Estimates of body sizes at maturation and at sex change, and the spawning seasonality and sex ratio of the endemic Hawaiian grouper (*Hyporthodus quernus*, F. Epinephelidae)

Edward E. DeMartini (contact author)[1]

Alan R. Everson[2]

Ryan S. Nichols[1]

Email address for contact author: edward.demartini@noaa.gov

[1] National Marine Fisheries Service
Pacific Islands Fisheries Science Center
Hawaii Research Center
99-193 Aiea Heights Drive, Suite 417
Aiea, Hawaii 96701

[2] National Marine Fisheries Service
Pacific Islands Regional Office
1601 Kapiolani Boulevard, Suite 1110
Honolulu, Hawaii 96814

Abstract—A case study of the reproductive biology of the endemic Hawaiian grouper or hapu'upu'u (*Hyporthodus quernus*) is presented as a model for comprehensive future studies of economically important epinephelid groupers. Specimens were collected throughout multiple years (1978–81, 1992–93, and 2005–08) from most reefs and banks of the Northwestern Hawaiian Islands. The absence of small males, presence of atretic oocytes and brown bodies in testes of mature males, and both developed ovarian and testicular tissues in the gonads of five transitional fish provided evidence of protogynous hermaphroditism. No small mature males were collected, indicating that Hawaiian grouper are monandrous (all males are sex-changed females). Complementary microscopic criteria also were used to assign reproductive stage and estimate median body sizes (L_{50}) at female sexual maturity and at adult sex change from female to male. The L_{50} at maturation and at sex change was 580 ±8 (95% confidence interval [CI]) mm total length (TL) and 895 ±20 mm TL, respectively. The adult sex ratio was strongly female biased (6:1). Spawning seasonality was described by using gonadosomatic indices. Females began ripening in the fall and remained ripe through April. A February–June main spawning period that followed peak ripening was deduced from the proportion of females whose ovaries contained hydrated oocytes, postovulatory follicles, or both. Testes weights were not affected by season; average testes weight was only about 0.2% of body weight—an order of magnitude smaller than that for ovaries that peaked at 1–3% of body weight. The species' reproductive life history is discussed in relation to its management.

Manuscript submitted 24 July 2010.
Manuscript accepted 3 December 2010.
Fish. Bull. 109:123–134 (2011).

Epinephelid groupers comprise about 160 species of economically valuable and ecologically important predatory fishes distributed worldwide in subtropical and tropical seas. They exhibit a variety of sexual patterns, including sequential hermaphroditism in which mature adults change sex (Erisman et al., 2010). The importance of information on adult sex ratio (Coleman et al., 1996; Heppell et al., 2006), spawning seasonality (Sadovy et al., 1994; Domeier and Colin, 1997), and body sizes at sexual maturity and at sex change (e.g., Pears et al., 2007) have been identified as important when developing management plans for sustainable extraction of sequentially hermaphroditic groupers. To date, however, comprehensive and self-contained evaluations based on all of these key life-history elements are rare. Few studies (e.g., Mackie, 2000; Chan and Sadovy, 2002; Erisman et al., 2010) have estimated body size at sex change, as well as size at first maturation for protogynous (female-first sex-changing) groupers, and few (e.g., Brule et al., 2003) have precisely quantified size at sex change. Only such comprehensive studies can provide the data necessary for meaningful exploration of patterns of sex and gonadal allocations that can provide additional insights into the behavioral responses of these fishes to fishing pressure (Alonzo and Mangel, 2005).

One of the more intriguing sex allocation patterns recently discovered for protogynous epinephelid groupers is the apparent covariation between adult body size and aggregation spawning. Medium- to large-body (>50 cm total length, TL) groupers of the genus *Epinephelus* and allied genera typically spawn in aggregations, while small-body species (e.g., within the genus *Cephalopholis*) tend not to spawn in aggregations (Sadovy et al., 1994; Sadovy, 1996). Some grouper species, moreover, have only one type of male, derived from sex-changed adult females (these species are termed "monandric"), whereas other species have an additional male type ("diandric") that is directly derived from the juvenile phase (Sadovy de Mitcheson and Liu, 2008).

The Hawaiian grouper (*Hyporthodus quernus*) (Seale) (Epinephelidae; Craig and Hastings, 2007; Smith and Craig, 2007) is endemic to the Hawaiian Islands and Johnston Atoll (a no-take zone within the Pacific Remote Island Area Monument since 2009, at 16°45'N lat., 169°31'W long.; Fig. 1) and is the only epinephelid indigenous to the Hawaiian Archipelago. The species has historically been a major component of both the North-

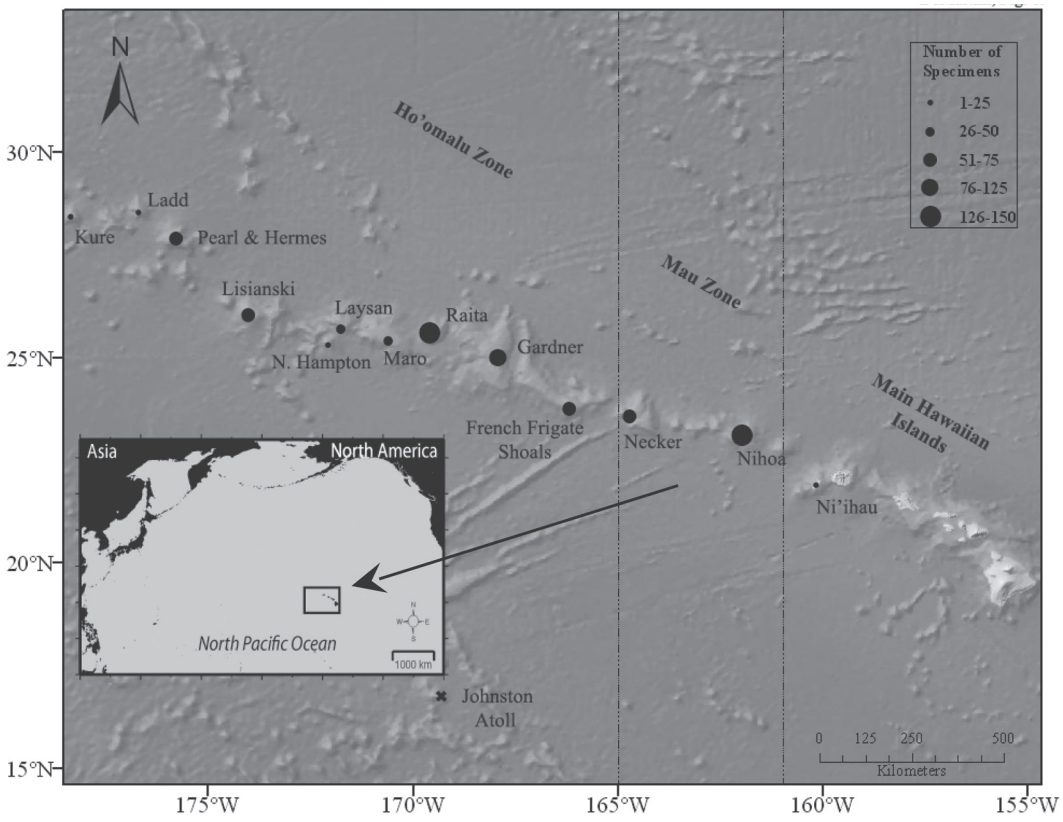

Figure 1

Map of the Hawaiian Archipelago and the North Pacific basin. The Main Hawaiian Islands bottomfish fishery zone and the Ho'omalu and Mau management zones of the former Northwestern Hawaiian bottomfish fishery are delineated by vertical dashed lines. Only fish collected from the Northwestern Hawaiian Islands and Niihau-Kauai (the island adjacent to and E-NE of Niihau)—i.e., west of about 160°W longitude—were evaluated for this study; >70% of these were from the Ho'omalu Zone, west of 165°W. The relative sizes of the black circles represent the number of Hawaiian grouper (*Hyporthodus quernus*) collected for analysis.

western Hawaiian Islands (NWHI) and Main Hawaiian Islands (MHI) handline bottomfisheries and, since closure of the fishery in both Ho'omalu and Mau Management Zones of the NWHI in June 2010, continues to be one of the targeted "deep-7" bottomfish species in the MHI. All deep-7 species have been protected by emergency fishery closures for 5 to 7 months during spring–summer of 2006–09 (http://www.hawaiibottomfish.info/index.htm). The MHI stocks of Hawaiian grouper have been identified as particularly stressed (Moffitt et al.[1]) based on the criterion of a low and recently declining SPR (spawning potential ratio: Goodyear, 1993) that has historically been used in Hawaii bottomfish stock assessments. Over the past half-decade, the catches of this grouper in the MHI have been variable and low compared to prior years, ranging from about 8000 to 16,000 pounds (3629–7257 kg; Moffitt et al.[1])—a small but valuable fraction of the recent (2007–2010) Total Allowable Catch limits set for MHI bottomfishes of 178–254 thousand pounds (80,740–115,200 kg) per year (http://www.hawaiibottomfish.info/BFnews_vol6_final.pdf; accessed July 2010). Like many large groupers (Huntsman et al., 1999), the Hawaiian grouper is a species of conservation concern (Morris et al., 2000). It is a slow growing, long-lived, and late maturing species (Nichols and DeMartini[2]), and preliminary evidence (Everson[3]) has indicated that, at least in the NWHI

[1] Moffitt, R., D. Kobayashi, and G. DiNardo. 2006. Status of Hawaiian bottomfish stocks, 2004. Pacific Islands Fisheries Science Center, National Marine Fisheries Service, NOAA Admin. Rep. NMFS-PIFSC-H-06-01, 45 p. Pac. Isl. Fish. Sci. Cent., 2570 Dole Street, Honolulu, HI 96822-2396.

[2] Nichols, R. S., and E. E. DeMartini. 2008. Preliminary estimates of age and growth for the endemic Hawaiian grouper (Hapu'upu'u, *Epinephelus quernus*, F. Serranidae). Pacific Islands Fisheries Science Center, National Marine Fisheries Service, NOAA Admin. Rep. NMFS-PIFSC H-08-06, 19 p. Pac. Isl. Fish. Sci. Cent., 2570 Dole Street, Honolulu, HI 96822-2396.

[3] Everson, A. R. 1992. Sexual identity and seasonal spawning of Hapu'upu'u, *Epinephelus quernus*, in Hawaii. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA Administrative Report NMFS-PIFSC H-92-13, 12 p. Southwest Fish. Sci. Cent., 2570 Dole Street, Honolulu, HI 96822-2396.

(Fig. 1)—the leeward low island region of the Hawaiian Archipelago—individuals are protogynous sequential hermaphrodites, like most epinephelids elsewhere (Shapiro, 1987; Sadovy and Domeier, 2005; Sadovy de Mitcheson and Liu, 2008). To date, however, data on body size at first sexual maturity, spawning seasonality, and adult sex ratio of Hawaiian grouper have not been adequately quantified, and information on its possible size-at-sex-change and sex and gonadal allocation patterns are lacking.

Our primary goal is to provide a comprehensive case study of the reproductive life history of Hawaiian grouper in the NWHI in which all of the aforementioned metrics are estimated (one of the few such studies so for any grouper). All of these metrics in combination are key elements necessary for conducting comprehensive reproductive studies of groupers elsewhere. We also evaluate the gonad indices of Hawaiian grouper in detail to explore possible relations among gonadal allocation pattern, male type, adult body size, and aggregation spawning.

Materials and methods

Fish collections and measurements

Hawaiian grouper were collected during three ("early," "mid-term," "recent") capture series. All captures were made with similar (hydraulic-powered) handline gear. Early specimens were caught by fisheries-independent research vessels during the period from May 1978 to August 1981. Mid-term specimens were obtained by fisheries-independent research cruises during August and December 1992 and June and September 1993. Most recent (October 2005–June 2008) specimens were purchased from contracted Hawaii-based commercial bottomfishing vessels and were fishery-dependent. All fish were collected from the NWHI, except 26 specimens (3.5% of all fish) collected from Niihau-Kauai at the northwestern edge of the MHI (Fig. 1). The limited availability of fish of a sufficient range of sizes from elsewhere in the MHI precluded separate analyses for fish from this region of relatively high fishing effort. For our analyses, we used a total 745 fish collected from the NWHI and Niihau-Kauai only (Fig. 1).

Body length (total length, TL, from tip of snout to posterior margin of caudal fin) was measured (mm) for each fish either aboard ship (research cruises) or at a fisheries laboratory ashore (for commercial specimens). The error of length measurements was about 0.5 cm. Fish were weighed (total round weight, RW, including all viscera, to 10 g) by using a bench scale. Additional details of specimen and shipboard data collection are provided elsewhere (Nichols and DeMartini[2]; Everson[3]) The Kolmogorov-Smirnov two-sample test (Zar, 1984) was used to compare body-length distributions and numbers of fish by month of collection between the total sample and a subsample used for histological examination.

Gonad extraction and processing

Both left and right lobes of gonads were either dissected from freshly caught specimens aboard the research ship or from iced fresh fish (commercial specimens) at a shore laboratory in Honolulu. Before further processing, gonads were weighed (to the nearest g). The gonads of 611 fish were examined microscopically. Histological slides were prepared for 604 fish including early and recent specimens (100 and 504 fish, respectively); no gonads from mid-term specimens were examined microscopically. In preparation for histological examination, gonads were first fixed in 10% (seawater buffered) formalin for a minimum of 2 months. A segment from the mid-region of one lobe (either left or right, random choice, including lumen and gonad wall) was then cut and placed in a histology cassette and sent to a contracted laboratory for further processing. The contractor then dehydrated tissues in an alcohol series, embedded them in paraffin wax, sectioned them at 6–7 microns, stained them with Harris's hematoxylin, and counter-stained them with eosin (Hunter and Macewicz, 1985). One slide of 2–6 (mode and median of 4) successive sections was prepared for each gonad specimen. The gonads of several ripe female specimens from the early series were prepared and examined for reproducibility of reproductive scores based on sections from the anterior, middle, and posterior regions of ovaries.

Sexual identity and reproductive status

Gonad appearance based on conventional macroscopic criteria (West, 1990) was insufficient to distinguish either sexual identity or maturation, and all determinations of sexual identity and maturation for Hawaiian grouper presented herein were based on standard microscopic criteria (West, 1990). After sexual identity was determined, each specimen was scored for reproductive stage by following a protocol (Table 1) similar to those used by others (Pears et al., 2007, and references therein) to evaluate gonadal stages of protogynous epinephelids. Key criteria included the presence of oogonia and primitive testicular tissue in the gonads of bisexual juveniles, the presence of brown body atretic structures in testes of mature sex-changed males as evidence of prior female function, and the occurrence of transitional individuals whose gonads contained both developing male and regressing female tissue types (Sadovy de Mitcheson and Liu, 2008). Fish were classified as mature females if ovaries were categorized at stages 3 through 6 (Table 1). The staging of oocytes was complemented by estimation of the median diameter of the largest mode of viable oocytes present in ovarian tissues by using a technique developed for Hawaiian bottomfishes (Lau and DeMartini, 1994). Size distributions of all yolked oocytes were described for a random subsample of ripe stage-5 fish and evaluated for multimodality by using the Kolmogorov-Smirnov one-sample test (Zar, 1984). Males were considered mature if at stage 7 or greater

Table 1

Diagnostic microscopic criteria (after Pears et al., 2007) and key to classification of sexual identity, maturation, and reproductive status of female and male Hawaiian grouper (*Hyporthodus quernus*), including gonadosomatic indices (*GSI*s) for each sex and diameter of the most advanced group of oocytes present in female ovaries. HYD=hydrated oocytes; POF=postovulatory follicles; *GFBW*=gonad-free body weight; *OD*=diameter of largest mode of viable oocytes; SE=standard error; *n*=sample size.

Gonadal maturity	Stage (0–10)	Microscopic characteristics	Mean ±SE (*n*) *GSI* (as % of *GFBW*)	Mean ±SE (*n*) *OD* (μm)	Mature? (yes/no)
Immature bisexual	0	Vestigial gonad: sex indeterminate; primitive sex cells of either one or both sexes present	0.08 ±0.009 (36)	44 ±9 (3)	no
Immature female	1	Undeveloped ovary: only primordial (weakly nucleate) oocytes present	0.08 ±0.007 (71)	76 ±3 (28)	no
Developing female	2	Ovaries contain perinucleolar (cortical alveoli-stage) oocytes with nucleolated nuclei	0.19 ±0.015 (85)	110 ±4 (22)	no
Ripening female	3	Oocytes largely previtellogenic; some eosin (pink) staining yolk vesicles	0.54 ±0.079 (13)	252 ±15 (13)	yes
Ripe female	4	Oocytes vitellogenic but not fully yolked; neither HYDs nor POFs present	0.70 ±0.129 (12)	313 ±13 (12)	yes
Imminent female	5A	Fully yolked oocytes; may have migratory nucleus; HYDs present	1.41 ±0.061 (151)	480 ±11 (29)	yes
Spawning female	5B	Fully yolked oocytes; POFs (or POFs and HYDs) present	0.23 ±0.281 (34)	533 ±12 (22)	yes
Resting female	6	Regressing or inactive, but previously spawned ovary: perinucleolar oocytes present; atretic oocytes, brown bodies, or both also present	0.48 ±0.031 (138)	111 ±6 (23)	yes
Transitional male	7	Gonad contains inactive ovarian tissue; brown bodies usually present along with developing testicular tissue (Sertoli cells in sperm crypts)	0.61 ±0.163 (5)	—	yes
Developing male	8	Recrudescing testes with spermatogonia; brown bodies may be present	0.21 ±0.016 (22)	—	yes
Active male	9	Ripe testes with spermatozoa in ducts or sinuses; brown bodies may still be present	0.21 ±0.015 (31)	—	yes
Resting male	10	Resting testes with evidence of prior spawning; sperm crypts present but no active spermatogenesis	0.32 ±0.102 (2)	—	yes

(Table 1). All histological preparations were examined by a single experienced reader (DeMartini).

Estimation of body sizes at maturation and at sex change

Nonlinear regression with maximum likelihood estimation was used to relate fish body lengths to sexual maturity and to size at sex change (Proc nlin; SAS for Windows, vers. 9.1, SAS Inst., Inc., Cary, NC). Specimens were binned by 10-mm length classes; the proportions mature (female, male) were then related to length class by using the 2-parameter logistic model,

$$P_x = 100 / (1 + \exp^{(a - b \cdot \mathrm{TL})}), \quad (1)$$

where a and b are fitted constants;
P_x = percentage mature at x TL; and
$L_{50} = (-a/b)$.

The proportions of mature and sex-changed fish were fitted by maximum likelihood by using Marquardt's algorithm. Mean proportions per 10-mm length class were weighted by square root of the respective sample size. Estimates of size at sexual maturity were further compared between early and recent collection series by using likelihood ratio statistics (Quinn and Deriso, 1999). A maximum likelihood chi-square test (Quinn and Keough, 2002) was used to compare adult sex ratios between collection series.

Spawning seasonality

Seasonality of spawning was gauged with a combination of gravimetric evidence of gonadal ripening and histological evidence of either recent or imminent spawning by females. A gonadosomatic index, $GSI=(GW/GFBW)\times100\%$, where GW=damp gonad weight and gonad-free body weight, $GFBW$=round weight minus GW, aggregated by month (years pooled) for each sex of adult fish, provided gravimetric evidence of gonadal growth. This simple (weight proportional) GSI was used to visualize temporal patterns. Because of the likely dynamic influence of reproductive stage on proportional gonad weight-to-body size, the calculation of a "relative gonad index" (RGI) was required in order to scale the relation across gonad maturation stages of mature females. This calculation removes the confounding influence of maturation stage on the gonad-to-body-size relation (Erickson et al., 1985). One-way ANCOVA (Zar, 1984) with month as a fixed factor (12 levels) and either TL or $GFBW$ as covariate was used to evaluate the potential effect of month on the RGI, controlling for fish size. ANCOVA was followed by a robust multiple comparison test (Ryan-Einot-Gabriel-Welsch [REGW] multiple-range test; Quinn and Keough, 2002) to quantitatively evaluate patterns among months. Plots of the proportional incidence for recently spawned and "imminent" (ready-to-spawn) females among all mature females constituted histological evidence of spawning by individual fish.

Results

Seventy percent of the Hawaiian grouper examined were from the Ho'omalu Zone, west of 165°W; another 26% were from the Mau Zone, between 165° and 161°W in the NWHI (Fig. 1). Fish from the early, mid-term, and recent series comprised about 18%, 11%, and 71%, respectively, of those collected. Fish were obtained in all months of the year (ranging from 20 in January to 108 fish in April); and the distribution of specimens among months of collection was indistinguishable between the parent sample and a subsample used for histological examination. The gonads of 20 (January) to 92 (April) specimens per month were examined microscopically. The mean and median body lengths of specimens was 661 mm TL (ranging from 241 to 1103 mm). Sizes of fish used for histological examination generally resembled those in the parent sample (mean=659 mm, median=652 mm, range 258 to 1103 mm TL; Kolmogorov-Smirnov two-sample test: $P>0.10$).

Comparisons of collection series

Estimated median body size at sexual maturation varied by barely 3 mm between the early (576.0 ±13.4 [standard error, SE] mm TL) and recent (579.1 ±4.0 mm TL) collection series. We considered this sufficient reason to pool samples, given the small magnitude of length measurement error, despite a marginally significant F-statistic ($F_{30,91}=1.97$, $P=0.02$). Estimates of adult sex ratios also were indistinguishable between these two collection series (maximum likelihood chi-square: $\chi^2=1.40$, df=1, $P=0.24$). Because neither metric differed between series, we pooled all fish collections and present single estimates for each of these and related variables based on all combined capture series.

Gonadal and sex allocation patterns

Gonadal patterns Four of the 604 specimens prepared for histological examination were alimentary tract tissues collected in error and were discarded. A small minority of the remaining 600 valid histological specimens were bisexual juveniles with vestigial gonads containing chromatin nucleolar oogonia, undeveloped spermatic tissue, or both (Fig. 2; Fig. 3, A and B). Most of these fish were <450 mm TL (Fig. 2). Among the remaining specimens whose gonads were more developed, about one-third of all females had immature but clearly all-female characteristics, another two-thirds were mature females, five were developing males with transitional gonads, and 55 (9.2%) were fully mature males (Table 1; Fig. 2; Fig. 3, C and D, E and F). Developing oocytes progressed from a cortical alveolus (perinucleolar) and previtellogenic stage through vitellogenesis and ovulation, with concomitant increases in ovarian mass and oocyte diameter (Table 1). The ovaries of each of three stage-5 females had multimodal size distributions of yolked or yolking oocytes (reject Ho: distribution unimodal normal; Kolmogorov-Smirnov one-sample test, all $P<0.001$; Fig. 3C). The gonads of all males contained from one to dozens of brown bodies; in many cases, these were recognizable as gamma-stage (Hunter and Macewicz, 1985) atretic structures (Fig. 3, E and F). No mature males smaller than 753 mm TL were encountered. Gonads were fused posteriorly and the gonads of fish of all sizes and each sex contained a membrane-lined ovary-like lumen (e.g., bisexual juveniles and mature males: Fig. 3, A and E). Maturation scores were identical and sizes of oocytes were indistinguishable from the respective anterior, middle, and posterior sections of the ovaries of three ripe females from the early collection series.

Body sizes at maturation and at sex change

Maturation Body size at first sexual maturation was estimated by using the pooled sample of all immature (bisexual and female) fish and all recognizably mature females. On the basis of Equation 1, proportional body length at 50% maturity was best described by the logistic equation

$$P_{50}=100 / (1 + \exp^{(0.0286 - 16.574 \cdot L_{50})}),$$

with $SE_a=0.003$, $SE_b=1.749$, $r^2=0.93$, $P<0.0001$, and $n=540$.

Body length (L_{50}) at female maturation was 580 ±8 mm TL (95% confidence interval [CI]) (Fig. 4). The respec-

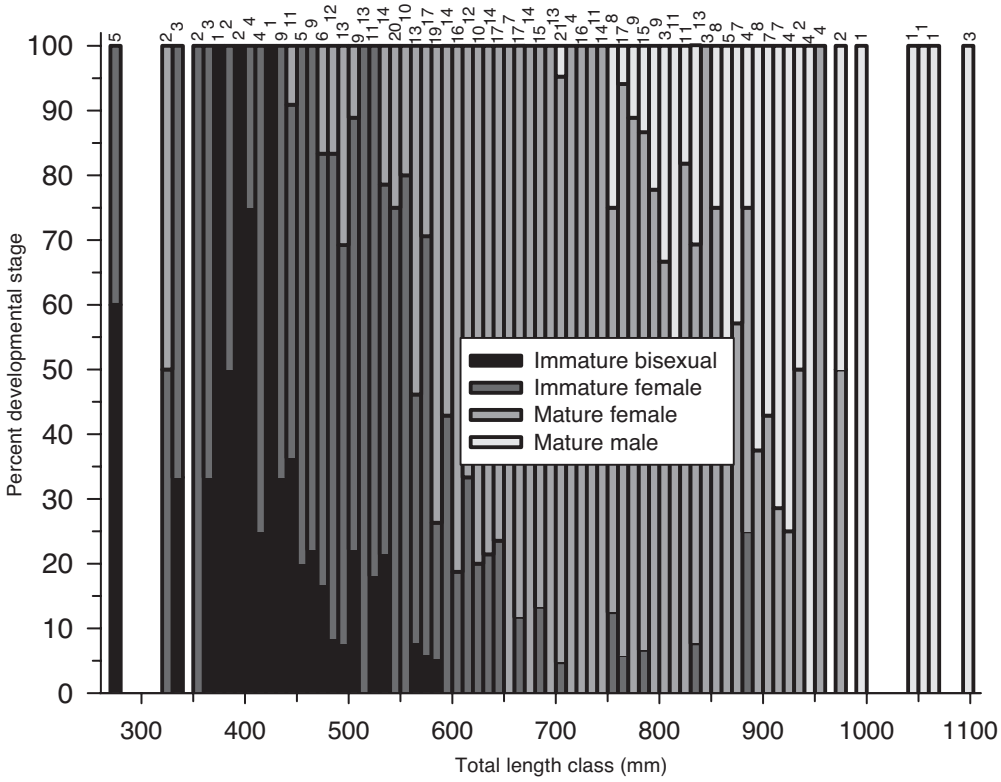

Figure 2

Proportions of immature bisexual, immature female, mature female, and mature male Hawaiian grouper (*Hyporthodus quernus*) present in each 10-mm length class of the 600-specimen subsample used for histological examination. Numbers above bars indicate numbers of specimens examined.

tive mean and median body lengths of all adult female specimens were 703 and 700 mm TL. The smallest and largest observed adult female individuals were 328 and 977 mm TL. The smallest female whose ovaries contained hydrated oocytes was 492 mm TL.

Sex change Body size at adult female-to-male sex change was appraised using all recognizably mature adult fish. The body length-at-female-to-male sex change relation was best described by the logistic equation

$$P_{50}=100 / (1+\exp^{(0.0153-13.653\cdot L_{50})}),$$

with $SE_a=0.002$, $SE_b=1.858$, $r^2=0.80$, $P<0.0001$, and $n=397$.

Body length (L_{50}) at female-to-male sex change was 895 ±20 mm TL (95% CI) (Fig. 5). The mean and median body lengths of all adult male specimens were 895 and 891 TL, respectively. The smallest and largest adult male fish encountered were 753 and 1103 mm TL. The five transitional fish ranged from 760 to 913 mm TL. Many (38%) of the mature specimens were between the sizes of the smallest male and the largest female fish.

Spawning seasonality

The slopes of the relations between gonadal and body weights and between gonadal weights and body lengths did not differ among females in prespawning, spawning, and postspawning maturation stages (ANCOVA of *GFBW* and maturation stage effects on *GW*: *GFBW*-by-maturation and TL-by-maturation interaction effects—$F_{2,327}=2.36$ and 2.30, respectively; accept H_o: slopes equal at $P=0.10$). The intercepts of these same relations, however, differed (ANCOVA: maturation stage effects—$F_{2,329}=114.0$ and 113.2, respectively; reject H_o: intercepts equal at $P<0.0001$). The relation between gonadal weight and body length provided the better fit and TL was used in subsequent analyses of monthly patterns. In these analyses we used *RGI*s, in which the pooled slope of the *GW*-to-TL relation (2.69266) was the scaling factor. *RGI*s indicated that most females began ripening in December and maintained elevated ovarian masses of 1–3% *GFBW* through April (1-way ANCOVA: month effect on *RGI*—$F_{11,324}=6.86$; reject H_o: all months equal at $P<0.0001$). Ovary weights peaked in January–March (REGW multiple-range test: January–March highest; $P<0.03$). Analyses with simple

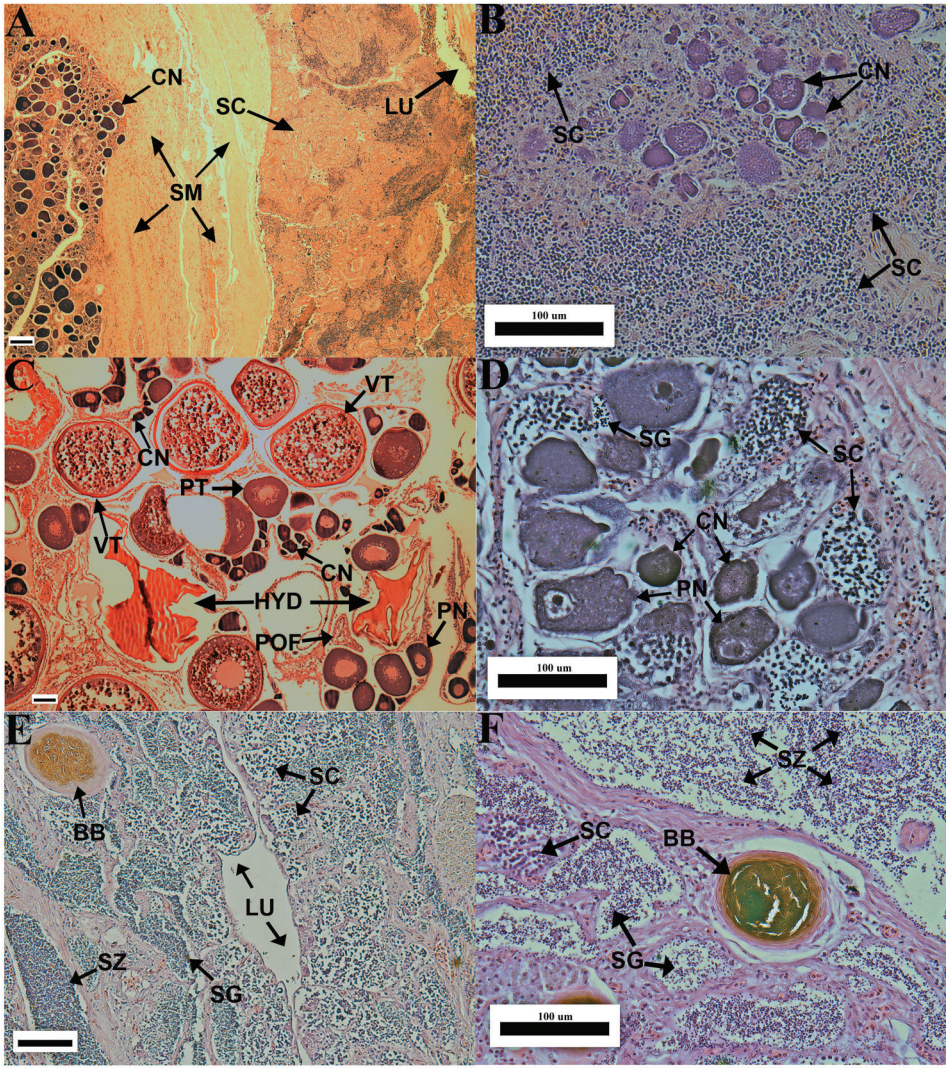

Figure 3

Photomicrographs of select representative gonads illustrating key reproductive stages of Hawaiian grouper (*Hyporthodus quernus*). (**A**) Vestigial bisexual, 333 mm total length (TL); (**B**) immature bisexual, 495 mm TL; (**C**) mature female, 624 mm TL; (**D**) transitional (female-male) mature fish, 913 mm TL; (**E**) mature male, 820 mm TL; and (**F**) mature male, 919 mm TL. CN=chromatin nucleolar oogonium; PN=perinucleolar oocyte; PT=previtellogenic oocyte; VT=vitellogenic oocyte; HYD= hydrated oocyte; POF= postovulatory follicle; SC=spermatocytes (Sertoli nurse cells); SG=spermatogonia; SZ=spermatozoa in sperm duct; SM=smooth muscle; BB=gamma-stage atretic "brown body"; LU=lumen. Black scale bars: 100 µm.

*GSI*s produced qualitatively similar results (Fig. 6) but the statistical clarity of monthly patterns improved when based on the more precise body-size–corrected *RGI*s.

The proportional incidence of active spawners, including "imminent" females ready to spawn (ovaries containing hydrated oocytes), recently spawned fish (ovaries containing recognizable [i.e., less-than-several-days-old; Fitzhugh and Hettler, 1995] postovulatory follicles), or both, also indicated a main February–June period of spawning with a peak in March (Fig. 7). Eleven (16%) of these 68 active spawners were captured outside of the February–June period (Fig. 7); nine of the 11 fish (caught during September–January) exceeded median body size at female maturity (range 550–833 mm). Adult female gonads averaged 1.35 and 1.15% of *GFBW* during the February–June peak and throughout the year, respectively (Fig. 6). Adult male gonads exhibited no seasonal change in weight and averaged 0.23% of *GFBW* throughout the year (Fig. 6).

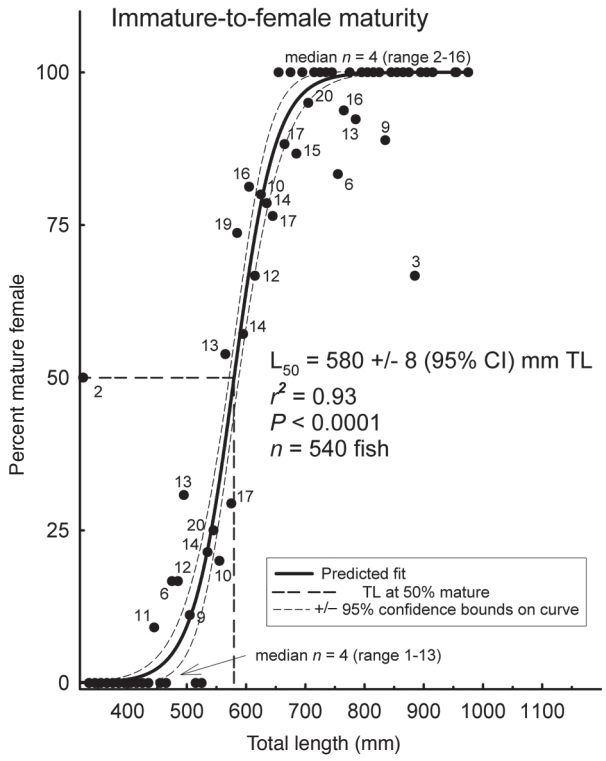

Figure 4

Estimated total length (TL) at which 50% of Hawaiian grouper (*Hyporthodus quernus*) first attain sexual maturity as females (L_{50}); estimates are for 540 fish: 36 immature bisexuals, 167 immature females, 337 mature females. Solid circles represent mean percentage mature by 10-cm length class; number of fish specimens in each length class is noted adjacent to its corresponding circle. Solid curved line represents the predicted best fit model; curved dashed lines enclose the 95% confidence bounds of the fitted line. The perpendicular dashed lines indicate estimated body length at median (50%) female sexual maturity.

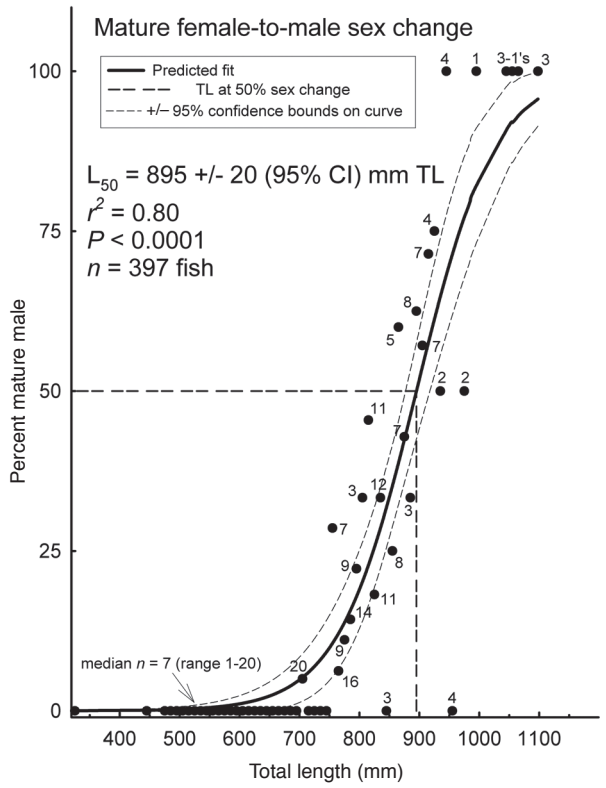

Figure 5

Estimated body size (length, TL) at which 50% of Hawaiian grouper (*Hyporthodus quernus*; 397 adult fish) changed sex from female to male. Key to symbols and lines are given as in Figure 4, except the perpendicular dashed lines indicate body length at median (50%) female-to-male sex change.

Sex ratio

For all specimens whose sex was verified histologically, the adult sex ratio was highly female biased (6.1 females per male; χ^2=205, df=1, P<0.0001). The ratio became progressively less female biased at body lengths approaching the estimated median length at adult sex change from female to male.

Discussion and conclusions

Gonadal and sex allocation

Hawaiian grouper from the NWHI are protogynous sequential hermaphrodites. This conclusion is based on three lines of evidence: 1) the presence of undeveloped bisexual gonads in small (generally <45 cm TL) fish; 2) a total lack of small mature males; and 3) the presence of a lumen, posteriorly fused gonadal lobes, and brown body remnants of yolked oocytes in the gonads of relatively large mature males (Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008). The Hawaiian grouper might be expected to be a functional gonochore (i.e., sexes separate in the adult without a postmaturational sex change) because the species has a subtropical distribution and nontropical species within primarily tropical, sex-changing lineages of serranids are often gonochores (DeMartini and Sikkel, 2006). There was no evidence to indicate this, however. Many primitive serranines and even some epinephelines (e.g., Nassau grouper [*Epinephelus striatus*]: Sadovy and Colin, 1995) are functional gonochores (Sadovy and Domeier, 2005; Sadovy de Mitcheson and Liu, 2008).

The total absence of small mature male Hawaiian grouper, despite the large number of sample fish collected in all seasons, over multiple years, and throughout much of its geographic distribution, further indicates that it is most likely monandrous. All males appear to be sex-changed females; primary males derived from bisexual juveniles were never encountered.

Diandrous species of epinephelines have been described (*Plectropomus leopardus* and *P. maculatus* [Adams, 2003]; *Epinephelus coioides* [Grandcourt et al., 2009]; *E. andersoni* [Fennessy and Sadovy, 2002]; *Cephalopholis boenak* [Liu and Sadovy, 2004]; *C. taeniops* [Siau, 1994]) but seem to constitute a minority of species within the subfamily Epinephelinae. Diandry appears to be better represented among small-bodied species and genera, whereas monandry exemplified by Hawaiian grouper is consistent with the general pattern for large-bodied groupers. Most known species of medium to large epinephelid groupers also are aggregation-spawners (Samoilys and Squire, 1994; Sadovy et al., 1994), but nothing is known of the spawning habits of Hawaiian grouper. Large (e.g., 7–12% [Erisman et al., 2007]) male *GSI*s are typical in groupers in which sperm competition occurs within multiple-male spawning aggregations. The relatively small (<1%) testes weights (compared to ovaries) of Hawaiian grouper are typical of protogynous species (Molloy et al., 2007) and indicate that it spawns in single-male spawning groups that lack intense sperm competition (Sadovy et al., 1994). Although inconsistent with multiple-male spawning groups, relatively small testes size might reflect pair-spawning within aggregations and cannot be used as evidence either for or against aggregation spawning in the species (Domeier and Colin, 1997). Growing evidence indicates that monandry and pair-spawning within aggregations are the norm for epinephelids that are large enough to migrate and can monopolize females while pair-spawning at low male densities. Diandry and multiple-male group-spawning is relatively prevalent in smaller-bodied species that cannot tolerate the predation risk of migration to aggregation sites and that experience sperm competition at relatively high male densities.

We caution that our evaluation of sex allocation patterns for Hawaiian grouper is limited to fish in the NWHI. Other sex and gonadal allocation patterns may exist for populations in the windward, high main Hawaiian Islands where, among other

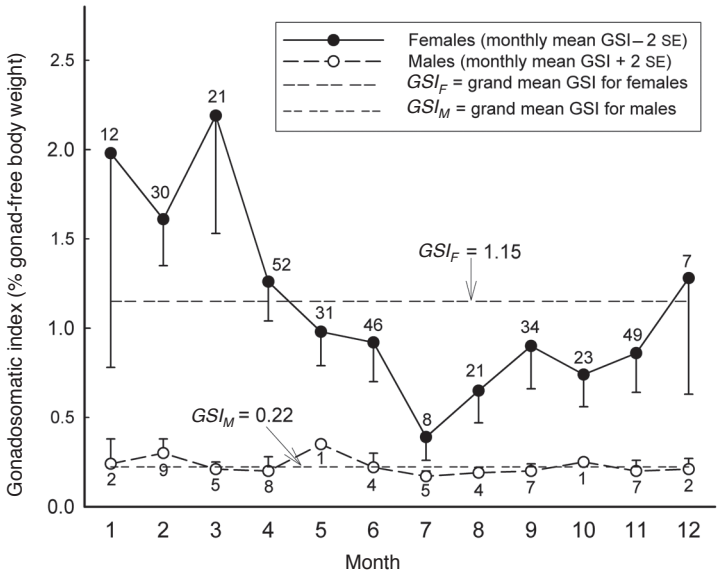

Figure 6

Monthly gonadosomatic indices for female (top: solid circles and line) and male (bottom: hollow circles and coarse dashed line) Hawaiian grouper (*Hyporthodus quernus*). The mean gonadosomatic indices for females (GSI_F) and males (GSI_M) are indicated by medium and fine dashed lines, respectively. The number either above or below each data point indicates sample size (number of fish). Vertical lines represent 2 standard errors (SE).

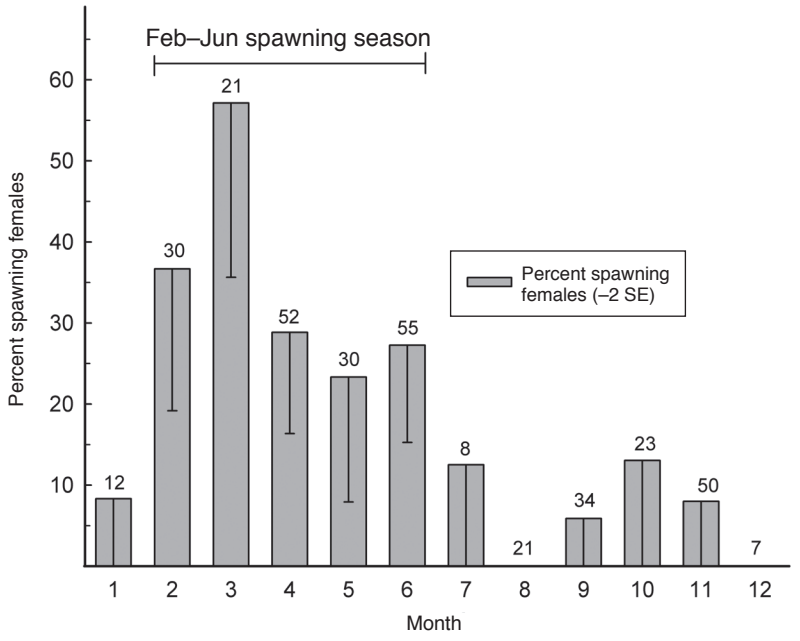

Figure 7

Proportional monthly incidence of actively spawning female Hawaiian grouper (*Hyporthodus quernus*) (i.e., those whose ovaries contained hydrated oocytes, postovulatory follicles, or both). The number above each histogram bar indicates sample size (number of fish). Vertical lines represent 2 standard errors (SE).

things, the species' depth distribution is appreciably deeper (DeMartini and Friedlander, 2004) and depth-related differences in benthic habitat are likely.

Body sizes at maturation and at sex change

All Hawaiian grouper apparently first mature as females at about 58 cm TL. Our estimate is close to a preliminary estimate (570.5 mm TL [Everson[3]]) based only on the same early series of histological slides. Maturation at 58 cm is equivalent to about 52% of a maximum body length of 110.6 cm TL (Seki, 1986). Most groupers mature at about 40–60% of maximum body length (Shapiro, 1987). A preliminary growth estimate for Hawaiian grouper (Nichols and DeMartini[2]) indicates that the median length at female maturation corresponds to an age of 6–7 yr.

On average, adult Hawaiian grouper change sex from female to male at about 89–90 cm TL. A preliminary growth curve indicates that this would be equivalent to an age of >20 yr, but size-at-age estimates for fish this large are imprecise (Nichols and DeMartini[2]) and any firm conclusion must await pending validations of older age estimates. Sex change thus occurs at about 81% of maximum body length. Our estimate of relative size at sex change approximates (perhaps coincidentally) the 80% predicted by using an empirical relationship between maximum body length and length at sex change derived by Allsop and West (2003) and based on data for diverse protogynous fish lineages.

Spawning seasonality and sex ratio

Evidence of spawning from gonad indices, together with estimates of the proportional incidence of spawning females, convincingly illustrates that Hawaiian grouper spawn in the NWHI during the first and second quarters of the calendar year, peak ripening occurs in December–April, and peak spawning follows several months later (in February–June). Relatively little reproduction occurs outside these months, although large females may begin to spawn in the fall. The multimodal size distribution of yolked oocytes indicates that individual females spawn more than once during a spawning season. Male Hawaiian grouper seem capable of spawning throughout the year. Most species of groupers spawn during a restricted period of year (Shapiro, 1987).

The highly female-biased adult sex ratio of Hawaiian grouper is typical of protogynous sequential hermaphrodites in which adult sex ratios are almost never male biased (Allsop and West, 2004; West, 2009). Species that do not change sex usually have adult sex ratios approximating unity (Charnov, 1982; Molloy et al., 2007). Our estimated 6-to-1 adult sex ratio for Hawaiian grouper is surely conservative because the targeting of larger adults by commercial fishermen is likely (i.e., catches are male biased), and most of our recent collection series were fishery-dependent samples. Fishery-dependent catches that are sex biased cannot be used to argue whether a fishery either has or has not induced changes in the operational sex ratio of a resource population.

Implications for fishery management

The life history information needed to better manage the Hawaiian grouper fishery in the MHI includes data on possible regional differences between fish in the MHI and NWHI. Little is known about geographic variation in sizes at maturity and at sex change among intraspecific populations of commercial species of sequential hermaphrodites, even though both potential and actual temporal changes reflecting varying magnitudes of exploitation are recognized (Heppell et al., 2006, and references cited therein). Our finding of equivalent body sizes at female maturation for the early versus recent NWHI collection series indicates that this fundamental aspect of the species' reproductive dynamics has not changed between the early 1980s and the mid-2000s in this region. This finding implies that the NWHI bottomfishery has not quantitatively altered the reproductive life-history of the species over the past several decades in the NWHI—perhaps reflecting the strongly regulated and capped levels of take in this region of the archipelago. In the MHI, however, body size has on average been smaller and the proportion of presumed immature fish has been greater for Hawaiian grouper caught in the MHI than in either the Mau or Ho'omalu Zone of the NWHI during the past two decades (Moffitt et al.[1]). The body sizes at female maturation and at female-to-male sex change of this species in the MHI, and evidence that they have recently declined, are currently unknown. Because of the likely but presently unquantified targeting of large Hawaiian grouper, there is a clear need to estimate body sizes at maturation and at sex change for the species in the MHI and to integrate such findings with those of this study. Characterizations of the growth rate and longevity of the Hawaiian grouper also need to be completed for each region and for the regions collectively.

Protogynous fishes, epinephelid groupers in particular (e.g., Adams et al., 2000; Coleman et al., 1996), are especially sensitive to changes in size and sex distributions as a result of harvesting (Alonzo and Mangel, 2004, 2005; Alonzo et al., 2008). Regional variations in key reproductive life history traits like sex ratio, body sizes at maturity and at sex change, and the occurrence of aggregation-spawning can profoundly influence population dynamics and the consequent effective management of protogynous stocks (Vincent and Sadovy, 1998). This is true regardless of whether the management scheme incorporates the use of no-take zones (e.g., the entire NWHI since 2010 and Johnston Atoll since 2009) or is limited to more conventional management measures like bag and size limits (Molloy et al., 2008). The latter unfortunately is impractical for a deep-water multispecies line fishery like the MHI bottomfishery in which barotrauma is a serious issue for all sizes of most species caught (Haight et al., 1993). Regional variation in stock structure may be especially important for

management of the MHI fishery because of the genetic differentiation of Hawaiian grouper that has been documented within the archipelago (Rivera et al., 2004).

Acknowledgments

We thank the many NOAA Fisheries biologists and technicians who processed specimens used in this study and the captains and crews of NOAA research vessels for their assistance. We acknowledge Federal Disaster Relief Project no. 657787, and the Pelagic Fisheries Research Program (University of Hawaii), for funding the purchase of recent specimens; A. Andrews and R. Humphreys for reviewing the draft manuscript; and M. McCracken for statistical advice. This article is dedicated to the memory of Julia P. Leung DeMartini, who photo-edited the final version of Figure 3; may her many memes continue to spread throughout the biological research community.

Literature cited

Adams, S.
2003. Morphological ontogeny of the gonad of three plectropomid species through sex differentiation and transition. J. Fish Biol. 63:22–36.

Adams, S., B. D. Mapstone, G. R. Russ, and C. R. Davies.
2000. Geographic variation in the sex ratio, sex specific size, and age structure of *Plectropomus leopardus* Serranidae) between reefs open and closed to fishing on the Great Barrier Reef. Can. J. Fish. Aquat. Sci. 57:1448–1458.

Allsop, D. J., and S. A. West.
2003. Constant relative age and size at sex change for sequentially hermaphroditic fish. J. Evol. Biol. 16:921–929.
2004. Sex-ratio evolution in sex changing animals. Evolution 58:1019–1027.

Alonzo, S. H., T. Ish, M. Key, A. D. MacCall, and M. Mangel.
2008. The importance of incorporating protogynous sex change into stock assessments. Bull. Mar. Sci. 83:163–179.

Alonzo, S. H., and M. Mangel.
2004. The effects of size-selective fisheries on the stock dynamics of and sperm limitation in sex-changing fish. Fish. Bull. 102:1–13.
2005. Sex-change rules, stock dynamics, and the performance of spawning-per-recruit measures in protogynous stocks. Fish. Bull. 103:229–245.

Brule, T., X. Renan, T. Colas-Marrufo, Y. Hauyon, and A. N. Tuz-Sulub.
2003. Reproduction in the protogynous black grouper (*Mycteroperca bonaci* (Poey)) from the southern Gulf of Mexico. Fish. Bull. 101:463–475.

Chan, T. T. C., and Y. Sadovy.
2002. Reproductive biology, age and growth in the chocolate hind, *Cephalopholis boenak* (Bloch, 1790), in Hong Kong. Mar. Freshw. Res. 53:791–803.

Charnov, E. L.
1982. The theory of sex allocation. Princeton Univ. Press, Princeton, NJ.

Coleman, F. C., C. C. Koenig, and L. A. Collins.
1996. Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environ. Biol. Fish. 47:129–141.

Craig, M. T., and P. A. Hastings.
2007. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. Ichthyol. Res. 54: 1–17.

DeMartini, E. E., and A. M. Friedlander.
2004. Spatial patterns of endemism in shallow-water reef fish populations of the Northwestern Hawaiian Islands. Mar. Ecol. Prog. Ser. 271:281–296.

DeMartini, E. E., and P. C. Sikkel.
2006. Reproduction. *In* The ecology of marine fishes: California and adjacent waters (L. G. Allen, D. J. Pondella, and M. H. Horn, eds.), p. 483–523. Univ. Calif. Press, Berkeley.

Domeier, M. L., and P. L. Colin.
1997. Tropical reef fish spawning aggregations: defined and reviewed. Bull. Mar. Sci. 60:698–726.

Erickson, D. L., J. E. Hightower, and G. D. Grossman.
1985. The relative gonadal index: an alternative index for quantification of reproductive condition. Comp. Biochem. Physiol. 81A:117–120.

Erisman, B. E., M. L. Buckhorn, and P. A. Hastings.
2007. Spawning patterns in the leopard grouper, *Mycteroperca rosacea*, in comparison with other aggregating groupers. Mar. Biol. 151:1849–1861.

Erisman, B. E., M. T. Craig, and P. A. Hastings.
2010. Reproductive biology of the Panama graysby *Cephalopholis panamensis* (Teleostei: Epinephelidae). J. Fish Biol. 76:1312–1328.

Fennessy, S. T., and Y. Sadovy.
2002. Reproductive biology of a diandric protogynous hermaphrodite, the serranid *Epinephelus andersoni*. Mar. Freshw. Res. 53:147–158.

Fitzhugh, G. R., and W. F. Hettler.
1995. Temperature influence on postovulatory follicle degeneration in Atlantic menhaden. Fish. Bull. 93:568–572.

Goodyear, C. P.
1993. Spawning stock biomass per recruit in fisheries management: foundation and current use. *In* Risk evaluation and biological reference points for fisheries management. (S. J. Smith, J. J. Hunt, and D. Rivard, eds.), p. 67–81. Can. Spec. Publ. Fish. Aquat. Sci. 120.

Grandcourt, E. M., T. Z. Al Abdesalaam, F. Francis, A. T. Al Shamsi, and S. A. Hartmann.
2009. Reproductive biology and implications for management of the orange-spotted grouper *Epinephelus coioides* in the southern Arabian Gulf. J. Fish Biol. 74:820–841.

Haight, W. R., J. D. Parrish, and T. A. Hayes.
1993. Feeding ecology of deepwater lutjanid snappers at Penguin bank, Hawaii. Trans. Am. Fish. Soc. 122:328–347.

Heppell, S. S., S. A. Heppell, F. C. Coleman, and C. C. Coleman.
2006. Models to compare management options for a protogynous fish. Ecol. Appl. 16:238–249.

Hunter, J. R., and B. J. Macewicz.
1985. Measurement of spawning frequency in multiple spawning fishes. *In* An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy, *Engraulis mordax* (R. Lasker, ed.), p. 79–94. NOAA Tech. Rep. NMFS 35.

Huntsman, G. R., J. Potts, R. W. Mays, and D. Vaughan.
1999. Groupers (Serranidae, Epinephelinae): endangered apex predators of reef communities. *In* Life in the slow lane: ecology and conservation of long-lived marine animals (J. A. Musick, ed.), p. 217–231. Am. Fish. Soc. Symp. 23, Am. Fish. Soc., Bethesda, MD.

Lau, B. B., and E. E. DeMartini.
1994. An evaluation of oocyte size in multiple regressions predicting gonad weight from body weight: a test using *Etelis carbunculus*. NOAA Tech. Memo. NMFS-SWFSC-212, 17 p.

Liu, M., and Y. Sadovy.
2004. Early gonadal development and primary males in the protogynous epinepheline, *Cephalopholis boenak*. J. Fish Biol. 65:987–1002.

Mackie, M.
2000. Reproductive biology of the halfmoon grouper, *Epinephelus rivulatus*, at Ningaloo Reef, Western Australia. Environ. Biol. Fish. 57:363–376.

Molloy, P. P., N. B. Goodwin, I. M. Cote, J. D. Reynolds, and M. J. G. Gage.
2007. Sperm competition and sex change: a comparative analysis across fishes. Evolution 61:640–652.

Molloy, P. P., J. D. Reynolds, M. J. G. Gage, I. Mosquiera, and I. B. Cote.
2008. Links between sex change and fish densities in protected areas. Biol. Conserv. 141:187–197.

Morris, A. V., C. M. Roberts, and J. P. Hawkins.
2000. The threatened status of groupers (Epinephelinae). Biodiv. Conserv. 9:919–942.

Pears, R. J., J. H. Choat, B. D. Mapstone, and G. A Begg.
2007. Reproductive biology of a large, aggregation-spawning serranid, *Epinephelus fuscoguttatus* (Forsskal): management implications. J. Fish Biol. 71:795–817.

Quinn, G. P., and M. J. Keough.
2002. Experimental design and data analysis for biologists, 537 p. Cambridge Univ. Press, Cambridge, UK.

Quinn, T. J., II, and R. B. Deriso.
1999. Quantitative fish dynamics, 542 p. Oxford Univ. Press, New York.

Rivera, M. A. J., C. D. Kelly, and G. K. Roderick.
2004. Subtle population genetic structure in the Hawai'i grouper, *Epinephelus quernus* (Serranidae) as revealed by mitrochondrial DNA analyses. Biol. J. Linn. Soc. 81:449–468.

Sadovy, Y.
1996. Reproduction of reef fishery species. *In* Reef fisheries (N. V. C. Polunin and C. M. Roberts, eds.), p. 15–59. Chapman and Hall, London.

Sadovy, Y., and P. L. Colin.
1995. Sexual development and sexuality in the Nassau grouper. J. Fish Biol. 46:961–976.

Sadovy, Y., and M. L. Domeier.
2005. Perplexing problems of sexual patterns in the fish genus *Paralabrax* (Serranidae, Serraninae). J. Zool. (Lond.) 267:121–133.

Sadovy, Y., A. Rosario, and A. Roman.
1994. Reproduction in an aggregating grouper, the red hind, *Epinephelus guttatus*. Environ. Biol. Fish. 41:269–286.

Sadovy, Y., and D.Y. Shapiro.
1987. Criteria for the diagnosis of hermaphroditism in fishes. Copeia 1987:136–156.

Sadovy de Mitcheson, Y., and M. Liu.
2008. Functional hermaphroditism in teleosts. Fish Fish. 9:1–43.

Samoilys, M. A., and L. C. Squire.
1994. Preliminary observations on the spawning behavior of coral trout *Plectropomus leopardus* (Pisces, Serranidae), on the Great Barrier Reef. Bull. Mar. Sci. 54:332–342.

Seki, M. P.
1986. Serranidae (*Epinephelus quernus*). *In* Fishery atlas of the Northwestern Hawaiian Islands. (R. N. Uchida and J. H. Uchiyama, eds.), p. 82–83. NOAA Tech. Rep. NMFS 38, 142 p.

Shapiro, D.Y.
1987. Reproduction in groupers. *In* Tropical snappers and groupers: biology and fisheries management (J. J. Polovina and S. Ralston, eds.), p. 295–327. Westview Press, Boulder, CO.

Siau, Y.
1994. Population structure, reproduction and sex-change in a tropical East Atlantic grouper. J. Fish Biol. 44:205–211.

Smith, W. L., and M. T. Craig.
2007. Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes. Copeia 2007:35–55.

Vincent, A. C. J., and Y. Sadovy.
1988. Reproductive ecology and the conservation and management of fishes. *In* Behavioral ecology and conservation biology (T. Caro, ed.), p. 209–245. Oxford Univ. Press, Oxford.

West, G.
1990. Methods of assessing ovarian development in fishes: a review. Austr. J. Mar. Freshw. Res. 41:199–222.

West, S.
2009. Sex allocation. Monographs in population biology. Princeton Univ. Press, Princeton, NJ.

Zar, J. H
1984. Biostatistical analysis, 2nd ed., 718 p. Prentice-Hall, Englewood Cliffs, NJ.