

Detailed demographic analysis of an *Epinephelus polyphkadion* spawning aggregation and fishery

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ABSTRACT: The demographic profile of camouflage grouper *Epinephelus polyphkadion* (Bleeker, 1849), a widely distributed, commercially important, and Near Threatened (on the IUCN Red List) reef fish species, was established in the present study by sampling markets and a spawning aggregation in Pohnpei, Micronesia. Estimates of size at age, growth, and mortality were obtained from sectioned sagittal otoliths. Sections showed clear annuli whose formation coincided with decreasing, low-variability seawater temperature. Mean size, age, or growth rates did not differ between sexes. The maximum age (t_{max}) was 22 yr. Estimated growth and mortality parameters resembled those of other tropical epinephelines that display low population turnover (instantaneous growth coefficient [K] = 0.251 yr⁻¹; total mortality [Z] = 0.227 yr⁻¹; and natural mortality [M] = 0.144 yr⁻¹). Juveniles recruited to the fishery at Age 2, but adults were not present in the aggregation until Age 4. The sexual pattern for this species was resolved by complementing detailed histological analyses with age data to show functional gonochorism with the potential for protogynous sexual transition. Following intense aggregation fishing in 1999, females averaged 30 mm less in size and nearly 3 yr younger than those caught in 1998. Since 1999, aggregation abundance has declined from several thousand to a few hundred individuals and a significant mean size reduction has been detected in marketed samples. Relative to other regional locales, camouflage grouper in Pohnpei has a substantially truncated age and size structure, all suggesting that the species is experiencing fisheries-induced demographic changes. Proactive and adaptive management is needed to reduce the perceived impacts to spawning adults and juveniles and improve spawning stock biomass.

KEY WORDS: Epinephelidae · Age and growth · Mortality · Gonochorism · Fish spawning aggregations · Micronesia

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INTRODUCTION

The camouflage grouper *Epinephelus polyphkadion* (= *microdon*) is a commercially valuable epinepheline with a large contribution to local and foreign commercial catch (Sadovy et al. 2003, Rhodes & Tupper 2007, Rhodes et al. 2008). The species ranges from the eastern coast of Africa through the Red Sea to the islands of the eastern Pacific, south to Lord Howe Island, and north to the Ryuku Islands of Japan (Heemstra & Randall 1993). Like many of its congeners, *E. polyphkadion* is highly vulnerable to overfishing and has experienced range-wide declines

in abundance (Russell et al. 2006). The species is among the primary targets of the Southeast Asia-based live reef food fish trade (LRFFT) (Sadovy et al. 2003) that targets fish spawning aggregations throughout much of the Indo-Pacific to supply regional Asian seafood markets and restaurants. Much of the observed regional grouper decline may be attributed to these activities (Warren-Rhodes et al. 2003). *E. polyphkadion* is a voracious, high-order predator that feeds throughout the aggregation period, thereby elevating its vulnerability to a wide variety of gear types (Rhodes & Sadovy 2002b, Rhodes et al. 2008).

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Similar to a number of other epinephelins, *Epinephelus polyphekadion* has several reproductive life-history characteristics that increase its risk of being subject to overexploitation. Among these is the formation of large (100s to 1000s of individuals), often brief, yet temporally and spatially predictable spawning aggregations (Johannes et al. 1999, Rhodes & Sadovy 2002b, Robinson et al. 2008). In parts of the species' range, the spawning season persists over only 2 to 3 reproductive months annually, with fish present at the aggregation site for ca. 2 wk mo⁻¹ (Johannes et al. 1999, Rhodes & Sadovy 2002b, Robinson et al. 2008). During aggregation periods, spawning typically occurs on the final 1 to 2 d (Rhodes & Sadovy 2002b). This limited window for reproduction increases the risk of fisheries-induced declines in abundance, particularly when the aggregation fishery is commercialized (e.g. Beets & Friedlander 1998, Coleman et al. 1999, Whaylen et al. 2004, Sadovy & Domeier 2005a). Additionally, sex-specific variations in movement and residency have been documented (Rhodes & Sadovy 2002b) that can increase the potential for sexual selection and subsequent reductions in reproductive output (Koenig et al. 1996).

Although the species is an important component of local and regional fisheries there remain large data gaps in our understanding of its life history. These include, but are not limited to, estimates of age and growth, mortality, habitat use and home range, ontogeny, operational sex ratios, and sexual pattern. A few studies have attempted to resolve sexual pattern for the species. The first evidence of protogynous sex change (the development of mature males via sexual transition of mature females) was demonstrated from a tag-recapture study whereby 2 tagged females were later recaptured as males (Johannes et al. 1999). Other indications of sex-change potential came from hormonal induction under laboratory conditions (Debas 1989, Bruslé-Sicard et al. 1992). However, induction of sex change is not considered tangible evidence of a species' natural sexual development (Sadovy & Domeier 2005a). A more extensive study of 1073 wild-caught individuals (in 1998 to 1999) found direct development of mature males and females from juveniles and a wide size overlap between sexes, traits that often characterize gonochores (non-sex-changing species) (Rhodes & Sadovy 2002a, but see Sadovy & Shapiro 1987, Sadovy de Mitcheson & Liu 2008). Based on the combined evidence from these studies, Rhodes & Sadovy (2002a) considered the sexual pattern for this species to be unresolved.

Recently, combining sex-specific size, age, and histological data with information on growth has gone some way towards clarifying the sexual pattern in groupers (e.g. Sadovy et al. 1992, Sadovy & Colin 1995,

Fennessy & Sadovy 2002, Pears et al. 2006). For example, growth data can help elucidate both the timing and pattern of reproductive development for males and females, particularly in cases where histological evidence is unclear or absent. For sex-changing species, such detailed analyses are often necessary, since transitional individuals (those undergoing sexual transition) are typically rare in samples (e.g. Crabtree & Bullock 1998, Wyanski et al. 1999, Fennessy & Sadovy 2002). Additionally, early-stage transition often occurs within highly localized areas of the gonad and is easily overlooked. Since the response to fisheries impacts by populations is affected by sexual pattern, effective management is dependent on understanding the sexual pattern (e.g. Bannerot et al. 1987, Huntsman & Schaaf 1994, Armsworth 2001, Alonzo & Mangel 2004). Generally speaking, gonochoristic species are more resilient to fishing pressure, since size selection tends to have less impact on operational sex ratio and, therefore, fertilization and reproductive output (Vincent & Sadovy 1998).

In Pohnpei (Micronesia), 2 camouflage-grouper spawning aggregations have been documented: 1 at Ant Atoll and 1 in Kehpara Municipality (Pohnpei Island). Of the 2, only the Kehpara aggregation has been examined in detail (Rhodes & Sadovy 2002b). Each of these aggregations is considered to entertain separate reproductive populations, based on previous indications of high spawning-site fidelity for this and other aggregating groupers (Zeller 1998, Johannes et al. 1999, Bolden 2000, Starr et al. 2007). In late 1999, the Kehpara aggregation experienced intense fishing pressure that resulted in the removal of thousands of individuals (Rhodes & Sadovy 2002b). This event prompted the lead author (K.L.R.) to make recommendations to the state in 1999 to protect the aggregation through a no-take zone. In 2010, following continued lobbying, complete area protection was afforded to the Kehpara camouflage grouper (and the adjacent brown-marbled grouper *Epinephelus fuscoguttatus*) aggregation, but not until fishing had reduced the abundance of the former from an estimated several thousand fish in 1998 to only around 270 individuals in 2010 (Conservation Society of Pohnpei unpubl. data). In addition to noted declines in abundance, an observed truncation (to 1 mo) in the typical 2 mo spawning season (Rhodes & Sadovy 2002a) was documented in 2009 (K. L. Rhodes pers. obs.). Variations in the length of the spawning season have also been noted elsewhere for the species (Johannes et al. 1999). In addition to observed impacts on the aggregation, anecdotal reports of declining stocks and mean sizes of coral reef fish stocks, in general, are now common among fishers, market owners, and consumers. These events prompted a more detailed assessment of the

coral-reef fishery (Rhodes et al. 2008) and the life history and behavior of camouflage grouper, including a thorough examination of its age, growth, and sexual pattern.

The objectives of the present study were: (1) to define the demographic characteristics of *Epinephelus polyphekadion* in Pohnpei using samples taken from the Kehpara aggregation and local markets in 1998 and 1999. This is the first known investigation to provide a detailed age-based demographic profile of a grouper spawning aggregation. (2) Sex-specific age and growth parameters, mortality, and size and age at sexual maturity were estimated. Age and growth parameters were then used to clarify the species' sexual pattern. (3) We used the existing 2 yr aggregation-derived data set, including known size-based fecundity estimates, to examine the short-term impacts of aggregation fishing on camouflage grouper. (4) We compared market samples from 1998–1999 to those taken in 2006 to examine long-term trends in overall and average size structure. All activities were designed to inform management decision-making, to further our current understanding of the species' sexual development and reproductive life history, and to gain insight into potential fisheries-induced impacts.

MATERIALS AND METHODS

Aggregation sampling. A sub-sample ($n = 697$) of several thousand reproductively active *Epinephelus polyphekadion* were obtained over 2 yr from the unprotected Kehpara fish spawning aggregation located in Pohnpei, Federated States of Micronesia ($6^{\circ} 55' N$, $158^{\circ} 15' E$) (Fig. 1). Previous estimates placed the abundance at ca. 10 000 individuals (Rhodes & Sadovy 2002b). At the time of sampling, individual weights (nearest g) and lengths (nearest mm total length [TL]) were measured. A regression analysis of standard length (SL) to TL confirmed a nearly uniform relationship ($r^2 = 0.98$) (Rhodes & Sadovy 2002b). Aggregation-based sampling was conducted over the 2 mo spawning season during 16 d in 1998 (10–14 March, 2–12 April) and 22 d in 1999 (22 February–2 March, 19–31 March). Total numbers of fish taken in 1998 included 94 in March and 160 in April; and in 1999, 114 in February and 321 in March (Rhodes & Sadovy 2002b). Sampling periods coincided with the typical spawning season for the species (February–March, or March–April). In Pohnpei, the initiation of the Kehpara spawning season may vary inter-annually, similar to other locales (Johannes et al. 1999, Robinson et al. 2008). Sampling focused on the days leading up to and including spawning. Sampling occurred a minimum of 9 d prior to and including full moon in April 1998 and

February–March 1999, but only 4 d in March 1998. Sampling was concluded in each aggregation month when fish had dispersed from the spawning site (Rhodes & Sadovy 2002b). During all months, fish were collected from ca. 25 to 40 m depth throughout the entire aggregation area, which extends along a narrow (100 to 125 m linear distance) strip of the reef wall and slope on the seaward edge of the barrier reef. The aggregation is estimated to extend seaward along the seaward-facing reef slope to at least 50 m depth. Sampling at the aggregation was conducted diurnally and nocturnally by a single patriarch fisher who collected the available size range of individuals using (barbed-circle) hook and line baited with cut skipjack tuna (*Katsuwamus pelamis* or *Euthynnus affinis*). Hook-and-line fishing represents one-quarter of all fishing effort by gear type in the commercial Pohnpei coral reef fishery (Rhodes et al. 2008) and is the exclusive method of commercial capture of reproductive camouflage grouper at known aggregation site(s), owing to

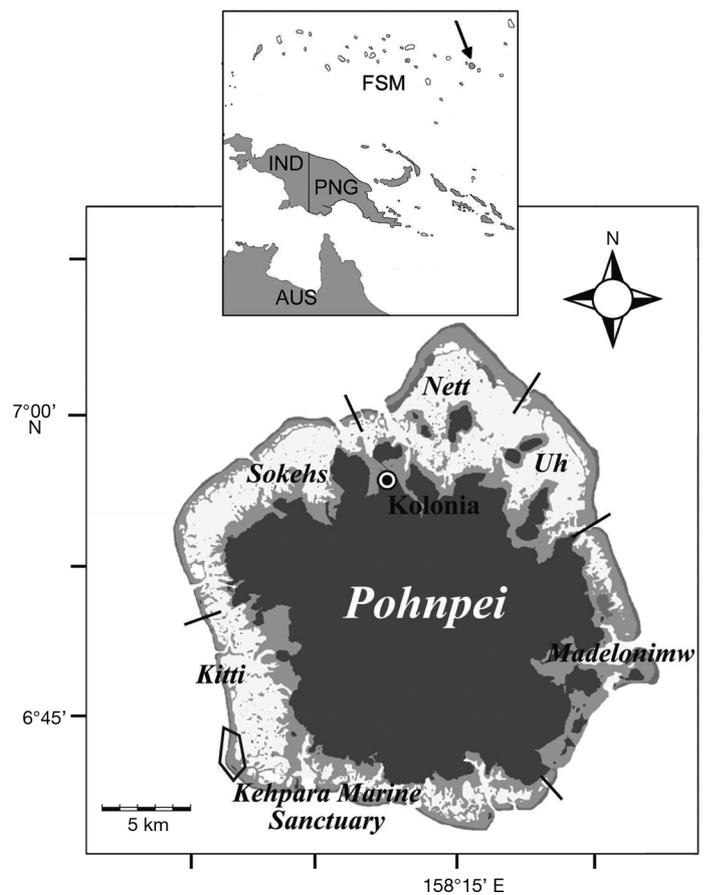


Fig. 1. Map of Pohnpei showing the location of the Kehpara aggregation site (Kehpara Marine Sanctuary, Kitti Municipality) and Kolonia, Nett Municipality, where local markets were sampled. AUS: Australia, FSM: Federated States of Micronesia, IND: Indonesia, PNG: Papua New Guinea. Black lines mark municipal boundaries

depth constraints using other methods (e.g. spear and net). In 1998, spearfishing on SCUBA by the lead author (K.L.R.) contributed to 5% of samples, with 15% of samples in 1999 taken by spear. A comparison between gears showed no significant difference in the sex ratio of catch (Rhodes & Sadovy 2002b). Sampling by both methods was haphazard, yet considered non-selective for size or sex. In contrast to aggregation fishing, nighttime spearfishing dominates commercial fishing effort during non-reproductive periods (~70% of the total).

Market sampling. Between May 1998 and January 1999 (inclusive), an additional 366 individuals were obtained by haphazardly sampling ca. 30 to 40 individuals monthly from multiple fish markets in Kolonia, Nett Municipality (Fig. 1), with a focus on collecting fish monthly throughout the year and across the size spectrum. The protocol was developed to allow a comprehensive demographic age, growth, and reproductive assessment of *Epinephelus polyphkadion* from commercial catch, including immature individuals not represented within the reproductive population. The origin of marketed samples is considered exclusive to the main island (Pohnpei), since 97.8% of all commercial reef fishing is conducted there (Rhodes et al. 2008). The remaining 2.2% of commercial catch is from nearby Ant and Pakin Atolls. Approximately 90% of commercial effort is focused on Kitti (~50%), Nett/Kolonia (~30%), and Sokehs (~10%) municipalities, which together represent ca. 80% of the total reef area (Fig. 1). In Uh and Madelonimw municipalities, fishing is primarily for subsistence purposes, yet contributes to ca. 10% of the marketed volume in Kolonia (Rhodes et al. 2008). While marketed mature fish likely represent >1 reproductive population, those from Kehpara are considered to be from a single reproductive population, based on documented high spawning-site fidelity of this and other aggregating groupers (Zeller 1998, Johannes et al. 1999, Starr et al. 2007, Rhodes & Tupper 2008).

To compare trends in the commercial fishery from 1998–1999, a comparable number of *Epinephelus polyphkadion* were sampled during a 10 January to 25 May 2006 coral reef fish market survey. Market sampling in all years excluded the period 1 March to 31 April, when the annual grouper sales ban occurs. During the 2006 survey, a total of 445 individual catches were examined from an average of 21.7 ± 3.3 markets daily. All individuals were weighed (nearest 100 g) and measured to nearest mm TL and SL (Rhodes & Tupper 2007). Fish sampled during the 2006 market were not sexed nor were gonads or otoliths collected. Fisher interviews were taken for all examined catch to document catch location (area effort) and method as part of a broader coral reef fish market survey (Rhodes et al. 2008).

Gonads. For all aggregation and all 1998–1999 market samples, gonads were extracted, weighed, and stored in a 10% formalin solution before being transferred to 70% ETOH (Rhodes & Sadovy 2002a). All 1998–1999 market-derived samples were fixed, stained, and sectioned prior to microscopic examination, while aggregation-derived individuals were sexed microscopically from either sectioned gonads (sub-sample) or tissue squashes.

Otoliths. Following sample collection, the otoliths (pair of sagittae) were removed, cleaned, and weighed (nearest 0.001 g) prior to sectioning. Sub-samples of otoliths were randomly selected in such a manner as to match the size distribution of original market and aggregation samples. The otoliths were prepared using 2 methods and read by author B.M.T. For the first method, 1 of the sagittal pair was affixed to paper tabs and coated in fiberglass resin ($n = 391$). These embedded samples were sectioned through the nucleus using a Buehler low-speed Isomet saw fitted with twin diamond-edged blades separated with a 0.5 mm plastic spacer. Sections were then transferred to a clean microscope slide and set in Cytoseal® acrylic resin for final polishing using P600 grit wet and dry paper prior to reading. To improve the resolution for individuals whose increments were faint, sections were left on a hot plate at 60°C for 1 to 2 h until dark brown in color, which has been shown to accentuate incremental patterns (Grandcourt et al. 2006).

For the second method, 1 sagittal otolith was set in Crystalbond® resin on the edge of a glass microscope slide ($n = 200$). Using a modified grinding wheel, each otolith was ground to the nucleus using P600 wet and dry paper (Choat et al. 2003). The otolith half was then repositioned in the middle of the slide, polished-side face down, and ground to the nucleus, producing a thin section (ca. 300 μm thick). Sections were then heated on a hot plate as described in the previous paragraph. For all otoliths, individual ages (in years) were assigned by counting the annual increments using transmitted light at 15 to 25 \times magnification. Using a double-blind method, all otoliths were read twice (Russ et al. 1998) with a 2 wk period between reads. If the 2 readings disagreed by >1 increment, the otolith was read a third time. The otolith was eliminated from the analysis if the third reading was different to the previous 2.

Analysis. Edge-type analysis (Manickchand-Heileman & Phillip 2000) was used to confirm the annual deposition of increments. The optical characteristic of the otolith margin was recorded for individuals as either opaque or translucent. The frequency of otoliths with opaque margins was plotted against month to determine when deposition took place. The monthly frequency of opaque-edge occurrence was

compared to a uniform distribution using a χ^2 goodness-of-fit test for circular distributions (Zar 1999).

Total mortality (Z) was estimated using an age-based catch curve from market-derived age-based data, where the natural logarithm of the number of fish in each age class was plotted against its corresponding age. Z is the absolute value of the slope from a line fitted to the descending limb of the curve (Beverton & Holt 1957, Ricker 1975). Only market-derived samples were used as these samples represent the island-wide commercial fishery for *Epinephelus polyphekadion*. Estimates of natural mortality (M) were obtained using the general equation of Hoenig (1983): $\log_e Z = 1.46 - 1.01(\log_e t_{\max})$, where t_{\max} is maximum age and Z is analogous to M in an unexploited population. In this instance, $t_{\max} = 31$ yr, based on age data for *E. polyphekadion* from New Caledonia (K. L. Rhodes & B. M. Taylor unpubl. data) and the Seychelles (Grandcourt 2005). The rate of exploitation (E) is the proportion of fishing mortality (F) relative to Z ($E = F/Z$) where F is derived from the relationship $F = Z - M$.

Age frequency distributions were plotted separately for males and females, and between annual spawning aggregations and market samples from the non-spawning period. The von Bertalanffy growth function (VBGF) was fitted to length-at-age estimates for all fish combined (aggregation and market samples) using non-linear least-squares estimation (von Bertalanffy 1938). The VBGF is represented by

$$L_t = L_{\infty} \{1 - \exp[-K(t - t_0)]\} \quad (1)$$

where L_t is the TL of a fish at age t , L_{∞} is the mean asymptotic TL, K is the growth coefficient, and t_0 is the theoretical age at which TL = 0. Because of the lack of fish aged <2 yr, the growth curve was constrained through the y-axis at 25 mm, which represents the size at settlement for *Epinephelus* spp. (Leis 1987, Pears et al. 2006). Using this technique increases the precision of the fitted VBGF curve by an order of magnitude (Kritzer et al. 2001). Potential differences in sex-specific growth parameters were investigated using bivariate 95% confidence ellipses surrounding the K and L_{∞} estimates (Kimura 1980).

Classification of gonad maturity stages follows that of Rhodes & Sadovy (2002a) and includes: F1 (immature); females: F2 (mature, inactive), F3 (mature, active), F4 (mature, ripe), F5 (post-spawn); males: M1/2 (immature; mature, inactive), M3 (mature, active), M4 (mature, ripe), M5 (post-spawn). F1 individuals were sub-categorized as F1-A and F1-B in Rhodes & Sadovy (2002a) to designate morphological differences observed among F1 (immature) individuals. F1-B are now presumed to be an intermediate developmental stage between the F1-A (sexually bipotential juvenile) stage and the M1/2 (immature;

mature, inactive) stage of male development. For the present analysis, F1-A and F1-B stages are combined.

Size and age at 50% female maturity was assessed using 2 methods following Pears et al. (2006) that described age and growth for the congeneric brown-marbled grouper *Epinephelus fuscoguttatus*. Method 1 involved calculating the percentage of mature (F3 and F4) and mature, inactive females (F2) by size and age class and fitting a logistic curve. Method 2 used only the percentage of mature (F3 and F4) females by size and age class within the aggregation during the spawning season. The latter method is termed 'effective maturity' (Pears et al. 2006), because it recognizes that sexually mature females may not reproduce every spawning season (i.e. skipped spawning). For camouflage grouper, there is no evidence to support skipped spawning in females. Nonetheless, we used both methods for comparison.

A 3-way ANOVA was used to test for differences in mean size and age between sexes and aggregation periods (1998 and 1999). Factors in the model included 'sex', 'month', and 'year'. This enabled a comparison of inter-annual and intra-annual (between months, each year) differences in these same variables. Because the aggregation forms twice annually, with 2 wk apart between aggregations, the factor 'month' was used to test between temporally distinct aggregations within years and to allow inference of observed size and age variations among sample times. All assumptions of homogeneity and equal variance were met prior to the analysis. A post hoc Holm-Sidak multiple comparisons test was used to determine differences among groups. Differences in mean age among the 1998 aggregation, the 1998 non-spawning period, and the 1999 aggregation were assessed using a 1-way ANOVA followed by a post hoc Holm-Sidak multiple comparison test comparing the mean age for each sample.

Female reproductive potential was investigated by plotting the mean ovary weight for mature females (F3 and F4) by size and age class. Only females sampled from the aggregation were used. The size-fecundity relationship for females (Fecundity = [gonad free body weight] \times 1350) reported in Rhodes & Sadovy (2002a) was used to model the effects of inter-annual changes in female size structure on the mean fecundity of the aggregation. To do this, the mean fecundity estimate per length class was multiplied by the proportional frequency of individuals for the respective length class. Values were summed across all length classes to estimate the mean fecundity per female in the aggregation. This was performed using the female size structures from the 1998 and 1999 aggregations separately for comparison to determine how changes in size structure between years affected population fecundity, irrespective of total aggregation size.

RESULTS

Of the 1034 fish examined for age and/or sexual development stage, 667 were taken from aggregations at Kehpara in 1998–1999 (380 assigned an age), while the remaining 367 individuals came from markets (211 assigned an age). All individuals were examined microscopically, including 465 individuals sexed and staged from thin-sectioned gonads (275 individuals in 1998: 135 immature, 91 female, 47 male; 190 individuals in 1999: 32 immature, 112 female, 46 male). The remaining samples were sexed and staged microscopically using tissue squashes.

Otolith validation

Age estimates from sectioned otoliths were obtained by counting annual increments (annuli) along the sulcal axis using transmitted light (Fig. 2). Annuli appeared as easily identifiable opaque zones that were considerably darker in color relative to adjacent translucent zones. Using this method, a 91.7% degree of accuracy was achieved between reads among individuals. Otolith weight was a good predictor of age for *Epinephelus polyphekadion* and accounted for 78% of the variability (Table 1). Edge-type analysis confirmed annual increment formation (Fig. 3), with significant differences among monthly frequencies of otoliths with opaque margins ($\chi^2 = 45.1$, $p < 0.001$). The formation of opaque or annual increments occurred from November to February just prior to the reproductive season (Rhodes & Sadovy 2002b) and during periods of decreasing, yet relatively stable mean sea-surface temperature (Fig. 3).

Growth, mortality, and maturity

For aggregation samples, sex-specific comparisons revealed significant differences in size ($F_{1,646} = 22.9$, $p < 0.001$) (males: 419.4 ± 1.8 mm TL, $n = 405$; females:

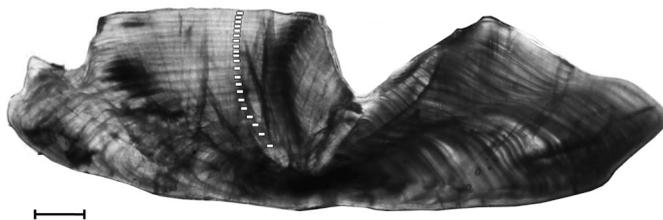


Fig. 2. *Epinephelus polyphekadion*. Photomicrograph of an otolith from a 21 yr old male (40.5 cm total length) taken from the aggregation in 1999. White bars represent annuli. Scale bar = 0.5 mm

Table 1. *Epinephelus polyphekadion*. (A) Growth and mortality parameters and (B) the relationship between otolith weight (OtWt) and corresponding fish age. TL: total length

(A)		
Parameter	Definition	Estimate
K	Growth rate	0.251 yr^{-1}
L_{∞}	Asymptotic length	447.1 mm TL
t_0	Age at which length = 0	-0.14 yr
Z	Total mortality	0.227 yr^{-1}
M	Natural mortality	0.144 yr^{-1}
F	Fishing mortality	0.083 yr^{-1}
E	Exploitation rate	0.366
(B)		
Relationship	Equation	r^2
Otolith weight by age	Age = $43.2(\text{OtWt}) + 0.45$	0.78

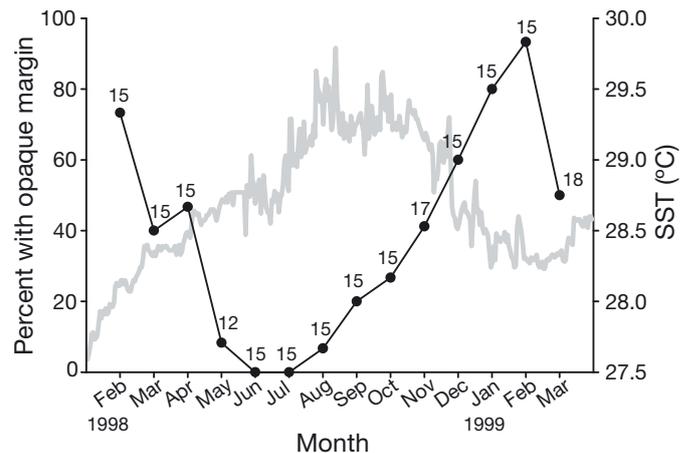


Fig. 3. *Epinephelus polyphekadion*. Comparison of the frequency of marginal increment formation (black line) to annual sea-surface temperatures (SST) (grey line) over the study period (data from Tropical Atmosphere Ocean [TAO] Project Office, NOAA Pacific Marine Environmental Laboratory). The frequency of increment formation in otoliths is highest during the spawning period (February and March) as temperatures decline. Numbers above data points indicate sample size

399.6 ± 2.5 mm TL, $n = 288$), as previously reported (Rhodes & Sadovy 2002b), but not age ($F_{1,331} = 0.8$, $p = 0.368$) (Fig. 4A,B). There was complete overlap in the age distribution, particularly in the older age classes (>16 yr), where both sexes were equally represented. The minimum age among aggregation samples was a 4 yr old female, while t_{\max} was 22 yr (2 males and 1 female). The t_{\max} for Pohnpei is well below the t_{\max} of 31 yr reported from the Seychelles (Grandcourt 2005) and New Caledonia (B. M. Taylor & K. L. Rhodes unpubl. data).

Like other epinephelids, *Epinephelus polyphemus* experiences moderate growth during its early life history until ~Age 6 ($K = 0.251 \text{ yr}^{-1}$), when 80% of L_{∞} is achieved (Fig. 5, Table 1). The combined L_{∞} for this species was 447.1 mm TL. This was ~10 cm less than the L_{∞} for *E. polyphemus* sampled from the Seychelles (Grandcourt 2005). Sex-specific growth

parameters did not differ significantly, with overlapping 95% bivariate confidence intervals surrounding estimates of K and L_{∞} (see inset in Fig. 5). Immature fish ranged from 2 to 10 yr old, but dominated the first 5 year-classes. Z , derived from an age-based catch curve, was 0.227 yr^{-1} , and M was 0.144 yr^{-1} (Table 1). This latter estimate was almost identical to that of fish from the Seychelles, with $M = 0.13 \text{ yr}^{-1}$ (Grandcourt 2005). The estimated E was 0.366.

The size (L_{50}) and age (t_{50}) at 50% female maturity was 352 mm TL and 6.4 yr using Method 1 and 327 mm TL and 5.4 yr using Method 2 (Fig. 6). The L_{50} result from both methods mirrored the L_{50} of ca. 335 mm TL reported previously for Pohnpei samples (Rhodes & Sadovy 2002a).

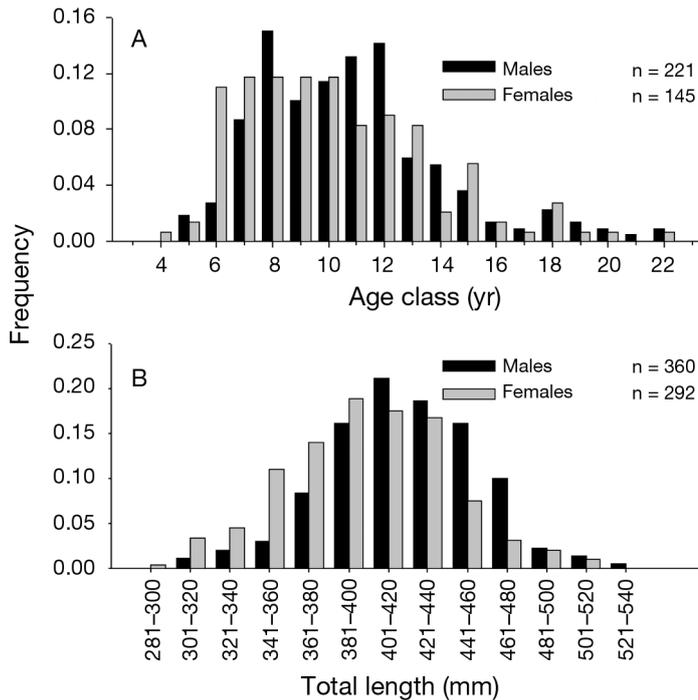


Fig. 4. *Epinephelus polyphemus*. Sex-specific (A) age frequency and (B) length frequency distributions taken from spawning aggregations in 1998 and 1999

Sexual pattern

Based on the present analysis, *Epinephelus polyphemus* conforms to a functionally gonochoristic sexual pattern with the potential for protogynous sex change. Support for gonochorism is provided by the overlapping, non-significant differences in growth, sex-specific size and age distribution, and the absence of transitional fish among the 1072 individuals examined. Indications of potential sex change are supported by both laboratory-induced sex change (Debas 1989, Bruslé-Sicard et al. 1992) and from prior tag-recapture experiments (Johannes et al. 1999). *E. polyphemus* matures directly from the juvenile state (Rhodes & Sadovy 2002a), similar to

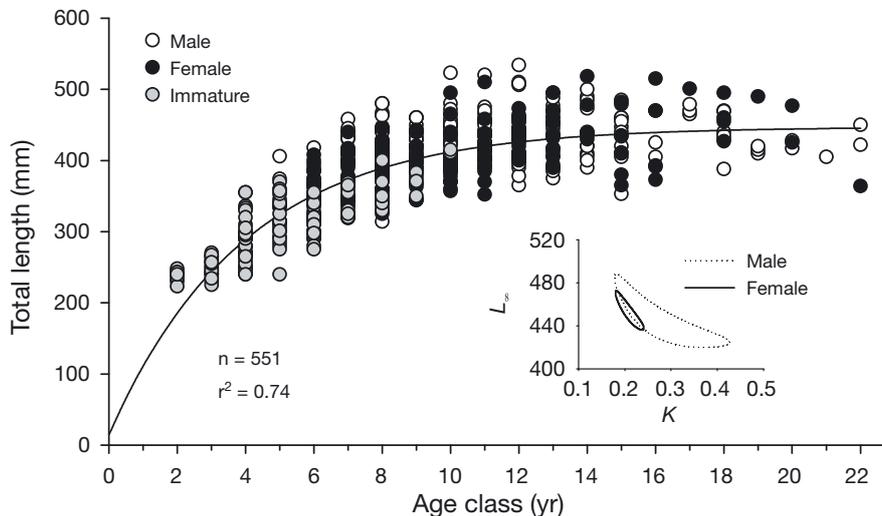


Fig. 5. *Epinephelus polyphemus*. Von Bertalanffy growth curve for combined market and aggregation-derived samples taken from 1998 to 1999. Inset: bivariate 95% confidence ellipses surrounding the growth coefficient (K) and mean asymptotic total length (L_{∞}) parameter estimates for males and females

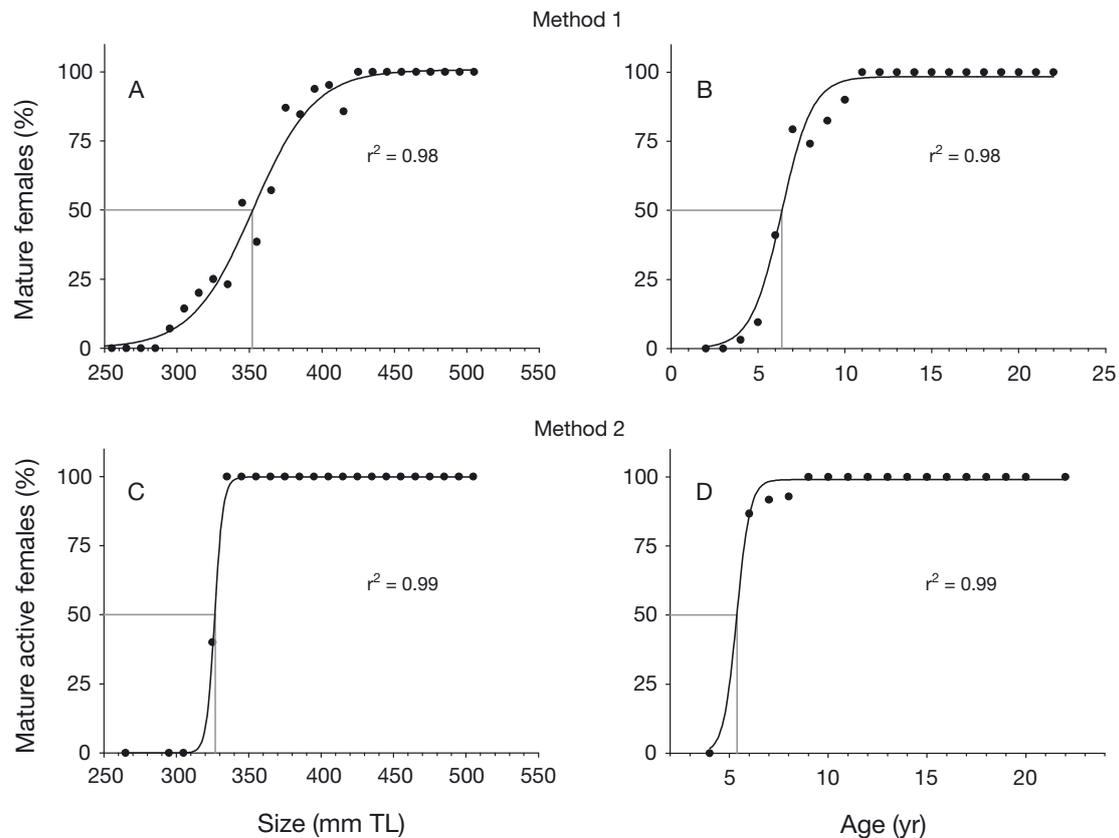


Fig. 6. *Epinephelus polyphekadion*. Two estimates of female maturity based on (A,B: Method 1) combined percentage of mature active and mature inactive females by size and age class and (C,D: Method 2) percentage of mature active females within the spawning aggregation during the spawning season. The 50% maturity estimates (grey lines) are indicated for (A,C) size (L_{50}) and (B,D) age (t_{50}). Sample sizes: (A) 297, (B) 273, (C) 128, (D) 125

other functionally gonochoristic epinephelines (e.g. Sadovy & Colin 1995, Posada 1996, Sadovy & Domeier 2005b, Erisman et al. 2008, Sadovy de Mitcheson & Liu 2008).

Inter-annual changes in reproductive potential

Female reproductive potential increased linearly with both size and age (Fig. 7), and concurs with previous findings using direct oocyte counts (Rhodes & Sadovy 2002a). A plot of mean ovary weight by age revealed no differences in female fecundity between years despite the decline in mean age (Fig. 7B). However, based on the shift in female size structure between 1998 (mean TL: 415.5 mm) and 1999 (mean TL: 390.7 mm) (Fig. 8A), the mean fecundity decreased by 19% (Fig. 8B), with a concomitant drop in total reproductive output of 35 to 43%. The latter is based on an estimated reduction of 20 to 30% in aggregation size between years.

Changes in size and age

An inter-annual comparison of body size showed significant inter-annual differences between combined-year aggregation samples, with 1998 fish larger on average than those in 1999 ($F_{1,646} = 28.3$, $p < 0.001$) (Fig. 9A, Table 2). In contrast, there was no intra-annual size difference between months ($F_{1,646} = 1.3$, $p = 0.257$) (Fig. 9A). Although males appeared older on average than females within each respective aggregation, these differences were not significant ($F_{1,331} = 0.8$, $p = 0.368$) (Table 3). Similar to body size, the overall mean age was significantly greater in 1998 than 1999 ($F_{1,331} = 23.4$, $p < 0.001$), with a substantial truncation in age structure observed in 1999 (mean age in 1999: 9.87 yr; in 1998: 11.55 yr). Based on aggregation fishing effects reported elsewhere (e.g. Beets & Friedlander 1998), it is plausible though not proven that this finding is a response to the intense aggregation exploitation in February 1999, with a resultant reduction in 13 to 18 yr old fish. Concomitantly, a substan-

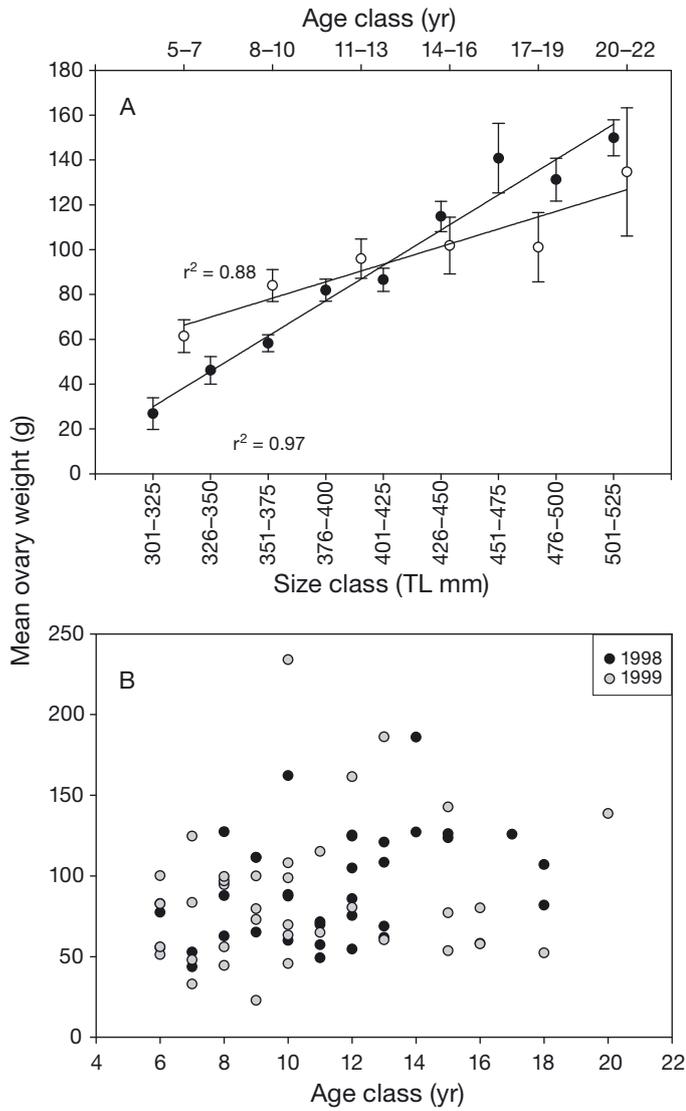


Fig. 7. *Epinephelus polyphkadion*. (A) Length (●) and age-specific (○) reproductive contribution (as mean ovary weight) for mature active females and (B) mean ovary weight plotted by age class for 1998 and 1999 aggregations. TL: total length

tially greater proportion of younger fish is represented in 1999 than in 1998 (Fig. 10). No such differences were evident among months within each of the 2 respective years ($F_{1,331} = 0.03$, $p = 0.852$). In lieu of fishing effects, such large differences would indicate substantial recruitment into younger age classes and high levels of mortality of older individuals that are not readily apparent in market or aggregation samples.

Market-aggregation comparisons

In addition to observed inter-annual size and age changes between years, the demographic profile of

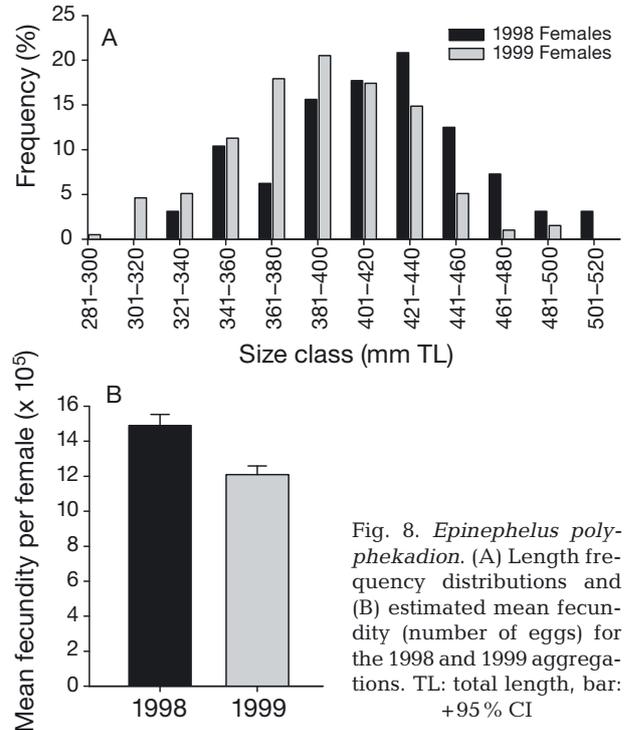


Fig. 8. *Epinephelus polyphkadion*. (A) Length frequency distributions and (B) estimated mean fecundity (number of eggs) for the 1998 and 1999 aggregations. TL: total length, bar: +95% CI

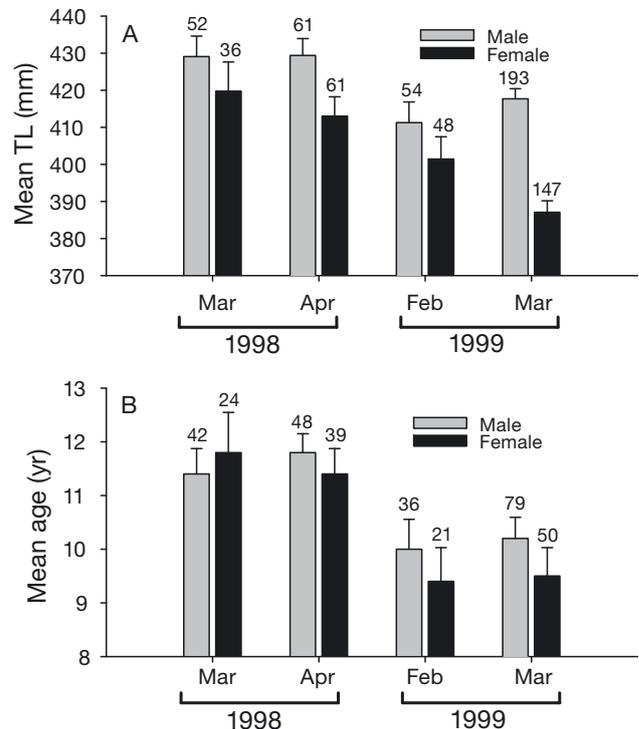


Fig. 9. *Epinephelus polyphkadion*. Inter- and intra-annual sex-specific aggregation dynamics for (A) length and (B) age at the aggregation site. Numbers above bars indicate sample size. TL: total length, error bars: + SE

Table 2. *Epinephelus polyphekadion*. 3-factor ANOVA showing the influence of year, month, and sex on the mean length of fish derived from the spawning aggregation. ***p < 0.001

Source of variation	df	MS	F	p
Year	1	43505.1	28.308	<0.001***
Month	1	1977.9	1.287	0.257
Sex	1	35155.6	22.875	<0.001***
Year × Month	1	0.1	<0.001	0.993
Year × Sex	1	1308.8	0.852	0.356
Month × Sex	1	5229.3	3.403	0.066
Year × Month × Sex	1	1799.5	1.171	0.280
Residual	646	1536.9		

Table 3. *Epinephelus polyphekadion*. 3-factor ANOVA showing the influence of year, month, and sex on the mean age of fish derived from the spawning aggregation. ***p < 0.001

Source of variation	df	MS	F	p
Year	1	247.6	23.415	<0.001***
Month	1	0.3	0.035	0.852
Sex	1	8.6	0.813	0.368
Year × Month	1	0.5	0.048	0.827
Year × Sex	1	7.0	0.663	0.416
Month × Sex	1	4.5	0.425	0.515
Year × Month × Sex	1	3.3	0.313	0.576
Residual	646	10.6		

fish taken from aggregations was dramatically different from those taken from markets (Fig. 11). For market samples, a significantly greater proportion of younger age classes (mean age: 6.97 yr) were represented than in aggregations ($F_{2,590} = 91.3, p < 0.001$). Juvenile camouflage grouper are typically absent from aggregations, yet represented 40% of market samples in 1998–1999 and increased to 48% in 2006 (Rhodes & Tupper 2007). Camouflage grouper first participate in the aggregation at Age 4 (both sexes combined), yet recruited to the non-aggregation fishery at Age 2.

Similar to the declines in size, age, and fecundity observed between aggregations, a significant decline in mean size ($F_{1,708} = 23.285, p < 0.001$) was observed between 1998–1999 and 2006 market *Epinephelus polyphekadion*. The mean (\pm SD) length in 1998–1999 was 29.2 ± 5.8 cm SL, while in 2006 it was 27.2 ± 5.0 cm SL. These results provide further support for the perceived population-level size reductions and the loss of larger-sized individuals.

DISCUSSION

Like many of its congeners, *Epinephelus polyphekadion* demonstrates a number of life-history traits that contribute to the potential for overfishing, including the formation of temporally and spatially predictable

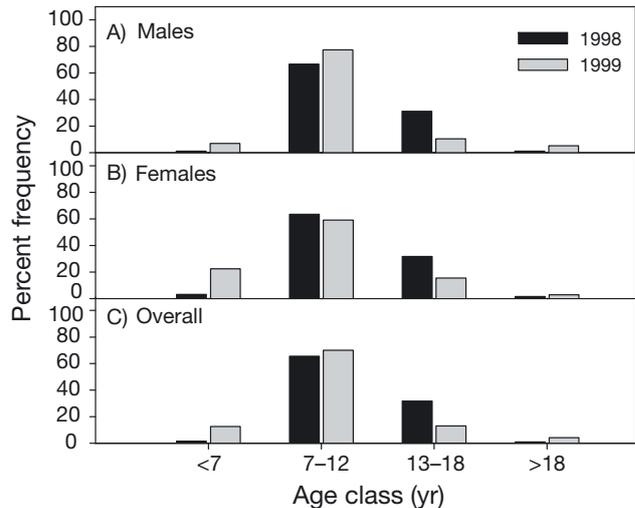


Fig. 10. *Epinephelus polyphekadion*. Age class frequencies for (A) males, (B) females, and (C) overall, showing the shift in prevalence from older individuals (13 to 18 yr) to younger individuals (<7 yr) between the 1998 and 1999 spawning aggregations

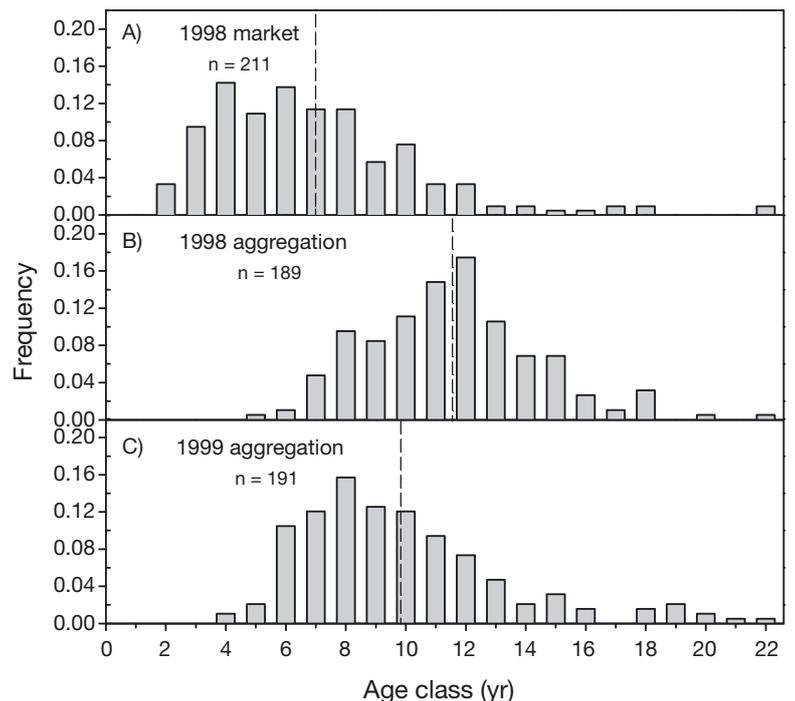


Fig. 11. *Epinephelus polyphekadion*. Age frequency distributions for the (A) 1998 market samples and the (B) 1998 and (C) 1999 spawning aggregations. Mean age is indicated (vertical dashed line)

spawning aggregations (Johannes et al. 1999, Rhodes & Sadovy 2002b, Robinson et al. 2008), late-onset reproduction (Manooch & Mason 1987, Pears et al. 2006), and relatively long lifespans (Manooch & Mason 1987, Bullock et al. 1992, Grandcourt 2005). In Pohnpei, the species is the largest component of the grouper catch among 24 species targeted (Rhodes et al. 2008) and has experienced heavy fishing pressure on its known spawning aggregations since at least 1999 (Rhodes & Sadovy 2002b). We documented heavy aggregation fishing pressure in February 1999 and observed a smaller mean size and declines in potential reproductive output among adults in comparison to 1998. In addition to documenting the demographic profile of aggregations, market samples provided a means to examine larger-scale (Pohnpei) population demography and highlight changes in mean size (7 yr span), a truncated age structure relative to some regional locales and what appears to be an increasing number of juveniles in catch (1998 to 2006). These combined findings suggest impacts that cannot be ascribed to changes in natural mortality or recruitment alone. Specifically, we suggest that the noted declines are likely a combined effect of aggregation fishing and the selection of juveniles and younger fishes as larger size classes become less available to the fishery over time.

Age, growth, mortality, and size at sexual maturity

The age and growth profile derived from analysis of *Epinephelus polyphekadion* shows that the species retains characteristics similar to other epinephelines, i.e. a moderate rate of growth during early development (to Age 6), late maturity (Age 4 to 5), and a (potentially) long life relative to its size (31 yr). Mortality estimates compare well with other regional fished populations, such as the Seychelles, where such parameters are known (Grandcourt 2005).

Sexual pattern

Using age and growth data, the present study provided the necessary evidence to confirm a gonochoristic sexual pattern for the species. Epinephelines exhibit a wide diversity of sexual patterns, including sequential and bi-directional hermaphroditism, and gonochorism (Shapiro 1987, Sadovy de Mitcheson & Liu 2008). Protogynous hermaphroditism has been the most commonly observed pattern, although functional gonochorism has now been confirmed in several species, including *Epinephelus polyphekadion* (the present study), *E. striatus* (Sadovy & Colin 1995), *Paran-*

thias furcifer (Posada 1996), *Paralabrax auroguttatus*, *Paralabrax nebulifer*, *Paralabrax clathratus*, and *Paralabrax maculatofasciatus* (Sadovy & Domeier 2005b), and *Mycteroperca rosacea* (Erisman et al. 2008). For *E. polyphekadion*, plausible evidence for protogyny was provided by a tag-recapture study, where 2 tagged females were later recovered as males (Johannes et al. 1999). In contrast, by combining evidence from previous microscopic gonad assessments with our age and growth results, we provide convincing evidence that the species is functionally gonochoristic, with the potential for protogynous sexual transition. The variable results at different locations imply regional plasticity in sexual pattern. Similarly, Nassau grouper has been classified as a functional gonochore with the potential for protogynous sex change (Sadovy & Colin 1995). The factors contributing to the regional variation observed in *E. polyphekadion* sexual pattern are unknown, but highlight both the complexity of reproductive development in epinephelids and the difficulties in designing regional management decision-making that is affected by sexual pattern, e.g. size limits. Although the species is gonochoristic and is presumed to have greater resiliency to fishing (e.g. Bannerot et al. 1987), evidence of aggregation loss, population declines, and changes to demography suggest a greater focus is needed on other life-history characteristics that appear to be elevating its vulnerability to overfishing. We discuss some of these factors below.

Differences in size and age

Observed differences in size and age were apparent between aggregation and market-derived samples as a direct result of variations in the life-history stages available to fishers. Specifically, spawning aggregations are composed almost exclusively of adults. In contrast, marketed camouflage grouper taken outside the March–April sales ban period come from the available size structure, in this case fish of Age 2 and upward. A potential contributor to the observed differences between aggregation and marketed fish is gear use and catch location, since 70% of commercial fishing is by (nighttime) spear(fishing) inside the lagoon (Rhodes et al. 2008). In contrast, aggregation sampling relied almost exclusively on hook-and-line fishing in seaward reef areas. However, no significant size or sex ratio differences were observed between gears or targeted locale (lagoon versus outside reef) (Rhodes & Sadovy 2002b), suggesting that aggregation and marketed size structures accurately represent those of the Kehpara reproductive and island-wide populations, respectively. Thus, the observed changes in size structure and reproductive output support our contention

that these differences are likely fisheries-induced. The stock level is currently unknown and estimates of sustainable yield are not yet available. Nonetheless, results clearly show a need for improved management that eliminates aggregation fishing and regulates size at catch and sale.

Aggregation fishing effects

The effects of aggregation fishing are widely known and include changes to abundance, size, total reproductive output, and genetic diversity (Chapman et al. 1999, Sadovy & Domeier 2005a). In extreme cases of overfishing, aggregations may cease to form (e.g. Sadovy & Eklund 1999) or become so highly skewed sexually that reproductive output is impaired (e.g. Koenig et al. 1996, Alonzo & Mangel 2004, Brooks et al. 2007). Similar to other aggregating grouper, *Epinephelus polyphekadion* has received tremendous commercial fishing pressure on aggregations throughout its range. This pressure increased substantially beginning in the 1980s when the LRFFT expanded throughout Southeast Asia and into the wider Indo-Pacific (Sadovy et al. 2003). The trade, which appropriates and maintains live groupers and wrasses for the Southeast Asia seafood industry, knowingly targets spawning aggregations and reportedly appropriates the entire annual sustainable grouper yield from Southeast Asia (Warren-Rhodes et al. 2003). Associated range-wide reductions in abundance and spawning aggregation loss for this and other targeted aggregating groupers have been noted as a result. In locations where this and other foreign commercial fishing is absent, aggregation fishing pressure comes from local, small-scale commercial or subsistence fishers, as profiled in the present study and other recent studies (e.g. Hamilton et al. 2005). The results of these localized efforts appear to be similar to that from large-scale commercial harvest and can occur quickly (Johannes et al. 1999, Robinson et al. 2004, Hamilton et al. 2005, Rhodes & Tupper 2007).

In Pohnpei, anecdotal reports suggest that aggregation fishing has continued for decades, although it was not until the mid-1900s that it began to be commercialized. Unfortunately, no historical details of the aggregation or commercial fishery are available. Nonetheless, we show the immediate effects of a single aggregation fishing event that appears to have reduced the mean size and age of the spawning population. Similarly, aggregation abundance declined from several thousand to only 260 or so individuals between 1998 and 2010 (Conservation Society of Pohnpei unpubl. data). While the former may be attributed to high incident adult mortality, the latter can only be

linked to the prolonged aggregation fishing known to have occurred during the period in question. If aggregation abundance can be considered a reliable indicator of population responses to fishing, other reported impacts from aggregation fishing are likely being experienced, such as island-wide reductions in population abundance, mean size, age, and reproductive output (e.g. Sadovy & Domeier 2005a).

Regardless of fishing impacts, populations of *Epinephelus polyphekadion* in Pohnpei appear to be comprised of relatively smaller and younger individuals than other regional stocks surveyed. Specifically, the maximum age for *E. polyphekadion* is 31 yr in both the Seychelles (Grandcourt 2005) and New Caledonia (B. M. Taylor & K. L. Rhodes unpubl. data), while the maximum age in Pohnpei appears to be 22 yr. Similarly, mean female size within a Seychelles aggregation was 12 cm TL greater than that from Pohnpei, while males were 16 cm TL larger (Robinson et al. 2008). In contrast to fished populations in Pohnpei and Seychelles, New Caledonia camouflage grouper are ciguatoxic and likely experiencing lower fishing pressure (M. Kulbicki pers. comm.). Since larger individuals are not targeted, the age structure likely reflects the species' true age maxima. The importance of preserving larger, older individuals within populations is well documented (Palumbi 2004, Birkeland & Dayton 2005). In general, older individuals are naturally less abundant within populations, but they nonetheless contribute disproportionately higher egg volumes (e.g. Pears et al. 2006) and have higher larval survivorship (Berkeley et al. 2004) than their smaller counterparts. While no historical demographic information exists for *E. polyphekadion* in Pohnpei, the observed truncation in size and age in the population relative to regional populations suggests a reduced potential to offset fisheries impacts and improve or maintain the population, particularly given the observed declines in aggregation abundance and continued fishing pressure.

Behaviors affecting overfishing and aggregation loss

In addition to late maturation and a relatively long lifespan, *Epinephelus polyphekadion* demonstrates a number of reproductive behaviors that appear to enhance the potential for large-scale fishing impacts. These behaviors include: (1) a reliance on aggregations to spawn, (2) egg release on or near the final day of the aggregation period (Rhodes & Sadovy 2002a), and (3) relatively brief reproductive seasons (Johannes et al. 1999, Rhodes & Sadovy 2002b, Robinson et al. 2008; but see Hamilton et al. 2005). While aggregation loss is common to all epinephelids using this reproductive approach, some species may buffer against popu-

lation diminution by distributing reproductive effort more broadly in time and space. Such spatial distribution is demonstrated by *Plectropomus areolatus*, which often co-aggregates in large numbers with *E. polyphkadion*, but may also form smaller, alternative aggregations away from larger primary spawning sites (Sadovy 2005). Similarly, leopard coral grouper *P. leopardus* form numerous small, spatially distributed aggregations across wide swathes of reef (Samoilys 1997, Zeller 1998). *P. areolatus* also demonstrates temporal distribution of reproductive activity by spawning over a relatively protracted season in comparison to other co-aggregating groupers (e.g. Johannes et al. 1999, Hamilton et al. 2005, Hutchinson & Rhodes 2010). Even where *E. polyphkadion* aggregates monthly, much of its reproductive output appears to occur within a few peak months (e.g. Hamilton et al. 2005). In other locales, *E. polyphkadion* aggregations form over only 1 to 2 mo annually (Johannes et al. 1999, Rhodes & Sadovy 2002b, Robinson et al. 2008), with egg release during 1 or 2 nights at the end of the aggregation period (Rhodes & Sadovy 2002b). Thus, the total annual reproductive output is produced within 1 or 2 d, thereby elevating the opportunity for large-scale impacts to populations from fishing, and limiting opportunities to replenish them. In contrast, Erisman et al. (2007) suggested that the propensity for overfishing of gonochoristic leopard grouper *Mycteroperca rosacea* increased from the formation of more frequent and predictable spawning aggregations. Until the advent and spread of the LRFFT, widespread Indo-Pacific species such as *E. polyphkadion* likely had a buffer against regional population depletion merely by being widely distributed, an advantage apparently now gone. A more detailed review of these and other factors that lead to increased vulnerability among individual aggregating groupers would be beneficial to management.

Management

The observed low rates of natural mortality ($M = 0.144$) for *Epinephelus polyphkadion* suggest that a limited annual harvest (~10% of the biomass) is appropriate (Walters & Pearse 1996, Coleman et al. 2000). Although there are many management options available to achieve this, in developing Pacific island communities, options are limited. For example, quotas and bag limits are generally unachievable or impractical due to resource constraints that limit fine-scale monitoring and record-keeping. Nonetheless, a number of measures to limit overfishing have been implemented that are focused on reducing harvest during reproduction, in some cases as a response to observed aggrega-

tion loss. For example, since the 1970s both traditional (*bul* = taboo) and modern fisheries-management measures have been used in Palau to protect 9 spawning sites and limit catch during the reproductive season (Johannes et al. 1999). These measures include both spawning-site protection and commercial restrictions, including sales and catch bans during much of the reproductive season. Prior to these measures being implemented, aggregation loss had occurred at 5 sites (including during the 1990s) from local and foreign overharvesting. Variability in the initiation of spawning season among target species has also diminished the effectiveness of commercial restrictions in both Palau and Pohnpei, underscoring the importance of understanding species' reproductive life histories when designing management strategies.

While precautionary management is urged to protect all spawning aggregations, adaptive management is also needed as additional life-history information becomes available. For example, in 1995, Pohnpei established the small-scale (1.46 km²) Kehpara Marine Sanctuary specifically to protect reproductive groupers. However, its initial boundaries excluded aggregations of *Epinephelus polyphkadion* and *E. fuscoguttatus* that, at least for the latter species, remain among the largest regionally. These boundaries were eventually expanded to include these aggregations in 2010, by which time the *E. polyphkadion* aggregation was severely impacted. The noted abundance declines could have been prevented with a quick-response adaptive management policy and the implementation of recommendations made a decade earlier. When precautionary and adaptive management are combined, management options such as those in Pohnpei and Palau have a greater chance of success.

Future research and the need for non-destructive aggregation sampling

Recently, the camouflage grouper has been designated by the IUCN Red List as Near Threatened (Russell et al. 2006), based on regional declines in populations and aggregation loss. Many of the observed impacts are a direct result of aggregation fishing. The present data set relied on aggregation samples derived independently of the fishery, with a total of only 254 individuals taken over 2 mo in 1998 and 114 taken in the first aggregation month in 1999, during the same period when intense commercial aggregation fishing removed an estimated 4000 of ca. 10 000 aggregating individuals. During the final month of the 1999 spawning season, we removed 321 fish that allowed us to examine *in situ* details of reproductive biology and demography that could only be taken during aggrega-

tion periods and that normally require destructive sampling (e.g. Rhodes & Sadovy 2002a,b). These published findings, combined with the observed impacts from commercial fishing and 10 yr of lobbying by the senior author (K.L.R.), directly resulted in improved management for the species, including extending the Kehpara Marine Sanctuary to protect camouflage and brown marbled (*Epinephelus fuscoguttatus*) grouper spawning aggregations, along with greater enforcement. While we submit that these relatively minor extractions would have contributed slightly to declines in aggregation abundance and reproductive output during those months, we ascribe the large-scale demographic changes within the reproductive population to commercial fishing endeavors. Nonetheless, given the current knowledge of this and other species vulnerability to overfishing, we suggest that future sampling be done with caution and in a manner that minimizes destructive impacts. Given the number and range of aggregating species at risk, a global scientific sampling protocol is now needed, particularly for sampling within aggregations. In the interim, aggregation sampling should attempt to rely on existing fisheries and include fisheries-independent protocols.

CONCLUSIONS

Given the recent Near Threatened designation for the species (Russell et al. 2006) and the continued regional declines in population abundance and aggregation loss, *Epinephelus polyphekadion* is in immediate need of improved management. Among management options, marine protected areas (MPA) focused on reproductive individuals remain one of the most useful options. While no data are currently available to provide estimates of effective MPA size for protecting this and other species during reproduction, the 1.46 km² Kehpara Marine Sanctuary is clearly insufficient (e.g. Rhodes & Tupper 2008, Hutchinson & Rhodes 2010). Expansion beyond its current size is politically and economically impractical. A more effective approach would be to expand the existing March–April sales ban to include February (when *E. polyphekadion* occasionally spawn) and combine with it a catch ban to fully protect the species during the entire reproductive season. Specifically, between 2001 and 2010, camouflage grouper aggregated in February in 6 of 10 spawning seasons, providing the potential for aggregation fishing. In addition, implementing a 350 mm TL size limit for catch and sale is an appropriate management option. Such a limit would reduce the impacts of overharvesting juveniles (Rhodes & Tupper 2007) and help promote population growth and/or recovery. Centralized markets, such as those in Pohn-

pei and Palau, offer suitable venues for monitoring and enforcement where resources are limited. Finally, a regional ban on the LRFFT is recommended, given the known impacts to populations throughout the range of this and other target species.

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