BULLETIN OF MARINE SCIENCE, 70(3): 851–869, 2002

REPRODUCTION IN THE CAMOUFLAGE GROUPER (PISCES: SERRANIDAE) IN POHNPEI, FEDERATED STATES OF MICRONESIA

Kevin L. Rhodes and Yvonne Sadovy

ABSTRACT

Reproduction in the camouflage grouper, *Epinephelus polyphekadion* (Bleeker, 1849) was determined from 1073 individuals collected during a July 1997 pilot study and a 14-mo period in 1998–99 in Pohnpei, Micronesia, with a view to understanding sexual pattern and reproductive seasonality and to inform fisheries management. Gonadosomatic indices, combined with histological analyses of gonads, show that fish spawn during a two consecutive month period between February and April, and that gonad maturation occurs 1–2 mo prior to spawning. Sexual pattern was unresolved: histological examination of 677 individuals revealed no fish undergoing transition from functional female to functional male, although direct development of some males from juveniles is suggested and males were significantly larger than females. Mean relative fecundity was estimated to be 1350 oocytes g^{-1} gonad-free body weight, actual fecundity increased linearly as a function of body weight and fecundity is determinate within each aggregation period. Females spawned an average of 83 ± 17% of their vitellogenic oocytes per month, with repeat spawning by individual females during a single aggregation period.

Reproductive patterns among members of the family Serranidae are highly varied and include several different sexual patterns and spawning modes: gonochorism, simultaneous and sequential hermaphroditism, pair-spawning and group spawning, aggregation and non-aggregation spawning (Smith, 1965; Thresher, 1984; Shapiro, 1987). Among the groupers and coral trout (sub-family Epinephelinae), protogyny is reported as the most common mode, with larger-bodied species typically forming spawning aggregations that are temporally and spatially predictable. While pair-spawning is common, group-spawning within aggregations has also been reported (Sadovy, 1996). Although the form of gonad development reflects phylogenetic relationships among serranids (Smith, 1965), the sexual patterns actually expressed can vary even within genera and detailed work is needed to distinguish developmental complexities of the serranid gonad from sexual pattern as reflected in reproductive function (e.g., Hastings and Petersen, 1986; Sadovy and Colin, 1995). Several previous studies indicating protogyny for the camouflage grouper, *Epinephelus polyphekadion,* for example, were inconclusive given what we now know of such complexities (AQUACOP et al., 1989; Debas, 1989; Debas et al., 1989; Bruslé-Sicard et al., 1992). In this study of reproduction in the camouflage grouper, we assess the reproductive cycle and examine the difficulties of diagnosing sexual pattern in *E. polyphekadion* in particular and serranids in general.

Understanding reproduction is important for effective fisheries management. For example, selective removal by a fishery of larger-sized individuals—males in protogynous species—may result in sex ratio imbalances that could lead to sperm limitation and reduced fertilization rates, while replacement of removed males by females in protogynous species could ultimately reduce mean female size, and hence, fecundity (Bannerot et al., 1987; Koenig et al., 1996; Vincent and Sadovy, 1998; Huntsman et al., 1999; Coleman et al., 2000). Therefore, conventional management measures, such as minimum size or catch

quotas, must be adapted to take into account those species which do not exhibit the typical gonochoristic sexual pattern of the majority of exploited species (Bannerot et al., 1987; Shapiro, 1987). Moreover, aggregating species, particularly those with short spawning seasons, and that tend to form relatively few large aggregations, may require a different management approach than those that do not aggregate, or that aggregate in small numbers at many sites (Coleman et al., 1999). Management for these fish specifically needs to incorporate direct protection of aggregations vulnerable to fishing, or protection during the aggregation period, during which the total annual reproductive output occurs (Shapiro, 1987; Coleman et al., 1999). Size of sexual maturation and fecundity are also important components of spawning stock biomass estimates (Goodyear, 1988).

The camouflage grouper, *E. polyphekadion* (= *microdon*) (Bleeker, 1849), is one of the most widely distributed of all Indo-Pacific groupers (Heemstra and Randall, 1993) and information on its biology is scarce. In many areas, it is known to form transient spawning aggregations that are targeted by both artisanal and commercial fishers (Johannes and Lam, 1999; Johannes et al., 1999; Rhodes, 1999). Given what is known of the impacts of aggregation fishing elsewhere (Sadovy and Figuerola, 1992; Sadovy, 1994; Koenig et al., 1996; Sadovy and Eklund, 1999), heavily exploited camouflage grouper aggregations may require immediate management attention (Bohnsack, 1989; Luckhurst, 1993). Indeed, aggregation loss and reductions in numbers of aggregating fish (Wase, pers. comm.; Johannes et al., 1999; Rhodes, 1999), mean size and catch-per-unit effort (Johannes and Lam, 1999) have been recorded as a direct result of overfishing, and managers in Pohnpei and Palau have implemented measures for their protection as a result.

The objectives of this study were to determine the reproductive parameters of camouflage grouper in Pohnpei, Micronesia, with a view to improving current management and conservation initiatives already in place; specifically, to determine (1) spawning seasonality, (2) sex-specific size distribution in catches, (3) gonadal development, (4) sexual pattern, (5) size at 50% sexual maturity, and (6) fecundity.

Methods

Camouflage grouper were obtained monthly through market purchases, hook-and-line and spearfishing in Pohnpei, Micronesia, in July 1997, and from February 1998 to April 1999, inclusive. All samples were of local origin, with market samples derived from the lagoon and outer reefs surrounding Pohnpei. Fish were also taken from a spawning aggregation site within Kehpara Marine Sanctuary (KMS) that formed in March and April in 1998 and February and March in 1999 (Fig. 1) (Rhodes and Sadovy, 2002). Morphometrics taken at the time of sampling include standard (SL) and total lengths (TL) to the nearest 1 mm and whole fish wet weight to the nearest 1 g. Gonads were removed, blotted dry and weighed to the nearest 0.1 g. Weights were later used to estimate spawning seasonality by use of the gonadosomatic index (GSI):

$$\text{GSI} = \text{gonad weight}/\text{gonad-free body weight} \times 100. \quad \text{Eq. 1}$$

Whole gonads were preserved in the field, after sex assignment, in 10% formalin and later transferred to 70% ethanol for long-term storage and histological sectioning. Tissues for sectioning were embedded in paraffin, sectioned at 7 μm, stained with Harris' haemotoxylin and counterstained with eosin. Small gonads (<1.0 g) were sectioned entire and longitudinally, while larger gonads were sub-sampled along both exterior-interior and anterior-posterior axes and sectioned transversely.

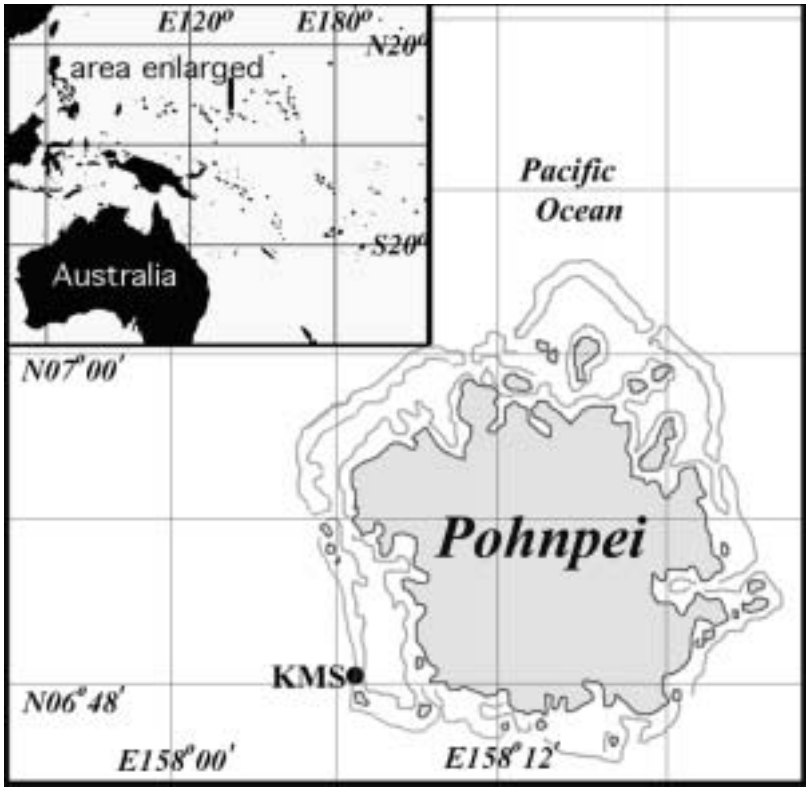

Figure 1: Map of Pohnpei, with regional inset, showing the aggregation sampling site at Kehpara Marine Sanctuary (KMS) and surrounding reefs (shaded lines).

Sex assignments were done both macroscopically and microscopically using the criteria outlined in Table 1. Oocyte development stages follow the definitions of West (1990) and Tyler and Sumpter (1996) as follows: (1) Stage 1- chromatin nucleolar stage oocytes; (2) Stage 2- perinucleolar stage of development to yolk vesicle or cortical alveolus formation; (3) Stage 3- yolk granule (early) through migratory nucleus (late) stage, vitellogenic; and (4) Stage 4- hydration. For sex assignment in the field, tissues were assessed macroscopically in 1998, and microscopically by tissue squashes following cannulation in 1999. Cannulation is the extraction of small samples through a narrow tube gently inserted into the gonopore. Both 1998 and 1999 samples were validated for sex assignment and stage of sexual maturation upon return to the lab. Histological sections were used to confirm field sex assignments and to categorize each gonad by development stage. Females were considered to be developing (F2) if they did not contain vitellogenic oocytes or exhibit previous signs of spawning, such as muscle bundle remnants (Shapiro et al., 1993). Such females may have been approaching maturity for the first time or may be resting between spawning seasons. Male development was based on the presence or absence of milt. An absence of milt characterized immature or inactive males, while the presence of milt denoted mature males.

Reproductive Seasonality, Gonad Classification and Sexual Pattern.—To determine functional sex (i.e., the sex expressed by an adult fish) and spawning seasonality, and to describe sexual development and sexual pattern in camouflage grouper, histological preparations were made of 267 preserved gonads taken from spawning aggregations (1998: n = 178; 1999: n = 89) and from all (n = 384) market samples. Determinations of sex and sexual maturity from histological preparations (Table 1: microscopic) were modified from the stages of sexual development of Moe (1969) for red

Table 1. Criteria used in macroscopic and microscopic evaluation of maturity stage in gonads of camouflage grouper, *Epinephelus polyphekadion*.

Maturity stage	Macroscopic	Microscopic
	Ovaries	
F1 (immature)	Small, strand-like tissue, compact, pink or cream; oocytes indiscernible; indistinguishable from M1 males	A: Gonad wall thin; tightly-packed previtellogenic Stage 1 (nucleolar) and 2 (perinucleolar) oocytes and gonia B: Stage 1 and early Stage 2 oocytes, gonad wall variously thick or thickening; oocytes widely dispersed within the gonia-packed stroma; no muscle bundles or other signs of previous spawning
F2 (mature, inactive)	Relatively small but rounded, greyish with thickened gonad wall; oocytes indiscernible or small (<0.4 mm). Indistinguishable from M2 prior to cortical alveolus stage when oocytes become discernible.	Stages 1 and 2 and cortical alveolus stage oocytes present; includes individuals recovering from spawning as indicated by distinct muscle bundles, thick gonad wall, and an absence of post-ovulatory follicles, and females maturing for the firts time.
F3 (mature, active)	Large and greyish with transparent gonad wall; large vitellogenic oocytes becoming clearly visible and tightly packed	Mainly vitellogenic (Stage 3) oocytes; gonad wall stretched and thin; Stage 1 and 2 oocytes relatively few; no large-scale atresia or post-ovulatory follicles
F4 (mature, ripe)	Ovary large, clear, hydrated oocytes visible through wall; typical of individuals just prior to spawning; egg release possible with application of light abdominal pressure	All stages of oocyte development, but dominated by Stage 4 (hydrated) and late Stage 3; yolk fusion and hydration are extensive
F5 (post-spawn)	Ovary flaccid with obvious capillaries; few oocytes visible	Post-ovulatory follicles numerous and large prominent muscle bundles scattered throughout the gonad; the gonad wall is thickened; few Stage 3 and/or 4 oocytes may be present and undergoing atresia
	Testes	
M1/2 (immature/inactive)	Indistinguishable from F1 females (see the description of F1)	Gonad filled with varying amounts of stroma; some early proliferation of 1º and 2º spermatocytes; sperm sinus present or not; seminiferous lobules may be evident; often with Stage 1 and 2 previtellogenic oocytes in varying amounts that may fill the gonad
M3 (mature, active)	Gonad expanding and becoming rounded and large; greyish in appearance. Early M2 individuals are indistinguishable from F2 until milt becomes evident in the sperm sinus	1º and 2º spermatocytes and early sperm formation, sperm crypts and seminiferous lobules; sperm/spermatids and 2º spermatocytes represent < 50% of the volume; sperm sinus present with thick or thickening tunica; Stage 1 and 2 oocytes often present in varying amounts.
M4 (mature, ripe)	Testes large and white with sperm visible in sinuses; milt release with light abdominal pressure	Seminiferous lobules extensive and filled with sperm; gonad volume > 50% sperm/spermatids and few gonia
M5 (post-spawn)	Testes flaccid and bloody; sperm release still possible on application of abdominal pressure	Sperm sinuses and seminiferous lobules largely empty with little or no sperm. Muscle bundles abundant and gonad empty of early stages of spermatogenic tissue

grouper, *Epinephelus morio,* and red hind, *Epinephelus guttatus* (Shapiro et al., 1993). Individuals were classified into five categories of sexual maturation. Stages F1–F5 characterize females and M1-M5 males (Table 1). Sexual pattern, based on histological evidence, was assessed using the criteria outlined in Sadovy and Shapiro (1987) for diagnosis of hermaphroditism in fishes.

SEXUAL MATURITY.—Size at 50% sexual maturity and minimum size at maturity were determined for all females taken from combined market and aggregation samples. Determinations were made by assessing the percent of females, for 10-mm size classes (220–460 mm SL), that were developing or sexually mature (F2-F5). All males with sperm were considered mature. However, given a wide range in the relative volumes of sperm/spermatids to spermatocytes, mature males were divided into two classes of >50% and <50% of sperm/spermatids to spermatocytes, respectively. The distinction between mature males, based on relative sperm volumes, was further examined by comparing mean GSI between the two classes using a Student's t-test ($\alpha = 0.05$).

FECUNDITY.—Fecundity was assessed by calculating potential fecundity corrected for the number of unspawned oocytes to estimate actual fecundity (see definition of correction factor below). Potential fecundity, or the total number of advanced (vitellogenic stage or hydrated) oocytes (Hunter et al., 1992), was determined from a sub-sample of females taken on the day of spawning (Rhodes and Sadovy, 2002). Assessments for fecundity were made using 2–5 sub-samples from each gonad, with a total combined weight of 0.1% whole preserved gonad weight, taken from each of 43 mature, active (F3) and mature, ripe (F4) females sampled from spawning aggregations in 1998 and 1999 (size range = 228–430 mm SL). The majority of females used in the analyses were mature, active (F3) females, since mature, ripe (F4) females were rarely sampled. Oocytes were separated from the supporting tissue and counts made directly under a dissecting microscope (100×). The total oocyte number from combined sub-samples of each individual (5000–25,000 oocytes) was used to estimate potential fecundity by extrapolating total combined sample counts per unit weight to the whole gonad weight.

In preparation for potential fecundity analyses, a pilot study was performed to test for homogeneity of advanced oocyte density and oocyte developmental stage along the length of the ovary. For this study, five similar-sized females, taken on the same day from an aggregation just prior to spawning in 1998, were used. Each gonad was divided into right and left lobes, the lobes divided into anterior, central and posterior sections and the sections subdivided into interior, central and exterior sub-sections along the longitudinal axis. A total of six randomly selected sub-samples, with a combined weight of 0.1% of the total preserved gonad weight, was taken from each lobe, two sub-samples from each sub-section. The test for homogeneity was performed using a nested ANOVA [$\alpha = 0.05$: Factor 1 (fish) - fixed, orthogonal, 5 levels; Factor 2 (lobes) - nested, fixed, 2 levels; Factor 3 (sections) - nested, random, 12 levels].

Actual fecundity, or the total number of oocytes successfully released by a female during a single spawning month, was determined by multiplying potential fecundity estimates by the correction factor. To determine the correction factor, stained and mounted histological sections were used. Counts of post-ovulatory follicles (POFs) and atretic advanced stage oocytes (AAO) were made at 400× magnification under a compound microscope, using an ocular quadrat, on 8–12 quadrats randomly selected from within each histological section, i.e., 16–24 quadrats per individual, representing approximately 50% of possible counts within each section. The correction factor thus represents the ratio of post-ovulatory follicles (POF) to atretic advanced stage oocytes (AAO) and was based on counts from 31 post-spawn (F5) females (260–377 mm SL) sampled the day after spawning, using the following equation:

$$\text{Correction Factor} = \text{POF}/(\text{AAO}+\text{POF}). \qquad \text{Eq. 2}$$

Since females used in the determination of the correction factor were taken within 24-h of spawning, the number of post-ovulatory follicles could be accurately assessed prior to atresia (Hunter and Macewicz, 1985). Application of this factor allows a more accurate representation of fecundity

since not all vitellogenic oocytes are hydrated and spawned by females. A correction factor pilot study was conducted using 15 individuals to estimate the optimal number of quadrats needed per histological section to accurately characterise each gonad and to test for homogeneity of relative proportions of AAO and POF across sections (2-way ANOVA, arcsine transformation) (Krebs, 1989). Sections within individuals were later combined and individuals grouped into size classes.

Results

Validation of Macroscopic Analysis.—Histological validation of samples assessed macroscopically in the field for sex assignments was conducted for all individuals. Validations were either by histological preparation (all market samples and 38.8% of aggregations samples) or by squashes of preserved tissue. All further assessments of reproductive parameters were conducted following these procedures.

Reproductive Seasonality.—Histological assessment of gonads, in combination with GSI analysis, confirmed spawning in camouflage grouper during March–April 1998 and February–March 1999 (Figs. 2,3). Vitellogenic oocytes become evident within the 1–2 mo prior to spawning. Of the 1073 camouflage grouper sampled, 47.5% were mature males (M3-5), 30.5% were mature or developing females (F2–5) and 22.0% were immature females or immature/inactive males (F1 or M1/2) (Table 2).

Size Frequency Distribution.—Size distributions by sex overlapped, although males (mean = 348.3 ± 32.5 mm SL, n = 552, range = 254–450 mm SL) were significantly larger than females (mean = 323.2 ± 38.3 mm SL, n = 327, range = 228–460 mm SL) (2-sample t-test, $\alpha = 0.05$, $P < 0.00$) (Fig. 4). Size comparisons between sexes for both market and aggregation samples, assessed separately, showed similar results, with males significantly larger than females (2-sample t-test, $\alpha = 0.05$, $P_{mar} < 0.00$, df = 170, $P_{agg} < 0.00$, df = 711). No significant sex-specific size differences were shown between market and aggregation samples (2-sample t-test, $\alpha = 0.05$, $P_{male} > 0.41$, df=547; $P_{female} > 0.12$, df = 334, respectively).

Immature Individuals.—Based on mean size differences, tunica morphology and the amount of connective tissue (stroma) present within the gonad, F1 individuals clearly fell into two distinct categories and were sub-classified as either F1-A or F1-B. Small amounts of scattered stroma, located among tightly packed previtellogenic oocytes, all held within a thin tunica characterized F1-A gonads (Fig. 5A). In contrast, a conspicuous gonia-packed stroma that surrounded disorganized and well-separated previtellogenic oocytes (Fig. 5B) and a thickened tunica (Fig. 5C) distinguished F1-B gonads. Size distributions of the two F1 sub-classes overlapped (168–350 mm SL), although F1-B individuals were significantly larger than F1-A individuals (2-sample t-test; $P = 0.01$, n = 194) (Fig. 6A). F1-A and F1-B individuals were common in market samples, but only four F1 (F1-A) individuals were sampled in aggregations.

Females.—Females were categorized as inactive (F2), mature, active (F3), mature, ripe (F4) or post-spawn (F5). F2 developing individuals with cortical alveolus stage oocytes only were common in samples 1–2 mo prior to spawning, but otherwise rare (Fig. 7A), while F2 inactive individuals with muscle bundle remnants, indicating previous spawning were evident for up to 7 mo after spawning (Fig 7B).

Whereas F2 females were common in samples during non-reproductive periods, mature, active (F3), mature, ripe (F4) and post-spawn (F5) females were only common within the aggregation. Gonads of all mature, active females (F3) sampled from all aggregations

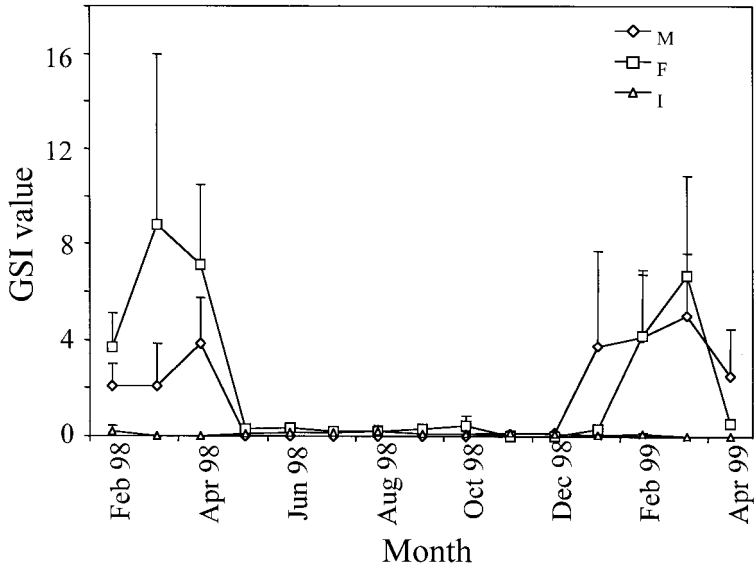

Figure 2: Monthly mean and standard deviation gonadosomatic index for camouflage grouper collected in Pohnpei, Micronesia between February 1998 and April 1999. Males = M; females = F; immature = I (M1/2, F1).

were dominated by late vitellogenic stage oocytes and cortical alveolus stage oocytes were absent, suggesting that additional recruitment of previtellogenic oocytes is negligible once females enter the aggregation (Fig. 7C). Mature, ripe individuals (Fig. 7D) containing hydrating and hydrated oocytes were rarely sampled and were taken only just prior to twilight on the day of spawning (Rhodes and Sadovy, 2002). Post-spawning individuals were taken only within a 1–2 d period immediately after spawning at the aggregation site. In these individuals (F5), previtellogenic oocytes were common, and all remaining vitellogenic oocytes were undergoing atresia (Fig 7E).

MALES.—Immature (Fig. 8A) and inactive (Fig. 8B) males (M1/2) (n = 42) were sampled throughout the non-reproductive period (Fig. 3B) from markets. Atretic previtellogenic oocytes were common in M1/2 individuals. A few M1/2 individuals were taken in February 1998 from markets prior to the spawning period, but never from aggregations. For M1/2 males, sizes (range = 275–380 mm SL, mean = 336.0 ± 28.2 mm SL) were significantly different from those of mature (M3-M5) males (range = 254–450 mm SL, mean = 348.1 ± 32.5 mm SL) (t-test, t = 1.96, P = 0.044, df = 551) (Fig. 6B).

M3 and M4 males were significantly different for mean GSI (M3: mean GSI = 3.24 ± 2.67, M4: mean GSI = 5.67 ± 2.21; t-test, df = 506, P << 0.000). This finding suggests that these two phases represent early and late phases of testicular development leading up to spawning. Further support for this separation comes from the presence of M3 males (Fig. 8C) in samples from 3 mo prior to spawning, whereas mature, ripe (M4) males were sampled 1 mo immediately before, and during, spawning (Fig. 8D). Post-spawn (M5) individuals were only taken immediately after spawning in each of 1998 and 1999 (Figs. 3B,8E). Males of all stages were absent from May 1998 samples.

SIZE AT SEXUAL MATURITY.—Size at 50% sexual maturity for female camouflage grouper in Pohnpei was ~270 mm SL, based on the examination of 327 (F2-F5) females and

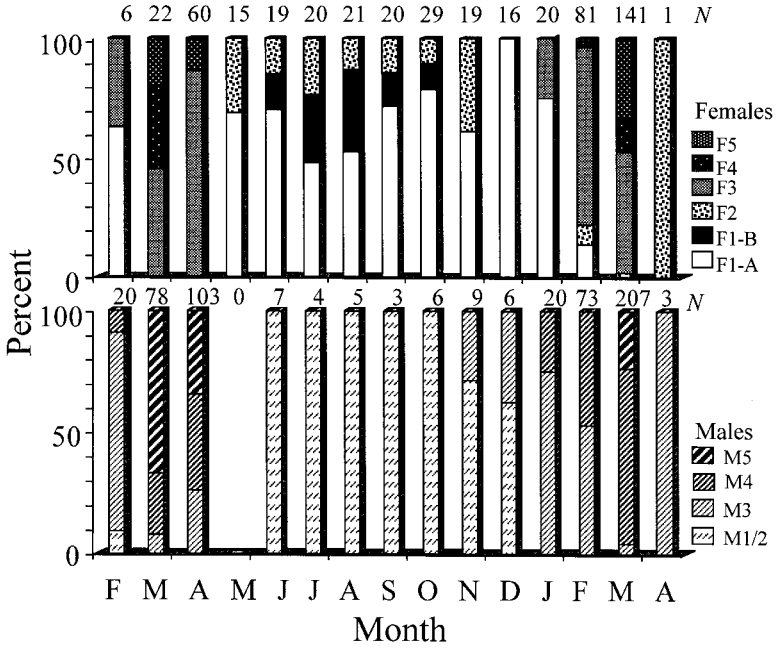

Figure 3: Gonad development trends (by stage of sexual maturity) expressed as percent per month for camouflage grouper collected in Pohnpei between February 1998 and April 1999. N = monthly total number of individuals. F1-A = immature; F1-B = immature; F2 = inactive or maturing; F3 = mature active; F4 = ripe; F5 = post-spawn; M1/2 = immature/inactive; M3 = mature, active; M4 = mature active; M5 = post-spawn.

Table 2: Summary by month and number of *Epinephelus polyphekadion* taken in Pohnpei from July 1997 and February 1998–April 1999. Sampling locales represented are markets (M), aggregations (A) and aggregation sites in non-spawning months (S).

Month	Males (M3-5)	Females (F2-5)	Immature (F1, M1/2)	Total	Mature (%)	Locale
Jul-97	0	7	29	36	19	M
Feb-98	18	3	5	26	81	M,S
Mar-98	78	21	1	100	99	A
Apr-98	103	60	0	163	100	A
May-98	0	5	10	15	33	M
Jun-98	0	2	24	26	8	M
Jul-98	0	3	22	25	12	M
Aug-98	0	2	24	26	8	M
Sep-98	0	2	21	23	9	M
Oct-98	0	4	31	35	11	M
Nov-98	4	7	17	28	39	M
Dec-98	2	0	21	23	9	M
Jan-99	20	4	16	40	60	M
Feb-99	73	68	14	155	91	A,M
Mar-99	207	138	3	348	99	A
Apr-99	2	1	1	4	75	S
Totals	507	327	239	1,073		

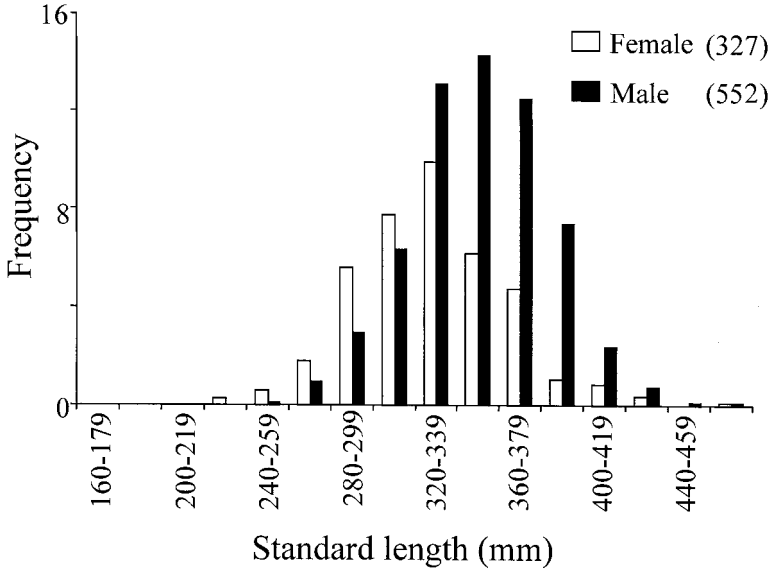

Figure 4: Size frequency distributions of male (M1-5) and female (F2-5) camouflage grouper collected in Pohnpei in July 1997 and between February 1998 and April 1999. Numbers in parentheses are numbers of mature individuals sampled.

194 immature (F1) individuals (Fig. 10). The smallest mature female was 228 mm SL and the smallest mature male was 254 mm SL.

Sexual Pattern and Sexual Development.—The sexual pattern in camouflage grouper remains unresolved with the data available from this study, based on the criteria outlined by Shapiro and Sadovy (1987). Evidence for sex change, best represented by transitional, or sex-changing (from functional female to functional male), individuals was not obtained. Some evidence suggestive of protogyny was found in the significantly larger males than females (Fig. 4). Two small males (290 and 292 mm SL) taken during the non-reproductive season suggest direct development from juvenile F1-B individuals into males, based on localized spermatogenesis within a gonad otherwise similar in appearance to F1-B individuals (Fig. 9B).

Fecundity.—In the pilot study, no significant differences in oocyte number along the length of the gonad (nested ANOVA) were shown (Table 3). Potential fecundity ranged from 392,000 oocytes in a 290-mm SL individual to more than 3.1 million oocytes in a 430-mm SL individual. The relationship between potential fecundity and female size was best described by the exponential equation $y = 0.0862^{0.0079x}$ ($r^2 = 0.52$, $F = 48.0$, $P << 0.00$, $df = 42$).

For the actual fecundity pilot study, no significant difference was found in the percent of spawned eggs among gonad sections for a given female (2-sample t-test, arcsine transformed, $0.13 < P < 0.80$, $n = 15$) and section counts were therefore combined. Minimal sample size for actual fecundity was 26 fish, with a minimum of 12.0 ± 12.8 quadrats ind^{-1}, based on a 10% relative error. Findings, based on 31 individuals, indicated that females spawn an average of 83 ± 17% of potential advanced stage oocytes. Size class comparisons, based on four size classes, showed no significant size-based difference in the percent of oocytes spawned (2-way ANOVA, arcsine transformation, $F = 0.634$, $P = 0.60$, $df = 3, 30$).

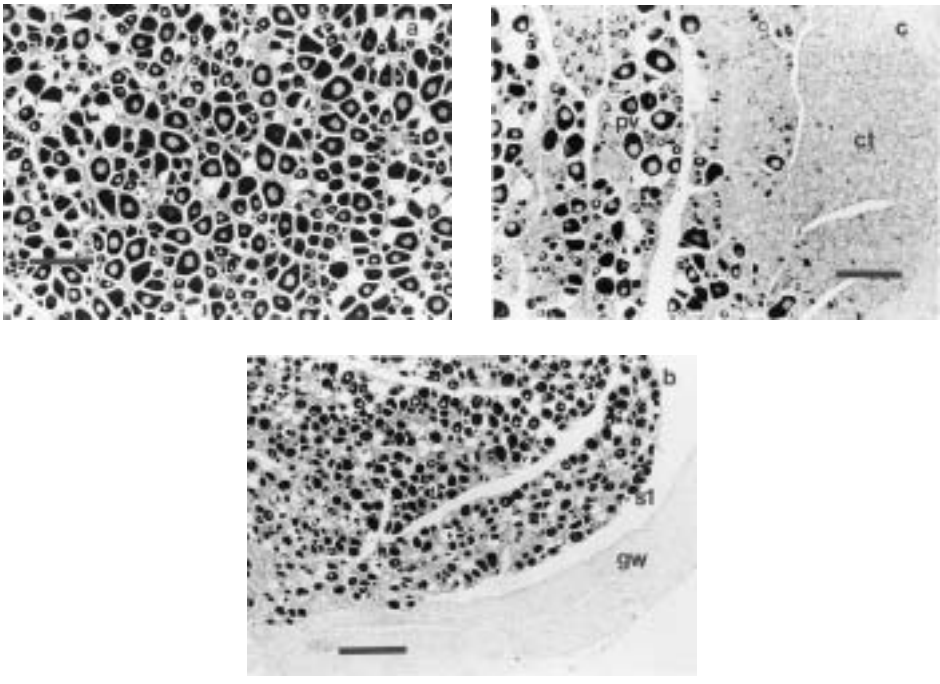

Figure 5: The two stages of development of immature individuals (F1) collected February 1999 in a market in Pohnpei: (a) F1-A; (b) F1-B (stroma) and;(c) F1-B (tunica). Individual F1-A: 310 mm SL (100×); individual F1-B: 330 mm SL (100×) (scale = 500 µm). ct = gonia-filled connective tissue (stroma), pv = previtellogenic oocytes, gw = tunica, s1 = nucleolar previtellogenic oocyte.

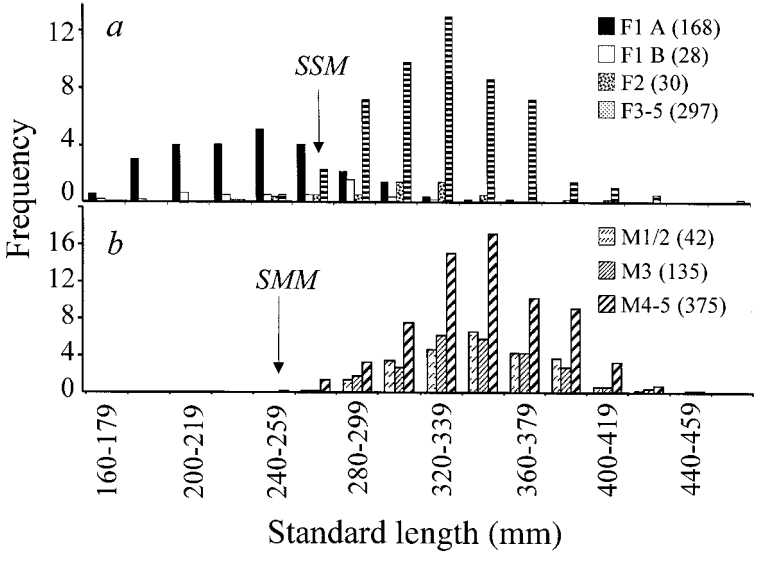

Figure 6: Size frequency distributions of female (a) and male (b) camouflage grouper, *Epinephelus polyphekadion,* sampled in July 1997 and between February 1998 and April 1999 in Pohnpei. Total number sampled is shown in parentheses. SSM = size at 50% sexual maturity for females; SMM = size of smallest mature male.

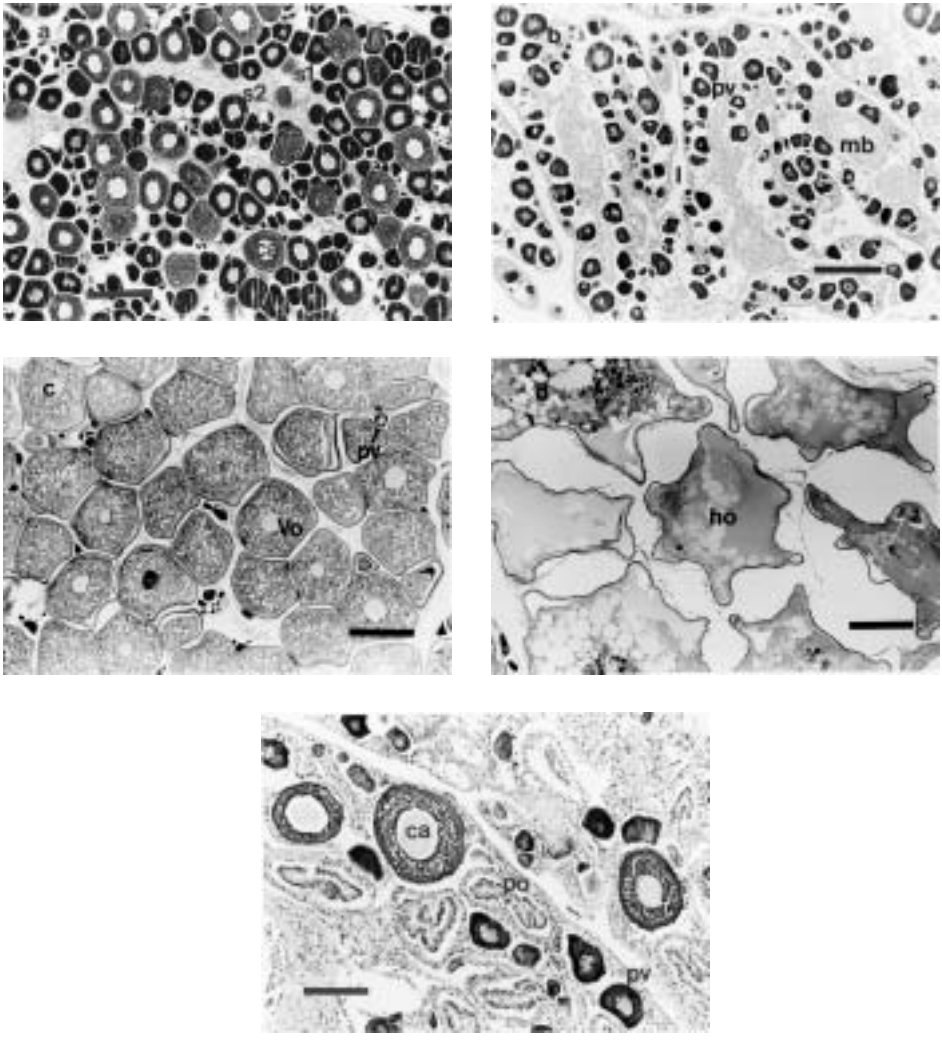

Figure 7: Stages of female maturation and development in camouflage grouper, *Epinephelus polyphekadion,*: (a) developing, inactive (F2), collected 22 December 1998, 370 mm SL (100×); (b) mature, inactive (F2) (recovering), collected 21 May 1998, 285 mm SL (100×); (c) mature, active (F3), collected 7 April 1998, 333 mm SL (100×); (d) mature, ripe (F4), collected 12 March 1998, 310 mm SL (100×); (e) post-spawn (F5), collected 10 April 1998, 340 mm SL (200×). ca = cortical alveolus stage oocyte, ho = hydrated oocyte, mb = muscle bundle, po = post-ovulatory follicle, pv = previtellogenic oocyte; s1 = previtellogenic oocyte, s2 = perinucleolar previtellogenic oocyte, Vo = vitellogenic oocyte. (scale = 500 μm in *a, b, c* and *d*; scale = 250 μm in *e*)

Following the adjustment for atretic unspawned oocytes, fecundity (actual) in camouflage grouper was best fitted by the linear function $y = 0.96x + 5.8$ ($r^2 = 0.58$, $F = 5.14$, $P = 0.03$, $df = 30$) (Fig. 9). Mean relative fecundity, defined as the number of oocytes g^{-1} gonad-free body weight, was found to be approximately 1350 oocytes g^{-1}. Relative fecundity was negatively correlated to size, but was not significantly different among size classes (ANOVA, $\alpha = 0.05$, $P >> 0.5$, $df = 42$). Fecundity measures presented here repre-

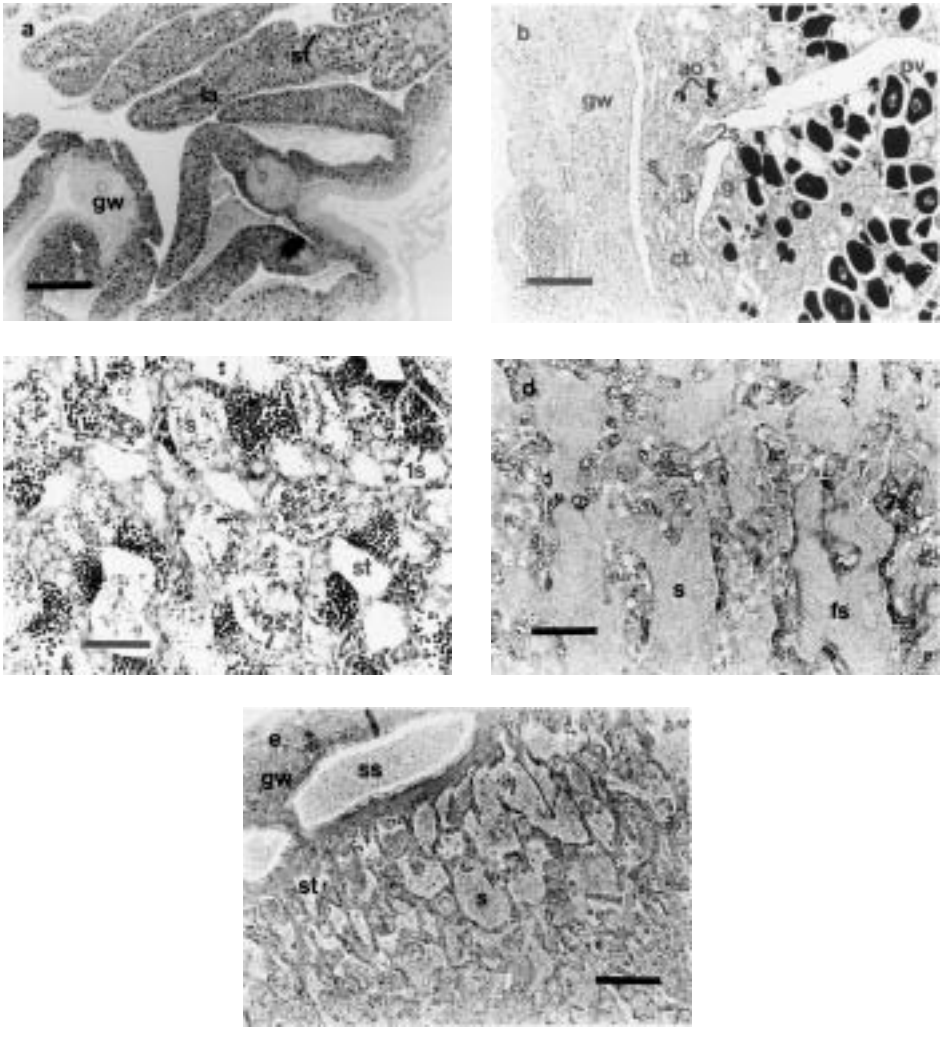

Figure 8: Stages of male maturation and development in camouflage grouper, *Epinephelus polyphekadion*. (a) Immature/inactive (M1/2) male collected 10 November 1998, 364 mm SL (40×) (scale = 1250 μm); (b) Early development of an M1/2 immature/inactive male, collected 1 December 1998, (200×) (scale = 250 μm); (c) mature, active (M3), collected 28 January 1999, 353 mm SL (400×) (scale = 125 μm); (d) mature active, ripe (M4) collected 4 April 1998, 367 mm SL (100×) (scale = 500 μm); (e) post-spawn (M5), collected 13 March 1998, 391 mm SL (100×) (scale = 500 μm). 1s = primary spermatocyte, 2s = secondary spermatocyte, ao = atretic oocytes, ct = connective tissue (stroma), fs = fused seminiferous tubule, g = gonia, gw = gonad wall, la = lamellae, pv = previtellogenic oocyte, s = sperm, ss = sperm sinus, st = seminiferous tubule.

sent monthly fecundity estimates, as all oocytes that have attained vitellogenesis or hydration prior to spawning in one month either are released or undergo degeneration. Therefore, fecundity for camouflage grouper is determinate, at least during a single aggregation period.

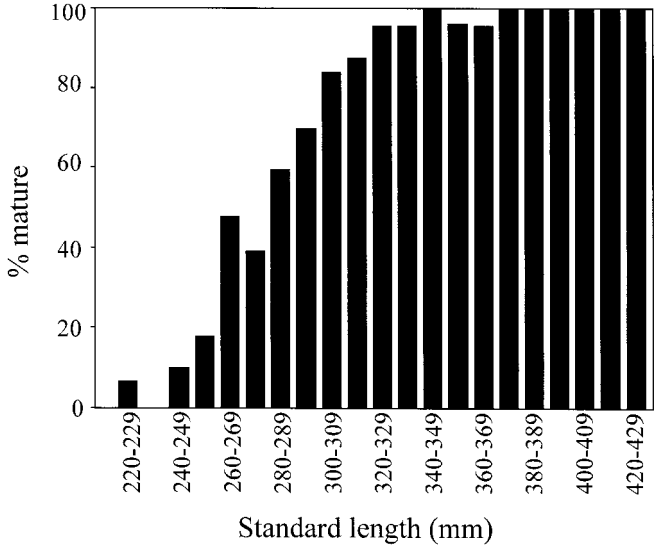

Figure 9: Size at 50% sexual maturity (~270 mm SL) for females taken in Pohnpei in July 1997 and February 1998–April 1999.

Discussion

The reproductive biology of *E. polyphekadion* is described in terms of spawning seasonality, sexual development, size at sexual maturity and fecundity. It was not possible to confirm sexual pattern, although development of males from juveniles was suggested from both size data and histological evidence. Camouflage grouper spawn during a two consecutive month period between February and April and form transient spawning aggregations that persist over approximately two weeks within each month. Histology suggested that relatively few, if any, additional vitellogenic oocytes were recruited into the oocyte population once females entered the spawning site, and there was evidence to suggest that individual females spawned more than once during each spawning month. No evidence was available regarding whether camouflage grouper spawn over more than one of the two spawning months. Following spawning, muscle bundles form as ovaries contract and persist over approximately seven months. Beyond this, a distinction of mature females from females becoming sexually mature for the first time is not possible.

Sexual Pattern.—Sexual pattern in the camouflage grouper in Pohnpei could not be resolved. Evidence to unequivocally support protogyny, such as gonads undergoing transition from a functional female to a functional male, or age data to link sex-specific size frequency differences with age were not available. Attempts to correlate fish length to age were unsuccessful, based on standard otolith ring counts because of poor legibility in sections of sagittae (n = 500). The presence of an 'ovarian' lumen and brown bodies are not considered reliable indicators of protogyny, as the former alone is not an indicator of functional sex change in families, such as the Serranidae, which have an hermaphroditic ancestry, and brown bodies may be produced by factors other than atresia of advanced stage oocytes (Roberts, 1978; Sadovy and Shapiro, 1987; Sadovy and Colin, 1995). It should be noted, however, that only 158 mature individuals were sampled during non-spawning months, typically the time during which sex change occurs (Smith, 1965; Moe,

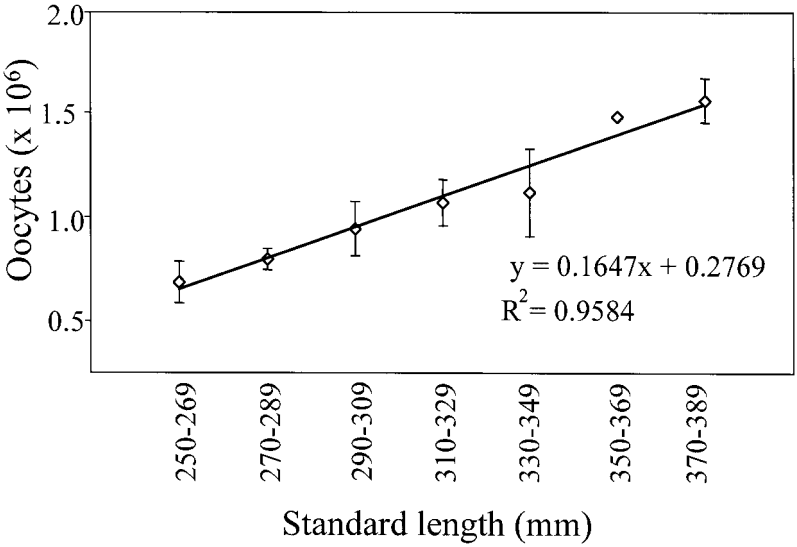

Figure 10: Size-related actual fecundity estimates for camouflage grouper, *Epinephelus polyphekadion,* (260–377 mm SL) sampled during aggregation periods in March and April 1998 and March 1999. Results are presented as mean ± standard deviation. n = 31.

1969; Chen et al., 1980; Sadovy and Shapiro, 1987; Stevenson et al., 1998). It is possible that transitionals (i.e., individuals undergoing sex change), if typically few or if transition is rapid, may have been missed, although similar study sample sizes were, in other studies, showing protogyny in serranids (e.g., Bullock and Murphy, 1994; Siau, 1994; Ferreira, 1995; Mackie, 2000). For example, transitionals have typically represented less than 1–2% of samples in hermaphroditic epinephelines (Moe, 1969; Collins et al., 1987; Hastings, 1989; Hood and Schleider, 1992; Shapiro et al., 1993; Bullock and Murphy, 1994; Koenig et al., 1996). However, since 'transitional' is not always defined, it is sometimes unclear whether such fish are undergoing sex change or simply exhibit gonads with both male and female elements.

While the sexual pattern could not be resolved in the present study, the presence of males below the 50% size of sexual maturity of females, the strong size overlap between sexes and the apparent difference between F1-A and F1-B individuals, suggest that at

Table 3. Results of the nested ANOVA showing differences among fish in the number of advanced stage oocytes within the ovaries of mature females (a) and homogeneity of individual ovaries [b (a)].

Source	Sum of squares	df	MS	F	P	F versus
a	185,058.43	4	46,264.61	21.88	0.0023	b (a)
b (a)	10,571.83	5	2,114.37	0.51	0.7665	c (a × b)
c (a × b)	83,172.33	20	4,158.62	1.22	0.3015	Residual
Residual	101,943.00	30	3,398.10			
Total	380,745.60	59				

least some males may develop from the immature, F1-B phase. This possible development pattern is further supported by histology in two small males that are identical to F1-B individuals in all aspects other than for small, localized areas of spermatogenesis.

E. polyphekadion has been diagnosed as protogynous elsewhere, based on histological, histochemical (Debas, 1989; Debas et al., 1989; Bruslé-Sicard et al., 1992) and tag-and-recapture methodologies (Johannes et al., 1999). Histological and size frequency data from French Polynesia sampled throughout the year, were similar to those of the present study, but do not show unequivocal evidence for functional sex change (Debas, 1989). Moreover, histochemical analysis of sex steroids (11-ketotestosterone, testosterone and estradiol-17) in the latter study show an almost identical relationship between immature females and 'intersexes' (term undefined) (n = 18), but little resemblance between 'intersexes' and immature males (Debas, 1989). Although sex inversion was hormonally induced in vitro, such evidence cannot conclusively demonstrate sex change because species that do not normally change sex can be induced to do so by hormones (Sadovy and Shapiro, 1987).

Bruslé et al. (1992) later conducted an ultrastructural examination of gonads from Debas' (1989) study and documented the presence of undifferentiated bipotential primordial germ cells (PGC) within the 'ex-ovarian lamellae'. Although PGC have been linked to sex change in other fishes (e.g., labrids) (Bruslé, 1983; Bruslé, 1987; Nakamura et al., 1989), these structures have also been found in gonochores and alone cannot be used as unequivocal evidence for protogyny.

Strong evidence for sex change was provided by a survey in Palau during which two mature females were tagged within an aggregation and later recaptured as males (Johannes et al., 1999). Although the tagging data provide support for female to male sex change in camouflage grouper, clear histological evidence of sex inversion, as in the present study, in the form of transitionals was not forthcoming. Since the present study also indicates that males can develop directly from females, putting the two studies together suggests that males of this species may have two developmental pathways, directly from the juvenile and through sex change. Further work on sexual pattern in this species will have to take this possibility into account.

The varied modes of gonad ontogeny in epinepheline serranids make evaluation of sexual pattern difficult. In particular, since all grouper testes bear an ovarian-like lumen, this gonad structure cannot be used, alone, in distinguishing between males that arise by sex change from a functional female from those that develop directly as males from the juvenile stage. Therefore, in epinepheline serranids, to accurately characterize sexual patterns, clear histological evidence of sexual transition in adults is needed, which may require relatively large sample sizes in order to improve the possibility of detecting sex change, should it occur. In addition, further evidence, such as that provided by age and growth or tag and recapture techniques may be needed in combination with histological assessment of gonads.

Spawning Periodicity.—Histological evidence suggests that repeat spawning within an aggregation month by individual females is likely, as determined by evidence in histological sections showing post-ovulatory follicles together with advanced stage and hydrating oocytes (>50% of the total number) taken on the eve of spawning (Rhodes and Sadovy, 2002). Repeat spawning within a single aggregation period has also been reported for camouflage grouper, and other epinepheline serranids, elsewhere (Sadovy et al., 1994; Samoilys and Squire, 1994; Ferreira, 1995; Johannes et al., 1999).

FECUNDITY.—For camouflage grouper, potential fecundity during one spawning month falls within the range of fecundity previously estimated for other serranids (McErlean, 1963; Burnett-Herkes, 1975; Chen et al., 1980; Bouain and Siau, 1983; Shapiro, 1987). Fecundity is determinate during a single aggregation month and all advanced stage oocytes observed from samples taken the day after spawning were atretic. Although females may spawn more than once during a single month, spawning frequency estimates were not possible as individuals stopped taking bait within the actual spawning period. Moreover, annual fecundity estimates were not possible, since individual spawners were not tracked over the reproductive season in Pohnpei. However, Johannes et al. (1999) reported 50 tagged camouflage grouper of unknown sex returning to aggregation sites in more than one lunar month, suggesting possible repeat spawning in some individuals.

MANAGEMENT OF AGGREGATIONS IN THE INDO-PACIFIC.—As spawning aggregations represent a highly vulnerable life history phase (Ralston, 1987; Shapiro, 1987), and as aggregation fishing seems to coincide with fisheries declines in some areas (Sadovy, 1994, 1999), identification, protection and monitoring of spawning sites and spawners are important to their long-term conservation in Pohnpei and elsewhere. Within the western Pacific, Pohnpei and neighboring Palau have taken proactive stances in conserving camouflage grouper stocks, as well as those of the confamilials *E. fuscoguttatus* and *Plectropomus areolatus*, by establishing aggregation-based marine protected areas and catch and sales bans during potential spawning periods (Johannes et al., 1999; Rhodes, 1999). In Pohnpei, enforcement at the aggregation site has improved recently and poaching is limited. Moreover, a long-term monitoring scheme was recently established at the Kehpara aggregation site to estimate changes in spawning seasonality, aggregation abundance, mean fish size and to describe patterns of spawning behavior (Pet et al., 2001). These data will be useful for local management and conservation agencies that seek to improve current measures leading to enhanced protection of aggregations and establish a baseline for estimating changes in populations from non-aggregation fishing mortality. Until sexual pattern is identified conclusively in camouflage grouper, a precautionary approach to management, based on marine protected areas for aggregations, nursery areas, and protection of spawners moving to and from aggregations and during the aggregation season, is warranted.

ACKNOWLEDGMENTS

The authors wish to thank the governments and peoples of the Federated States of Micronesia and Pohnpei State. Contributors to the success of this work were the Overseas Fishery Cooperation Foundation and Pohnpei Inshore Resources Management Project, and the Marine and Environmental Research Institute of Pohnpei, which was instrumental in sample collection during much of the year. Funding and support for the project were provided by the Department of Ecology and Biodiversity, University of Hong Kong (HKU), the Hong Kong Research Grants Committee (RGC HKU7246/97M) (YS) and the American Museum of Natural History Lerner-Grey Fund (KLR). B. Paul and A. Olpet provided invaluable field assistance and advice throughout the project. Additional thanks go to G. Williams, R. Saunders, histologists H. C. Leung and Y. S. Cheung at the University of Hong Kong and J. Parker, A. Andrews, G. M. Cailliet and J. Gellar of Moss Landing Marine Laboratories.

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DATE SUBMITTED: January 12, 2001. DATE ACCEPTED: August 31, 2001.

ADDRESS: *The University of Hong Kong, Department of Ecology & Biodiversity, Kadoorie Biological Sciences Building, Pokfulam Rd., Hong Kong, SAR, People's Republic of China. E-mail: <klrhodes_grouper@yahoo.com>.*