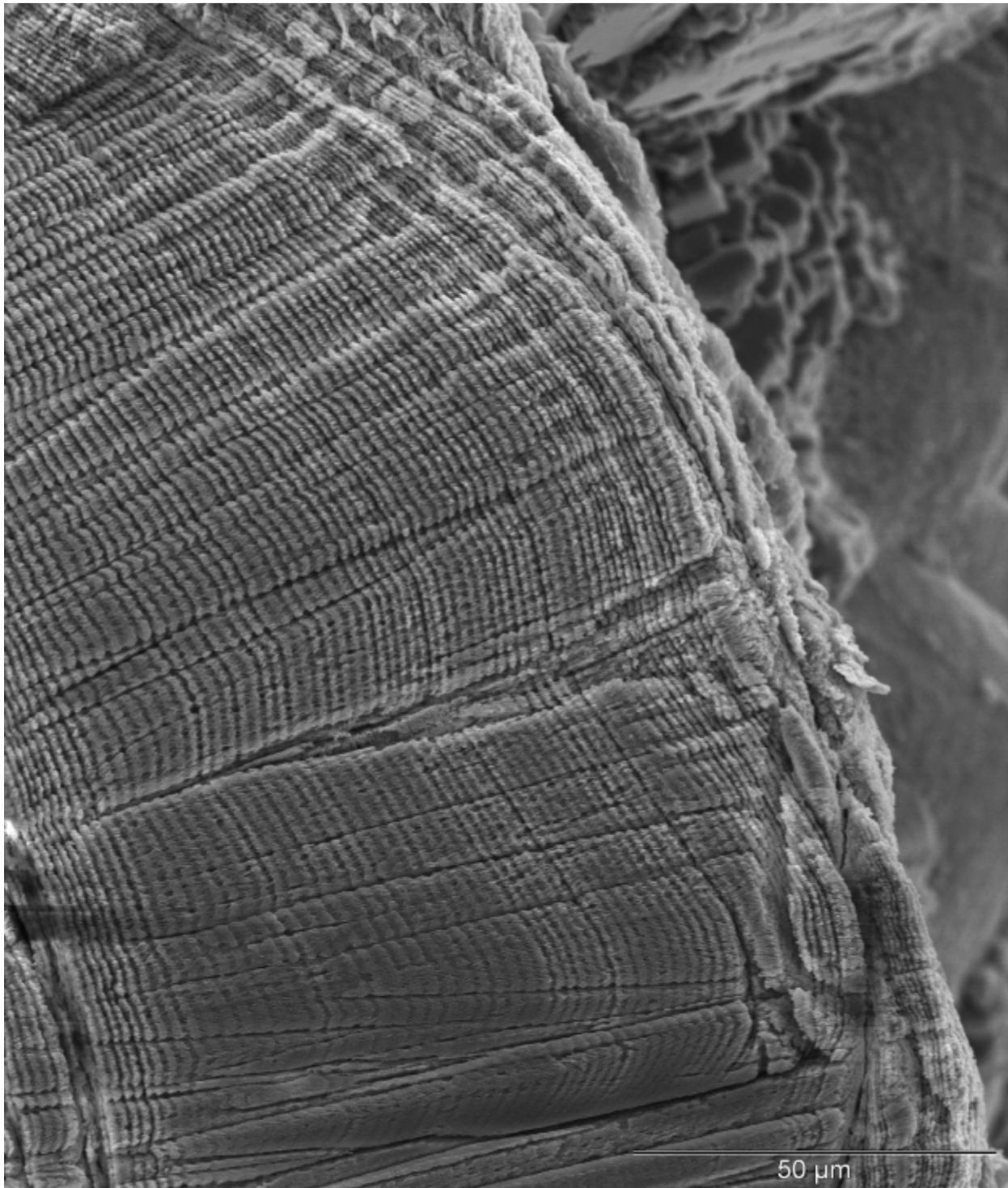


Figure 2 Scanning electron micrograph of the margin of a prepared *Acanthurus triostegus sandvicensis* otolith. We assigned a total age of 219 days to the fish that produced this otolith.

Mortality

We used laser videogrammetry to describe the size distribution of manini in marine reserves and nearby, comparable fished habitats (Figure 3). Here, we used closed-circuit rebreathers (Oahu) or open-circuit SCUBA (Maui and Hawaii) to swim two-meter-wide belt transects along a compass heading. A video camera fitted with parallel laser beams was used to capture images of individuals when they were oriented perpendicular to the laser beam axes. We then reviewed the video with Windows Movie Maker® and captured still frames where both lasers appeared on the fish. Because the beams are parallel, the lasers superimpose a reference scale on the side of the fish, allowing length estimates by solving for equivalent ratios. Still images were analyzed using ImageJ (National Institutes of Health). In most cases, we were able to estimate total or fork length. However on occasion, the only reliable length estimate was “body depth” (the distance between the origins of the dorsal and pelvic fins). In these cases, we used morphometric relationships to convert this measurement to total or fork length. Longenecker & Langston (2008) demonstrated a nearly 1:1 relationship between fish length estimated from laser videogrammetry versus actual fish length. Further, the prediction interval suggested 95% of estimates will be within 0.5 cm of the actual fish length (Figure 4).

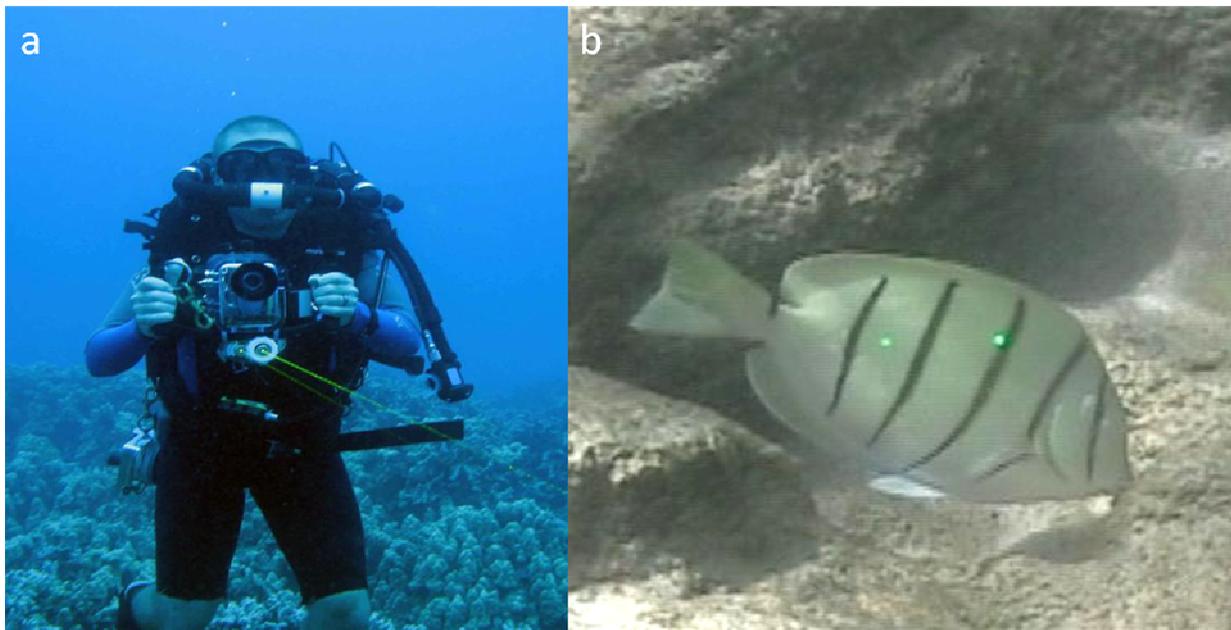


Figure 3 . Laser videogrammetry, a non-destructive technique to estimate fish length. (a) a diver operates a video camera fitted with two parallel laser beams. (b) the laser beams superimpose a measurement scale on the side of *A. triostegus sandvicensis*.

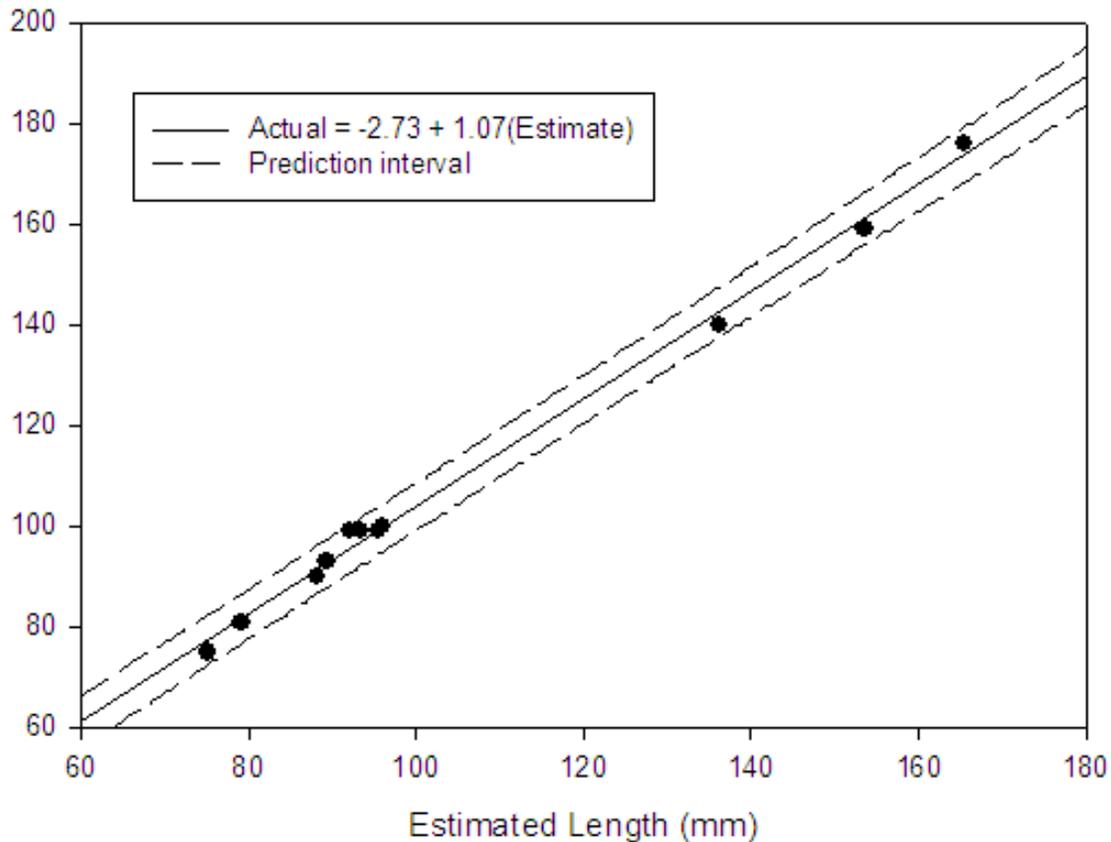


Figure 4 The relationship between estimated and actual lengths of specimens “captured” on videotape for laser videogrammetry and subsequently speared. The prediction interval suggests that 95% of length estimates will be within 0.5 cm of actual fish length (from Longenecker & Langston 2008).

We used the vonBertalanffy growth equation (above) to convert lengths to age estimates and constructed a cumulative age distribution for all marine reserves surveyed and for all fished sites surveyed. We then used regression analysis to describe the natural logarithm of the frequency of each age class as a function of age, and obtained total mortality (Z) from the negative slope of the line (Everhart & Youngs 1992). Because fishing is prohibited in Marine Life Conservation Districts, total mortality at these sites is equivalent to natural mortality (M). At comparison fished sites, total mortality is the sum of natural (M) and fishing mortality (F). Fishing mortality was estimated by subtracting total mortality in marine reserves from total mortality at fished sites. That is:

$$\begin{aligned} Z_{\text{reserves}} &= M \\ Z_{\text{fished}} &= F + M \end{aligned}$$

Therefore:

$$Z_{\text{fished}} - Z_{\text{reserves}} = (F + M) - M = F$$

RESULTS

Morphometric relationships

We collected 179 manini for life history analysis. The length-weight relationship was best described by a two-parameter power function where weight was an approximately cubic function of length (Figure 5). All length-to-length relationships were linear (Table 1).

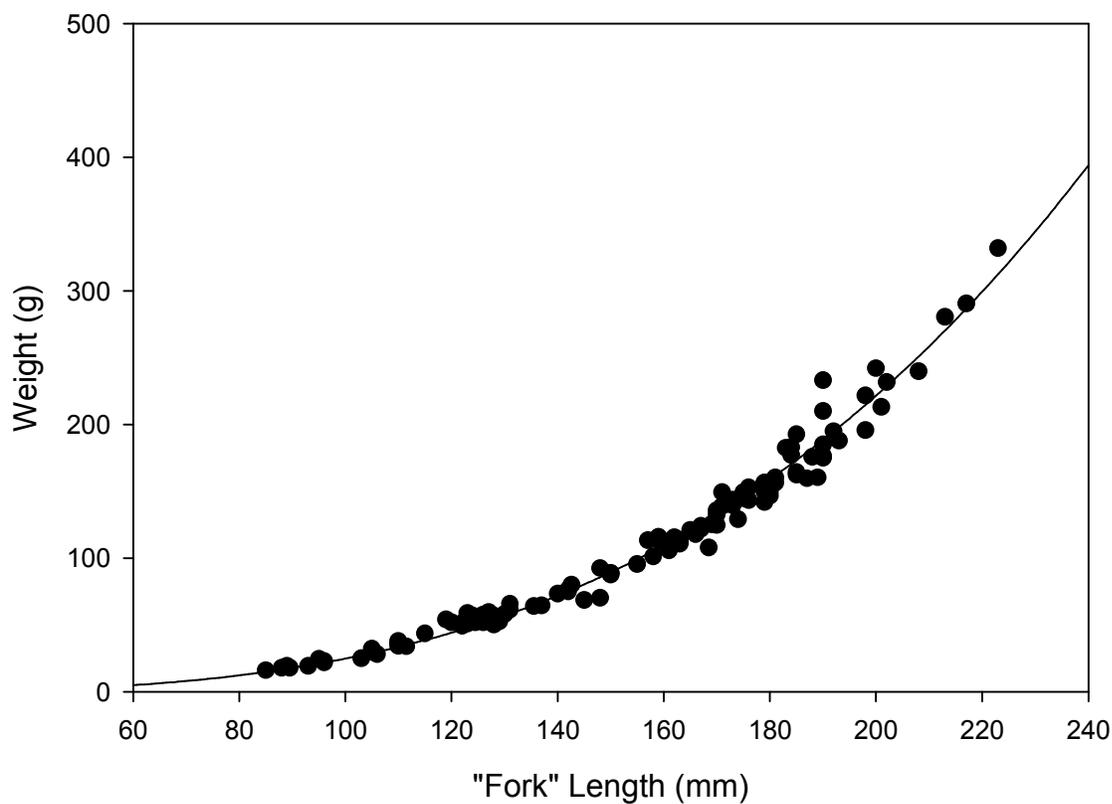


Figure 5 Length-weight relationship for *A. triostegus sandvicensis*. $W = 0.00001214(FL)^{3.1557}$; $n = 115$; $r^2 = 0.98$.

Table 1 Linear regressions predicting fork length (FL) *A. triostegus sandvicensis*. $FL = a + (X)b$, where X is a linear distance in mm. TL = total length; SL = standard length; BD = the distance between dorsal and pelvic fin origins.

| Variable | N | a | b | r ² |
|----------|-----|-------|-------|----------------|
| TL | 188 | 1.796 | 0.939 | 0.997 |
| SL | 187 | 1.922 | 1.196 | 0.995 |
| BD | 153 | 9.397 | 2.190 | 0.893 |

Growth

We obtained 45 readable otolith preparations. A vonBertalanffy growth equation fitted the observed data points well (Figure 6). The predicted asymptotic length is close to Randall’s (2001) observation that the species rarely exceeds 200 mm. There are no obvious differences in growth rate by sex or location.

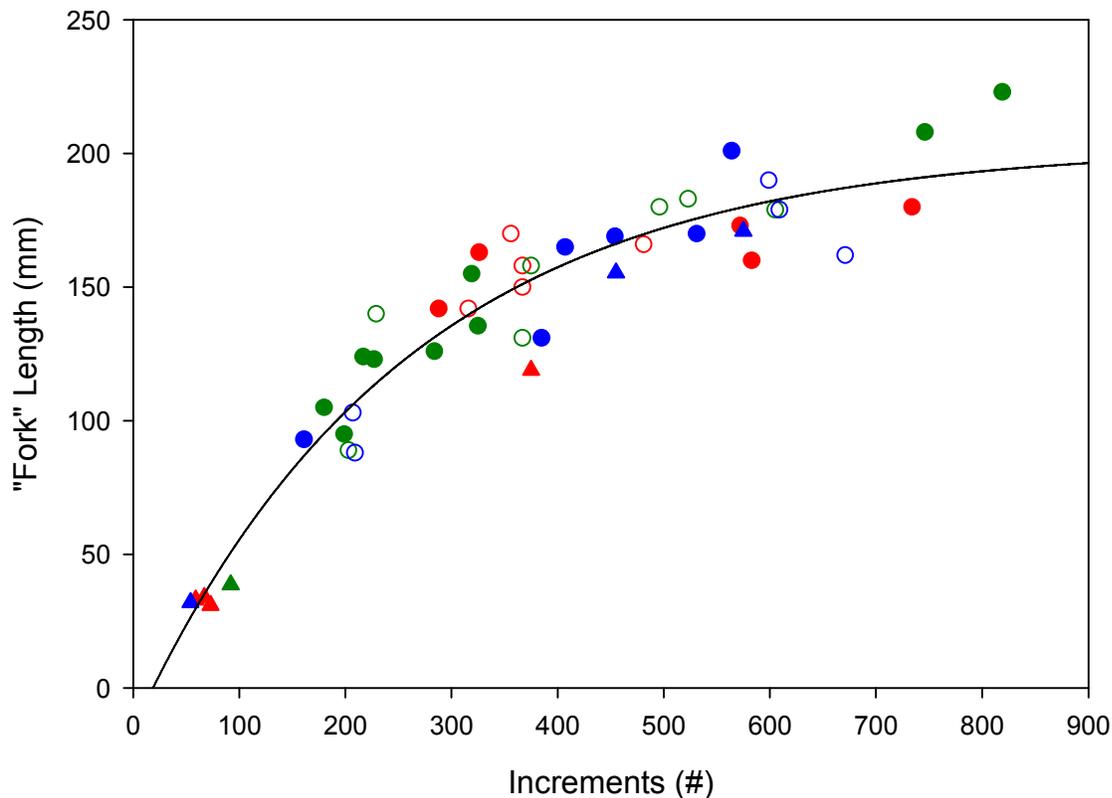


Figure 6 A scatterplot of age (assuming each otolith increment is equivalent to one day) versus total length for manini, *Acanthurus triostegus sandvicensis*. The curve represents the resulting vonBertalanffy growth equation: $l_t = 202.798(1 - e^{-0.0039276(t-18.6533)})$. Colors represent island: red = Hawaii, green = Maui, blue = Oahu. Symbols indicate sex: triangles = unknown, filled circles = females, open circles = males.

Reproduction

We histologically examined gonads from 122 individuals and classified each as mature or immature based on the stages of gametes present (Figure 7). Of these, 70 were ovaries and 52 were testes, yielding a physical sex ratio of 43:57 (M:F). Seventy percent of males were mature at 90 mm FL (the smallest size-class sampled) whereas 50% of females were mature by 164 mm (Figure 8). Because females mature at a larger size than males, the operational sex ratio varies with size structure of the population (Figure 9). Below 160 mm, the population consists entirely of mature males and immature females whereas above 180 mm it contains more females than males. The overall operational sex ratio for the populations sampled was male-biased (63:37).

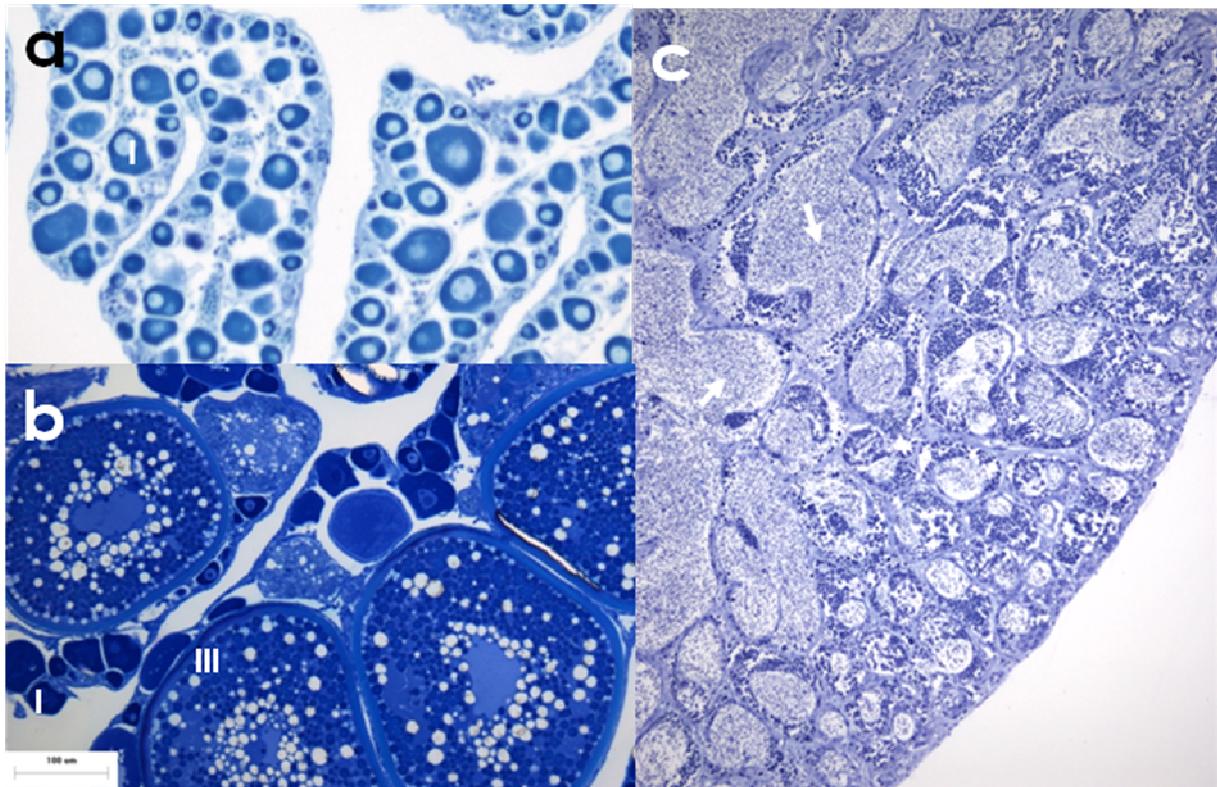


Figure 7 Histological sections of *A. triostegus sandvicensis* gonads (Toluidine Blue). Ovaries of immature females (a) contained only primary growth (I) and cortical vesicle stage oocytes whereas those of mature females (b) also contained vitellogenic (III) or mature oocytes. Testes of mature males (c) contained visible spermatozoa (arrows).

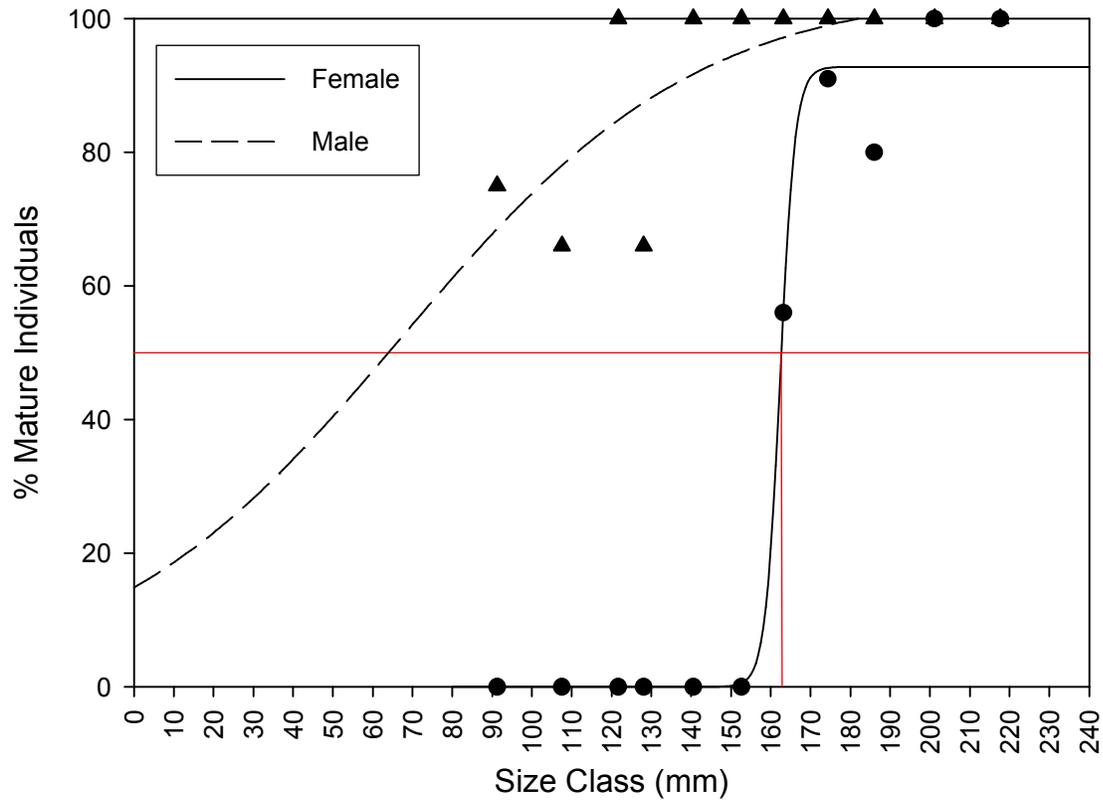


Figure 8 Size at maturity (L_{50}) for manini, *Acanthurus triostegus sandvicensis*. Triangles represent males, circles represent females.

Fecundity

Nineteen individuals were selected for batch fecundity analysis. Fecundity estimates ranged from 21,997 eggs/spawn for a 159 mm FL specimen to 362,293 eggs/spawn for a 213 mm specimen. The relationship between size and fecundity was best described by linear regression (Figure 10).

Mortality

We surveyed a total 48,704 m², 52% of this area was in marine reserves and 48% was in fished habitat (Table 2). We captured on video a total 297 individuals suitable for size estimation, 224 of these were in marine reserves and 73 were in fished habitat.

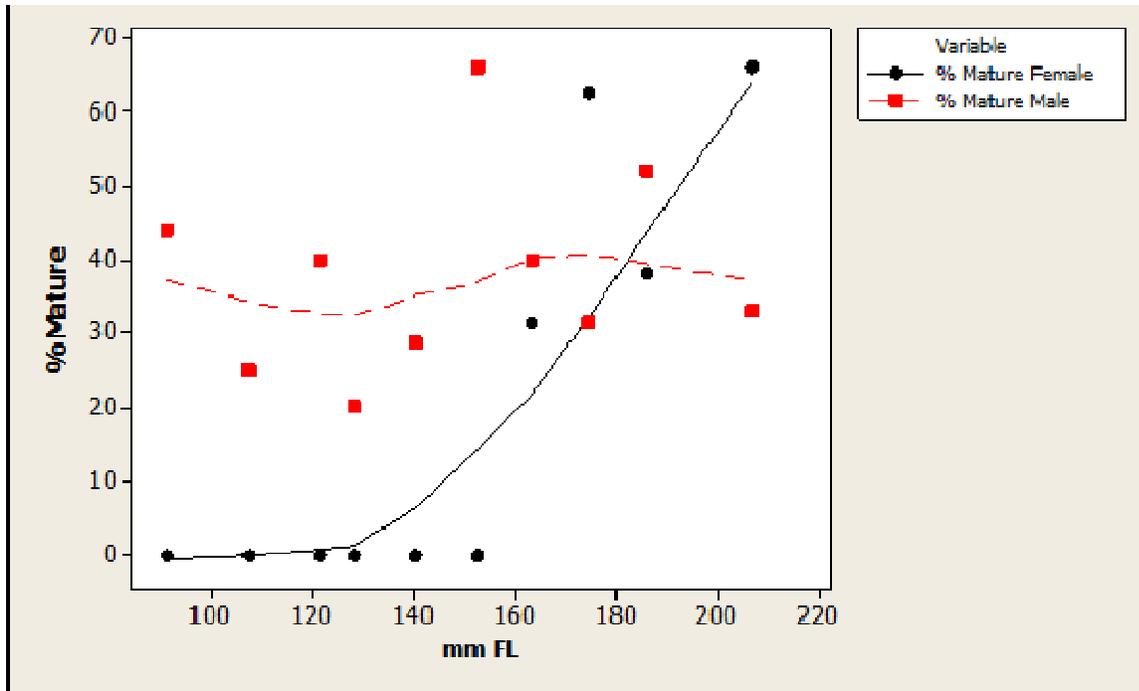


Figure 9 Proportion of mature males and females by size-class.

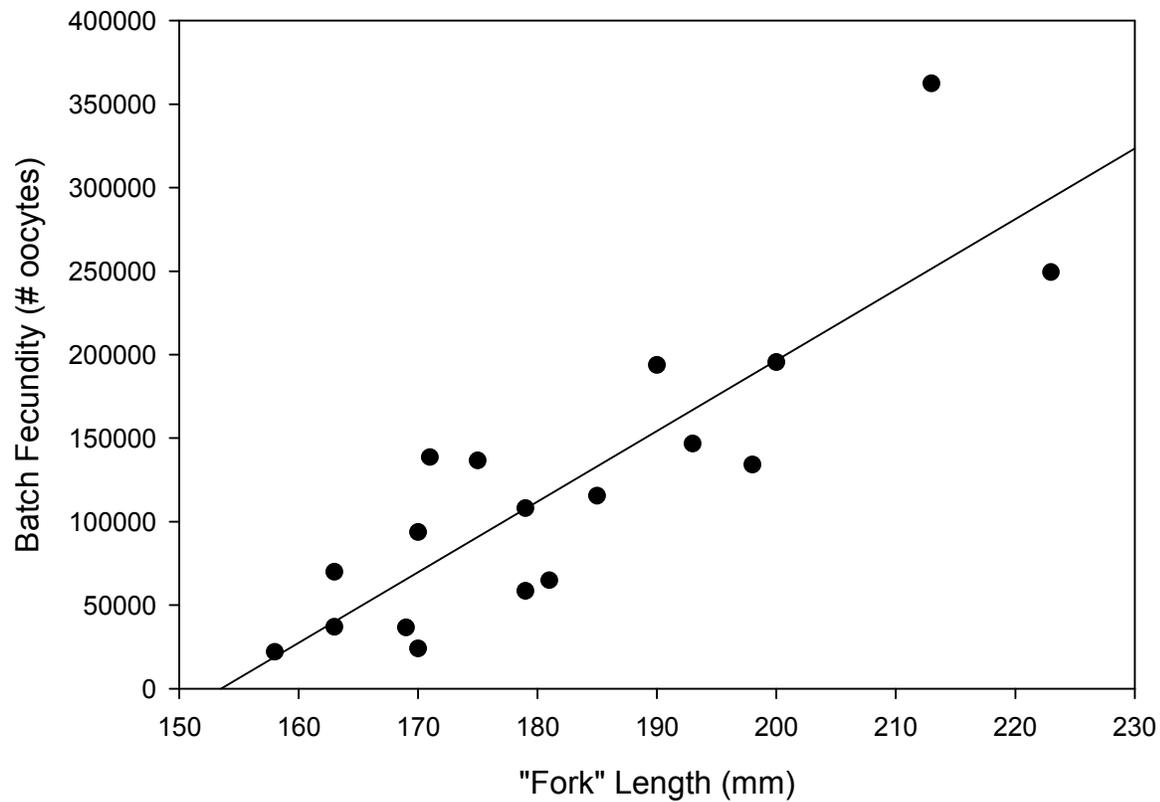


Figure 10 Size vs. batch fecundity. $BF = -648,588.17 + FL(4226.06); r^2 = 0.73.$

Table 2 Area surveyed in marine reserves and nearby comparison sites.

| Reserve Site | Area Surveyed (m ²) | Fished Site | Area Surveyed (m ²) |
|----------------------|---------------------------------|-----------------|---------------------------------|
| Hanauma Bay | 25,350 | Moanalua Bay | 23,354 |
| Honolua-Mokuleia Bay | 5,514 | Kapalua Bay | 3,201 |
| Lapakahi | 2,801 | Mahukona | 3,201 |
| Waialea Bay | 3,515 | Anaehoomalu Bay | 2,147 |
| Total | 25,350 | | 23,354 |

Fourteen individuals from marine reserves exceeded the asymptotic length of the vonBertalanffy growth equation, preventing back-calculation to age. These could not be included in a mortality estimate. Mortality plots for the remaining individuals are presented in Figure 11. A expected, total mortality in marine reserves was lower than that in areas open to fishing, thus allowing fishery-independent estimation of fishing mortality. Total quarterly mortality in marine reserves, which equals natural mortality, is 0.53549. Total quarterly mortality in fished areas is 0.68170. Quarterly fishing mortality is 0.14610, or about 20% of all mortality, in fish aged one through two years. The scatterplot for marine reserves, plus the additional 14 individuals exceeding the asymptotic length of the growth equation (which are presumably older than the individuals included in the scatterplot), suggests that mortality decreases after 2.5 years; however we have too few data to reliably estimate mortality in these older age groups. *Acanthurus triostegus sandvicensis* appears to have a maximum life span exceeding four years (Figure 11).

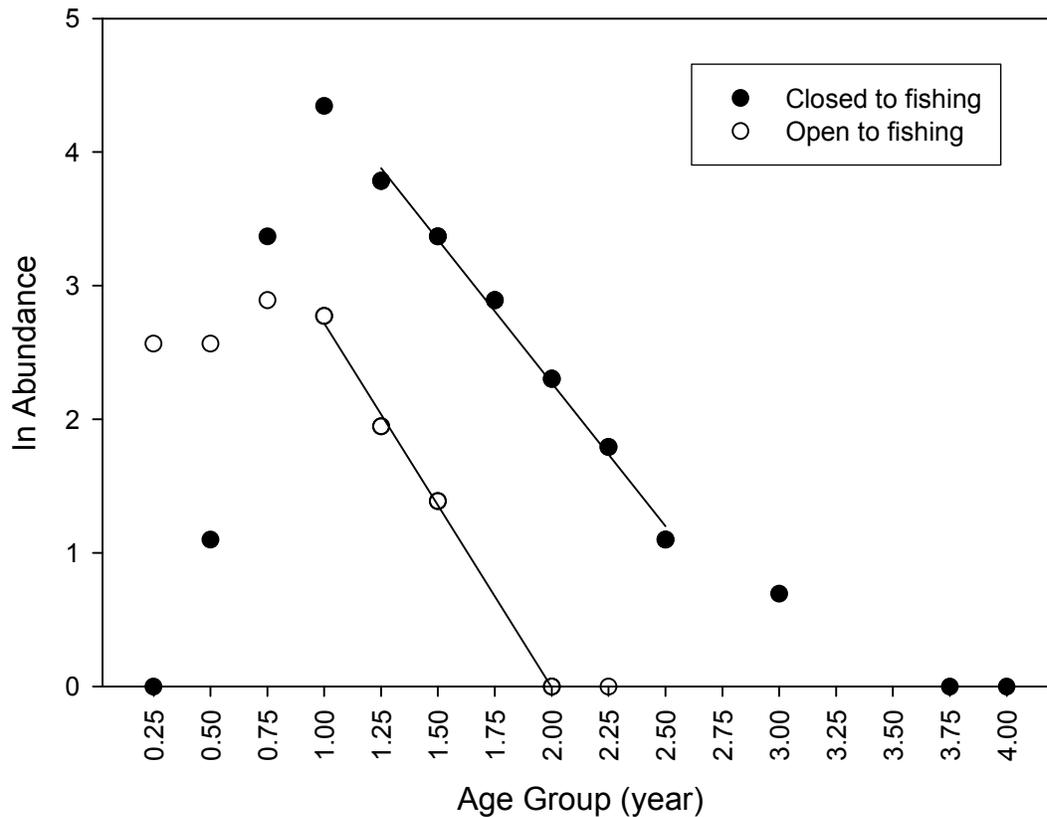


Figure 11 Quarterly mortality estimates for manini, *Acanthurus triostegus sandvicensis*, in reserves (filled circles) and fished (open circles) areas. Total mortality (Z) in reserves = 0.53549, in fished areas = 0.68170.

DISCUSSION

The overarching goal of this project was to provide the life history information necessary to estimate biomass production and reproductive output of manini under various management regimes. To obtain rough estimates of production, managers could combine the information presented here with density estimates, easily obtained from fish censuses, while assuming that age structure is the same as that reported here. Because of the low number of manini encountered during videogrammetry work in fished areas, however, we feel that more robust production estimates would result from further videogrammetry work while conducting fish censuses.

Certainly, the mortality estimates presented here would be improved with additional surveys. These estimates were based on the assumptions of constant survival and constant recruitment. These assumptions cannot be evaluated without larger sample size and/or a longer study period. Additional size structure data would also help resolve whether natural mortality declines after 2.5 years, as suggested by Figure 11.

Although we attempted to chemically mark otoliths, we were not able to validate the periodicity of increment formation in time for this report. We assumed the increments we observed were formed on a daily basis. Because the number of pre-settlement increments closely match those observed by Basch (unpublished data), we feel this assumption is reasonable.

The growth equation presented in Figure 6 is a composite of males and females from three islands. There are no obvious sex- or site-based differences in growth; however additional specimens should be examined before a hypothesis of no growth differences is rejected.

The oldest fish included in the growth analysis was approximately 2.25 years old. Age estimates obtained by transforming size estimates (from laser videogrammetry) suggest manini attain 4 years. The latter estimate is likely to be low because 14 individuals recorded during laser videogrammetry surveys were significantly larger (up to 17 mm) than the asymptotic length of the vonBertalanffy equation, thus preventing age estimates for the largest individuals observed.

The physical sex ratio observed here (43:57) contrasts with that observed by Randall (1961) who found about 66% of adults captured were males. Claisse et al. (in prep) found that males and females of the surgeonfish *Zebrasoma flavescens* occupy different habitats and depths. Thus, it is possible that the differences in sex ratio may reflect sex-specific habitat associations, as the majority of fishes collected in this study were caught from depths less than 20 feet whereas those of Randall (1961) were collected from 30 to 90 feet.

The operational sex ratio for fish collected in this study was male-biased (63:37). This bias can be attributed to the fact that males mature at a smaller size than females (<90 mm vs. 164 mm) and that the majority of specimens collected for life history analysis (154 mm) were below L_{50} for females but well-above that for males. Because the proportion of reproductive females changes predictably with length, resource managers may be able to roughly estimate sex ratio of surveyed *A. triostegus sandvicensis* populations based on size alone.

The linear size-fecundity relationship reported here (Figure 10) is unusual among fishes. More commonly, fecundity is an approximately cubic function of length. We obtained few gravid females in the larger size range, which should be expected given that individuals rarely exceed 200 mm (Randall 2001). However, additional large specimens have the potential to alter the shape of the size-fecundity relationship.

If the linear relationship holds, a small mature female would contribute proportionately more to lifetime reproductive output than would be expected for species with a cubic size-fecundity relationship. This possibility, combined with our estimate of female size at maturity suggests an issue for immediate consideration by resource managers: The current size limit for manini is 127 mm, which is well below the L_{50} of 164 mm. Although Randall (1961) reported a mature 122.7 mm FL female (as estimated from the morphometric relationships presented in Table 1), it appears that the vast majority of females enter the fishery before having had the chance to reproduce.

ACKNOWLEDGMENTS

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Heather Leba, Collin Olito, Erin Cox and Joanna Philippoff collected or donated specimens. Hanauma Bay Nature Preserve and the Hawaii Division of Boating and Ocean Recreation allowed us access to Hanauma Bay on days it was closed to the public. The Hawaii Division of Aquatic Resources (Tony Montgomery in particular), Leeward Community College (Frank Stanton in particular), and the University of Hawaii Dive Safety Program donated vessel support for field work in Hanauma and Maunalua Bays. The Hawaii Institute of Marine Biology provided access to vessels and SCUBA equipment for work in Kaneohe Bay. Russell Sparks and Darla White provided logistical advice/support on Maui. Lahaina Divers supplied SCUBA tanks at a reduced rate on Maui. Bill Walsh, Luisa Castro, and Dane Maikui provided logistical advice/support on Hawaii. Kassi Cole allowed the use of her laboratory. North Hawaii Community Hospital provided formalin and biopsy jars for our fieldwork on the Big Island. Otoliths were examined at the Biological Electron Microscopy Facility. Tina Carvalho provided SEM training to our assistants. The Hawaii Undersea Research Laboratory provided access to closed-circuit rebreathers. This work was conducted under the University of Hawaii IACUC protocol 06-058.

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