



Age-based demography and reproductive biology of three *Epinephelus* groupers, *E. polyphekadion*, *E. tauvina*, and *E. howlandi* (Serranidae), inhabiting coral reefs in Okinawa

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Abstract The demography and reproductive biology of three *Epinephelus* groupers (Serranidae), namely *E. polyphekadion*, *E. tauvina*, and *E. howlandi* in the Yaeyama Islands, Okinawa, were examined based on age assessment using otoliths and gonadal histology. The maximum ages for these three species were 26 year, 23 year, and 17 year. The von Bertalanffy growth functions were also determined for each species. The size and age at 50% female maturity were estimated to be 358 mm in total length (TL) and 6.0 year for *E. polyphekadion*, 371 mm TL and 6.7 year for *E. tauvina*, and 327 mm TL and 4.1 year for *E. howlandi*, respectively. Significant differences between the sexes in size and age frequencies were found in all three species, with males being larger and older than females, or transitional individuals. These results strongly indicated that the population of these three grouper species showed monandric protogynous hermaphroditism. The sex ratios of *E. polyphekadion* and *E. tauvina* were biased in favor of females, but that

of *E. howlandi* was equivalent between sexes. The relative sizes of ripe testes indicated that the intensity of sperm competition varied among species suggesting different mating system of each species. Reproductive seasonality was similar among species, with active vitellogenesis coinciding with the annual rise in water temperature. The active spawning period was determined to be between April and May for *E. polyphekadion*, in May for *E. howlandi*, and from March to June for *E. tauvina*.

Keywords Age and growth · Longevity · Sexual pattern · Spawning · Protogynous hermaphrodite

Introduction

Groupers (family Serranidae) are one of the most heavily exploited groups of fishes on coral reefs worldwide, because of their commercial importance (Heemstra and Randall 1993; Sadovy de Mitcheson et al. 2013). Consequently, there are growing concerns about the potential overexploitation of groupers (Sadovy de Mitcheson et al. 2013).

There are three aspects of the biological characteristics of groupers that increase their vulnerability to overfishing. Firstly, groupers are generally known to be long-lived, slow-growing, have relatively low rates of natural mortality, and take many years to mature sexually, indicating low resilience to fishing pressure (Manooch 1987; Sadovy de Mitcheson et al. 2013). Secondly, many groupers are known to exhibit protogynous hermaphroditism (Sadovy de Mitcheson

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and Liu 2008). Size-selective fisheries often target larger males and thus impact the stock dynamics of protogynous species (Huntsman and Schaaf 1994; Coleman et al. 2000; Armsworth 2001; Alonso and Mangel 2004). Thirdly, many groupers have a habit of forming spawning aggregations at specific times and places that are often associated with the lunar cycle (Johannes, 1978; Domeier and Colin 1997; Hamilton et al. 2005; Sadovy de Mitcheson and Colin 2012). The spawning aggregations are highly predictable and thus are often targeted by artisanal and commercial fisheries. As a result, species which form spawning aggregations are especially vulnerable to fishing pressure because of both increased catchability (lethal effects) as well as decreased reproductive output at lowered animal densities (nonlethal effects) (Sadovy de Mitcheson 2016).

Groupers have generally been thought to be long-lived fishes (e.g., Manooch 1987; Pears et al. 2006), but a previous study has argued that the longevity of groupers varies greatly from <10 to >40 year (Williams et al. 2009). Furthermore, groupers exhibit great diversity in their sexual patterns. Most species appear to be monandric protogynous hermaphrodites (Sadovy de Mitcheson and Liu 2008), though some species were also confirmed to be diandrous (Siau 1994; Fennessy and Sadovy 2002; Adams 2003; Marques and Ferreira 2011). However, some species were demonstrated to be gonochoristic (Sadovy and Colin 1995; Erisman et al. 2008; Rhodes et al. 2011). Thus, groupers display differential reproductive modes even among closely related species, and it is no longer acceptable to make generalizations about these important biological components (Sadovy de Mitcheson et al. 2013). Additionally, the Red List assessment for all 163 grouper species shows that 25% of the species are considered to be at risk of extinction or Near Threatened, but 30% of all species are considered to be Data Deficient (Sadovy de Mitcheson et al. 2013). These facts highlight a general lack of basic life history information on many grouper species and indicate that species-specific studies are needed (Sadovy de Mitcheson et al. 2013).

Three *Epinephelus* groupers, the camouflage grouper *E. polyphekadion*, the greasy grouper *E. tauvina*, and the blacksaddle grouper *E. howlandi* are distributed in the Indo-Pacific region and inhabit coral reefs (Heemstra and Randall 1993; Russell et al. 2006; Kulbicki, 2008; Rhodes et al. 2008). The former two species attains 75 cm in total length (TL) and the latter attains 44 cm TL (Heemstra and Randall 1993). The

biology of *E. polyphekadion* is relatively well studied, especially in terms of demography (Grandcourt 2005; Mapleston et al. 2009; Rhodes et al. 2011), reproduction (Bruslé-Sicard et al. 1992; Rhodes and Sadovy 2002b; Teruya et al. 2008), and spawning aggregation (Johannes et al. 1999; Rhodes and Sadovy 2002a; Robinson et al. 2008; Rhodes et al. 2014). However, their biological characteristics show great variations among localities. Their longevity varies from 22 year in the Pohnpei, Micronesia (Rhodes et al. 2011) to 44 year in the Great Barrier Reef (Mapleston et al. 2009). Moreover, the sexual pattern of *E. polyphekadion* also varies and has been reported as protogynous hermaphroditism in Tuamoto, French Polynesia (Bruslé-Sicard et al. 1992) and in Palau (Johannes et al. 1999), but as functional gonochorism with the potential for protogynous sexual transition in Pohnpei (Rhodes et al. 2011). On the other hand, little biological information is available for *E. tauvina* and *E. howlandi* (Kulbicki, 2008; Rhodes et al. 2008). Some studies of the reproduction (Abu-Hakima 1987; El-Sayed 1999) and demography (Edwards et al. 1985) of *E. tauvina* are questionable as a result of the misidentification of other species (Rhodes et al. 2008).

E. polyphekadion is one of the most commonly consumed live reef food fish in Hong Kong, and it is locally consumed throughout its distribution range (Russell et al. 2006). *E. tauvina* and *E. howlandi* are also important to artisanal and recreational fisheries (Kulbicki, 2008; Rhodes and Tupper 2007; Rhodes et al. 2008). These species have also been targets by local fisheries in the Yaeyama Islands, Okinawa, Japan, including the spawning aggregations of *E. polyphekadion* (Ohta 2008; Ohta unpublished data). The catches of these three species were the second, third, and fourth largest after that of the white-streaked grouper *E. ongus* among the 24 species of *Epinephelus* groupers caught in the Yaeyama Islands (Ohta 2007, 2008). The catch data of the three species showed a declining trend, indicating stock depletion (Ohta et al. 2007; Akita et al. 2015); therefore, the elucidation of their detailed biological characteristics in relation to rational fishery management action is urgently needed.

The broad objective of this study was to examine the age-based demography and reproductive biology of three species of *Epinephelus* groupers (*E. polyphekadion*, *E. tauvina*, and *E. howlandi*) by a combination of sectioned otolith analysis and histological assessment of the gonads. The specific aims were to determine: (1) growth

and longevity, (2) sexual pattern, (3) size and age at maturity and (4) spawning season for a better understanding of their biology and basic information for the purpose of fisheries management.

Materials and methods

Sampling and study localities

A total of 450 specimens of the three grouper species were collected between 2007 and 2016 (Table 1). All samples originated in the Yaeyama Islands ($24^{\circ}20'N$, $124^{\circ}03'E$), Okinawa, located in the most southwestern part of the Ryukyu archipelago, Japan (Fig. 1). Most of the samples were bought haphazardly during the course of the research twice a week in the two markets (Table 1), the local fish market of the Yaeyama Fishery Cooperative on Ishigaki Island, which is the only wholesale market in the Yaeyama Islands, or the central market at Tomari on Okinawa Island, which is 400 km northeast of the Yaeyama Islands. For all specimens, TL and body weight (BW) were measured to the nearest 1 mm and 0.001 kg, respectively.

Age and growth

Sagittal otoliths were removed from 156 *E. polyphekadion*, 137 *E. tauvina*, and 143 *E. howlandi*, and then cleaned and stored dry. Each pair of intact otoliths was weighed to the nearest 0.001 g. Because the otoliths were often broken by spears, the sagittal weight was given as the average of a pair of otoliths or as the values from a single intact otolith.

One of each pair of otoliths was embedded in epoxy resin and sectioned transversely through the core at a thickness of about 0.5 mm using either an EXAKT micro-cutting machine with a diamond saw or a diamond cutter. Each section was mounted on a glass slide with a medium (Eukitt: O. Kinder) and glass coverslip. Sectioned otoliths were examined under a microscope

using both reflected and transmitted light. Alternating opaque and translucent zones were visible, so it was assumed that one pair of opaque and translucent zones was laid down each year as one annulus. The number of opaque zones was counted along a consistent axis near the sulcus, where the zones were most visible, on at least two occasions with an interval of over two weeks between counts and without knowledge of the length of the fish. If the two counts coincided, that reading was accepted, but if they differed, the otolith was read once more, and any two coinciding counts were accepted. The precision of these counts was quantified by the coefficient of variance (CV; Campana 2001). Thus, the presumed age of each species was determined.

Edge type analysis was conducted to test the assumption that the increments were annuli. The edge of each otolith section was judged to be opaque or translucent. The monthly frequency of opaque edges was compared to a uniform distribution using a χ^2 goodness-of-fit test for circular distributions (Zar 1984). If it was difficult to determine the edge type, that otolith was excluded from the analysis. On the assumption that opaque zones were annual rings, the age for each individual was considered to be the number of opaque zones counted. A von Bertalanffy growth function (VBGF) was fitted to the TL at the presumed age by the non-linear least square method to estimate the growth characteristics of this species. The VBGF is represented by

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

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 $TL = 0$.

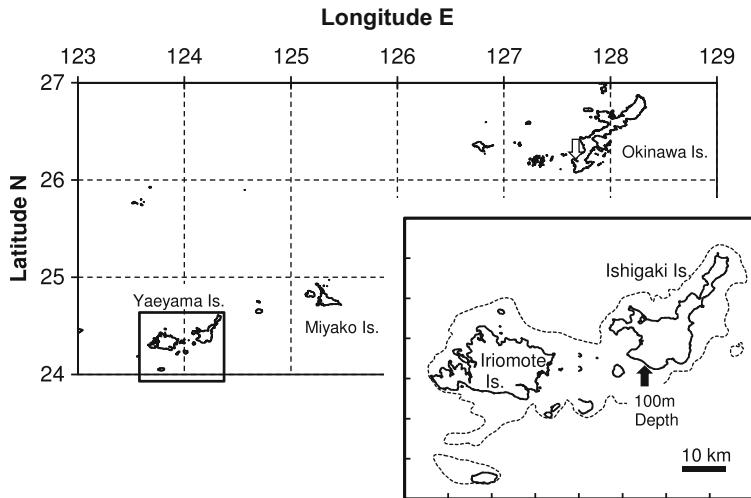
Sexual pattern and reproductive biology

Gonads from 143 *E. polyphekadion*, 139 *E. tauvina*, and 150 *E. howlandi* were weighed to the nearest 0.01 g (gonad weight: GW) and were fixed in Bouin's fixative.

Table 1 Number of monthly samples examined in this study

Species	Month												Total	Year
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
<i>E. polyphekadion</i>	9	14	17	14	14	19	12	6	8	23	16	10	162	2007-2011, 2013, 2015
<i>E. tauvina</i>	14	9	14	12	19	10	16	21	4	0	5	8	132	2007-2011, 2013-2016
<i>E. howlandi</i>	7	17	20	19	42	11	6	5	11	5	7	6	156	2007-2011, 2013-2015

Fig. 1 Map of Okinawa and the Yaeyama Islands, indicating the locations of the two fish markets where the specimens were collected, at the Tomari market on Okinawa Island (open arrow) and at a local fish market on Ishigaki Island (closed arrow)



The gonadosomatic index (GSI) was calculated as: $GSI = 100 \times GW / BW$. Within 24 h after fixation, the samples were transferred to 90% ethanol for long-term preservation and histological sectioning. The tissues for sectioning were embedded in paraffin, sectioned at 6 μm , and stained with Mayer's hematoxylin solution and 0.5% eosin aqueous solution. Based on the observations of the sectioned gonads, the sex was determined histologically.

The development of the gonads were classified into 6 stages: I) ‘immature’, II) ‘developing’, III) ‘spawning capable’, IV) ‘actively spawning’, V) ‘regressing’, and VI) ‘regenerating’, based on histological features outlined in Brown-Peterson et al. (2011). Females ranging from ‘developing’ (stage II) to ‘regenerating’ (stage VI) were defined as being mature. Careful examination of histological sections was conducted to determine if male germ cells were present in ovaries or ovarian structures present in testes (e.g. residual oocytes, a central lumen or gonadal lamella). The term ‘transitional’ referred to individuals which possessed a bisexual gonad containing developing testicular tissue, scattered previtellogenic oocytes and often muscle bundles as a sign of prior spawning (Shapiro et al. 1993; Sadovy de Mitcheson and Liu 2008).

Differences in the size and age structure between females and males in the samples were determined by the Kolmogorov-Smirnov test. Size and age at 50% female maturity was assessed by assuming binomial distribution (mature or immature) and fitting a logistic curve to these data: $y = 1/[1 + \exp. (-a - bx)]$. Parameters a and b were estimated by maximum likelihood method for each relationship. Confidence intervals at the 95%

level (CI) were estimated using parametric bootstrapping with 500 bootstrap resamples: $CI = [\theta_n \pm 1.96 \sqrt{V_{boot}}]$, where θ_n is the estimated value at 50% maturity based on the samples, $\sqrt{V_{boot}}$ is standard deviation of estimates based on 500 bootstrap resamples.

Sex ratios were calculated as mature females/males based on the samples. The sex ratios were considered to be closely related to the sex ratios of the adult population in the study area, because the samples were collected haphazardly in the fish markets throughout the year and mature individuals were fully recruited in the local fishery based on the size frequency in the markets (Akita et al. 2011). Statistical significances for the sex ratios were examined by chi-square test.

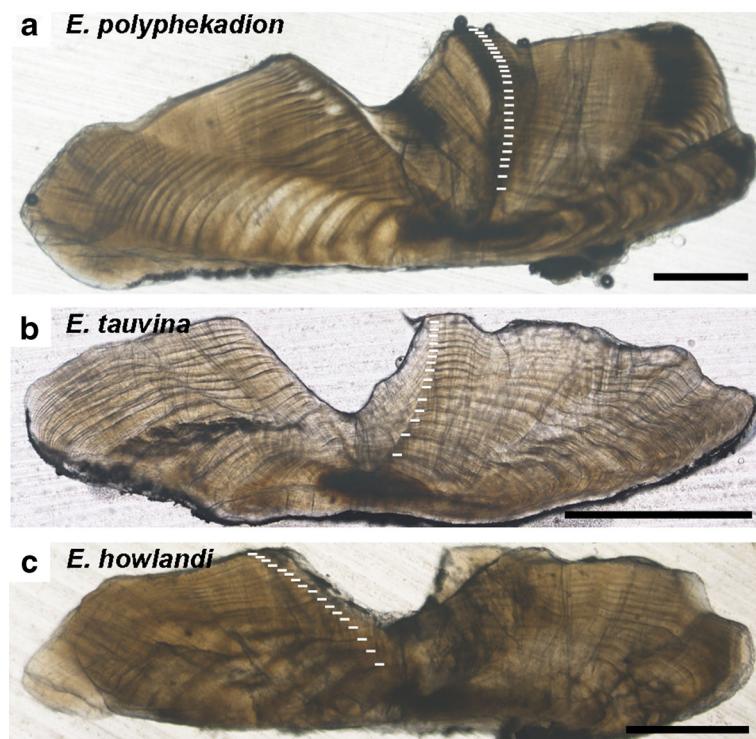
The relative testis size has been used as an index of sperm competition related to a mating system of fishes (Sadovy et al. 1993; Stockley et al. 1997; Molloy et al. 2007; Erisman et al. 2009). To infer a mating system of each species based on the relative testis size, the mean values of GSI of actively spawning male at the functional maturation stage, in which the seminal lobule and sperm sinus were filled with spermatozoa (Grier 1981), were calculated.

Results

Age and growth

Of the sectioned otoliths observed, 97% to 99% had visible increments and these were used for age determinations of the three species (Fig. 2, Table 2). Of these

Fig. 2 Transverse sections of sagittal otolith with annuli indicated by white bars. (a) *E. polyphekadion*, 26 yr. old female (618 mm TL). (b) *E. tauvina*, 15 yr. old male (460 mm TL). (c) *E. howlandi*, 17 yr. old male (455 mm TL). Scale bars = 1 mm



otoliths, 68% to 78% showed complete agreement between the first two counts of increments (Table 2). The mean CV was low, ranging from 2.3 to 2.9 (Table 2). Edge type analysis revealed an annual periodicity of increment formation with significant differences among

the monthly frequencies of opaque edges for all three species (Table 2). Increment formation occurred between February and August (Fig. 3). There were significant positive correlations between otolith weight and presumed age for all three species (Table 2). These

Table 2 Number of samples, statistics, and parameters to indicate accuracy and validity of age assessment

Data	Species		
	<i>E. polyphekadion</i>	<i>E. tauvina</i>	<i>E. howlandi</i>
N1: number of sectioned otolith observed	156	137	143
N2: number of age determined	155	136	139
N2/N1 (%)	99.4	99.3	97.2
N3: the first two counts coincided	106	92	109
N3/N2 (%)	68.4	67.6	78.4
Mean CV (coefficient of variance)	2.9	2.8	2.3
Annual cycle of increment formation			
goodness-of-fit test for circular distributions χ^2	65.6	35.7	114.7
	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)
Linear relationship between otolith weight (OW: g) and presumed age (Age: yr):			
Age = $a\text{OW} + b$			
a	41.79	259.58	151.13
b	0.98	-1.90	-2.43
r^2	0.85	0.83	0.89
P	< 0.001	< 0.001	< 0.001

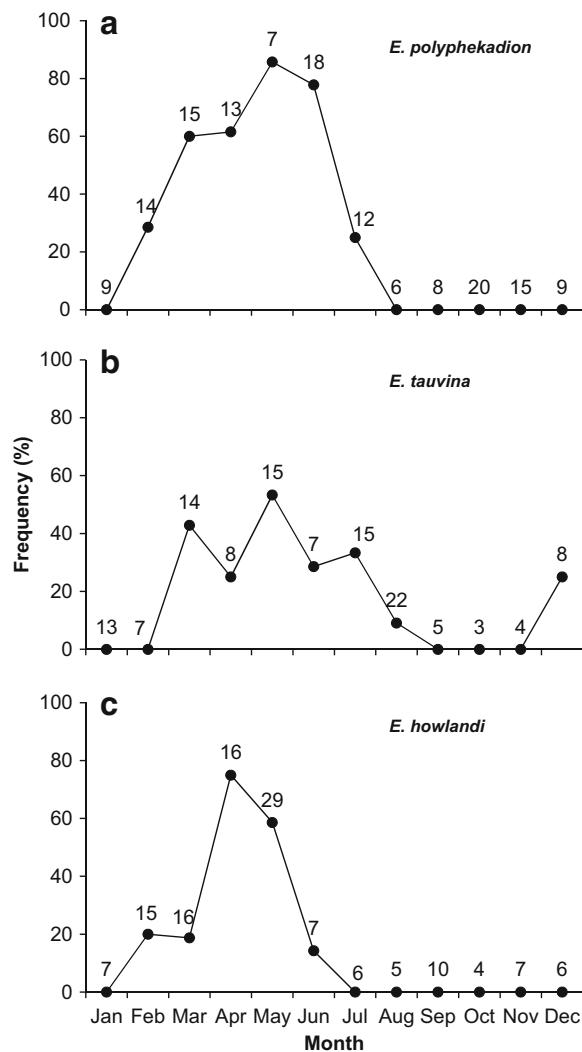


Fig. 3 Edge -type analysis for sectioned otoliths for (a) *E. polyphekadion*, (b) *E. tauvina*, and (c) *E. howlandi*, showing the percentage of otoliths with an opaque margin. Numbers above plots indicate sample sizes

results strongly indicated that the age determination of the three species done in this study was effective.

The growth curves of VBGF for three grouper species are shown in Fig. 4, and their parameters are listed in Table 3. The maximum ages (T_{\max}) for *E. polyphekadion*, *E. tauvina*, and *E. howlandi* were 26., 23., and 17 year., respectively.

Sexual pattern and maturity

For 143 *E. polyphekadion*, 139 *E. tauvina*, and 150 *E. howlandi*, sex was determined histologically. Females had ovaries with a central lumen and gonadal

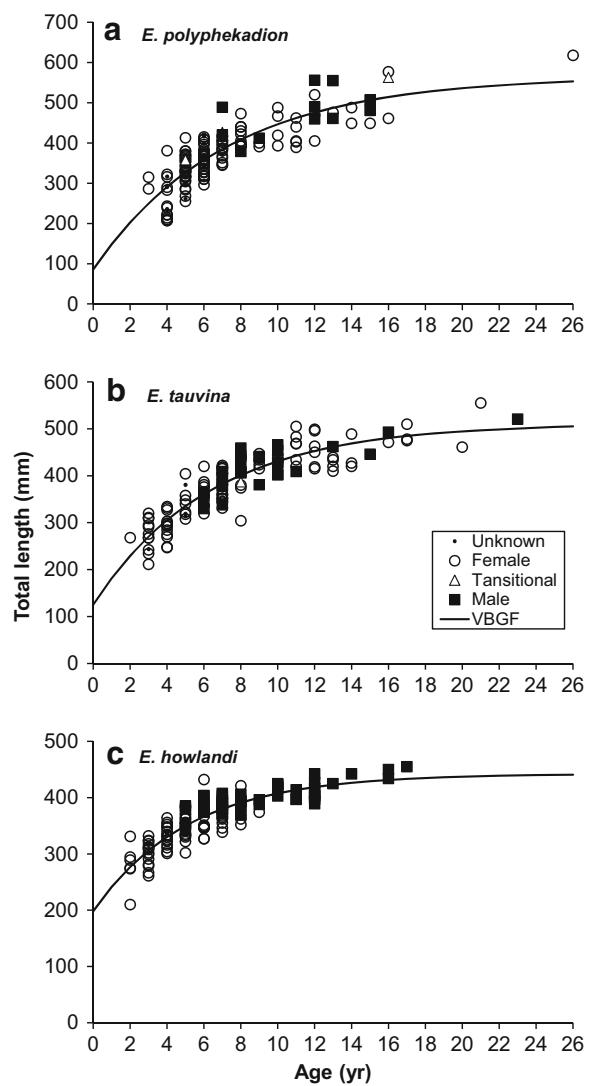


Fig. 4 Growth curves of (a) *E. polyphekadion*, (b) *E. tauvina*, and (c) *E. howlandi*. Von Bertalanffy growth function fitted to size- at age data for all sexual phases combined

lamellae (Figs. 5 and 6). Also, all of the males had testes with central lumen. In ripe testes, spermatozoa were present in the seminal lobules and the sperm sinus, but not in the central lumen, indicating that the lumen is not used for sperm transport (Fig. 7 and Fig. 8).

In all three species, significant differences between females and males were found in both size and age, with males being larger and older than females (Table 4, Fig. 9). For *E. polyphekadion*, females ranged from 207 to 618 mm TL (mean \pm SD: 370 ± 71 mm TL) and from 3 to 26 year (6.9 ± 3.2 year), while 70% were less than 390 mm TL and 7 year (Fig. 9). Males ranged from 379 to 556 mm TL (474 ± 53 mm TL) and from 7

Table 3 Parameters of von Bertalanffy growth function for three *Epinephelus* groupers

Species/Locality	L_{∞} (mm TL)	k (yr $^{-1}$)	t_0 (yr)	T_{max} (yr)	Citation
<i>E. polyphekadion</i>					
Yaeyama Is., Japan	565.8	0.139	-1.18	26	present study
Pohnpei, Micronesia	447.1	0.251	-0.14	22	Rhodes et al. (2011)
Seychelles	579.0	0.180		31	Grandcourt (2005)
GBR, Australia	562.3	0.194	-0.08	44	Mapleston et al. (2009)
<i>E. tauvina</i>					
Yaeyama Is., Japan	511.6	0.157	-1.79	23	present study
<i>E. howlandi</i>					
Yaeyama Is., Japan	442.2	0.195	-3.05	17	present study

to 15 year (11.2 ± 3.0 year) (Fig. 9). For *E. tauvina*, females ranged from 211 to 555 mm TL (mean \pm SD: 376 ± 72 mm TL) and from 2 to 21 year (7.8 ± 4.1 year), while 70% were less than 410 mm TL and 7 year (Fig. 9). Males ranged from 330 to 521 mm TL (414 ± 43 mm TL) and from 6 to 23 year (9.3 ± 3.5 year) (Fig. 9). For *E. howlandi*, females ranged from 210 to 432 mm TL (mean \pm SD: 343 ± 39 mm TL) and from 2 to 12 year (5.0 ± 2.0 year), while 70% were less than 360 mm TL and 6 year (Fig. 9). Males ranged from 341 to 455 mm

TL (400 ± 22 mm TL) and from 5 to 17 year (9.0 ± 3.0 year) (Fig. 9).

The size and age at 50% female maturity in each species was estimated to be 358 (CI: 346–370) mm TL and 6.0 (5.6–6.4) yr. for *E. polyphekadion*, 371 (361–381) mm TL and 6.7 (6.3–7.2) yr. for *E. tauvina*, and 327 (321–334) mm TL and 4.1 (3.7–4.6) yr. for *E. howlandi*, respectively (Table 4, Fig. 10). Most males of all three species were larger (older) than the smallest (youngest) mature female and 50% mature size (age) of

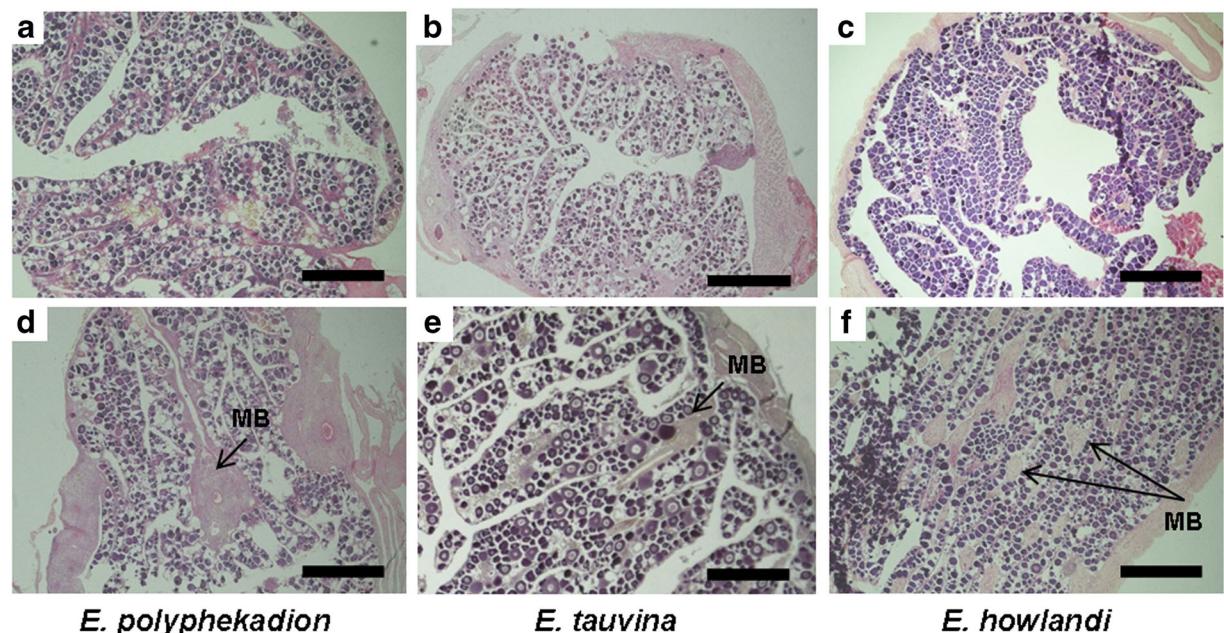


Fig. 5 Transverse sections of ovary at immature (a, b, c) and regenerating (d, e, f) stages: *E. polyphekadion* (left) [(a) 366 mm TL, (d) 415 mm TL], *E. tauvina* (center) [(b) 303 mm TL, (e)

423 mm TL], *E. howlandi* (right) [(c) 322 mm TL, (f) 325 mm TL]. Showing muscle bundle (MB). Scale bars = 0.5 mm

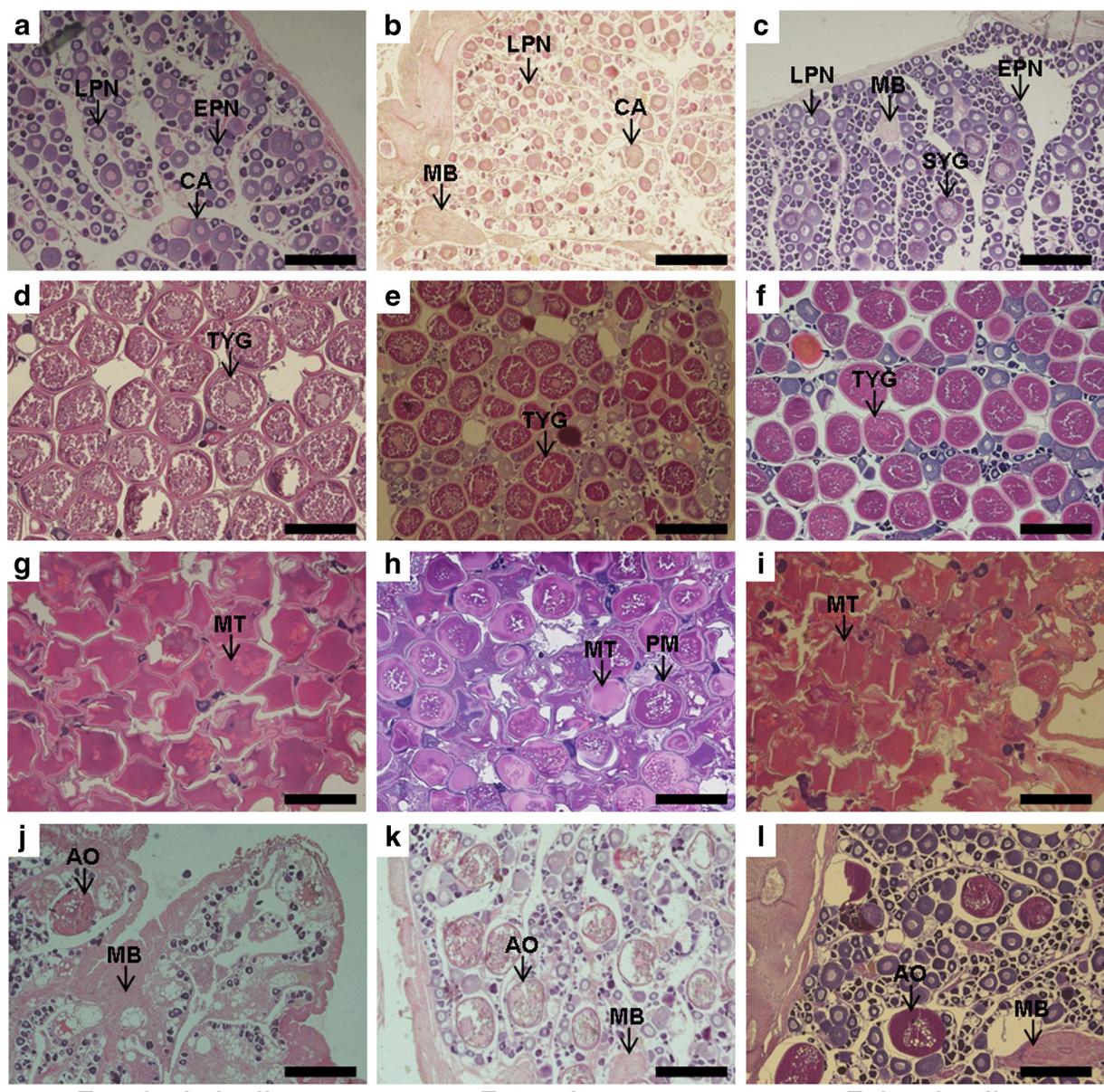
*E. polyphekadiion**E. tauvina**E. howlandi*

Fig. 6 Transverse sections of ovary at developing (a, b, c), spawning capable (d, e, f), actively spawning (g, h, i), and regressing (j, k, l) stages: *E. polyphekadiion* (left) [(a) 449 mm TL, (d) 389 mm TL, (g) 470 mm TL, (j) 462 mm TL], *E. tauvina* (center) [(b) 555 mm TL, (e) 391 mm TL, (h) 365 mm TL, (k) 463 mm TL], *E. howlandi* (right) [(c) 365 mm TL, (f) 384 mm TL,

(i) 340 mm TL, (l) 419 mm TL]. Showing oocytes at some developmental stages; early perinucleolus stage (EPN), late perinucleolus stage (LPN), cortical alveolus stage (CA), secondary yolk globule stage (SYG), tertiary yolk globule stage (TYG), pre-maturation stage (PM), maturation stage (MT), atretic vitellogenetic oocytes (AO), and muscle bundle (MB). Scale bars = 0.5 mm

female, whereas the smallest male was equivalent to the smallest mature female for *E. tauvina*.

Transitional individuals, which have gonads with many previtellogenic oocytes, developing testicular cells, and often muscle bundles (Fig. 8), were rare but were found in all species and sporadically through the

year in each species as follows: *E. polyphekadiion* ($n=3$, March, April, October), *E. tauvina* ($n=2$, February, August), *E. howlandi* ($n=2$, October, November). Most transitional individuals of all species were also greater than the smallest and the youngest females (Fig. 9). These results strongly indicated that the population of

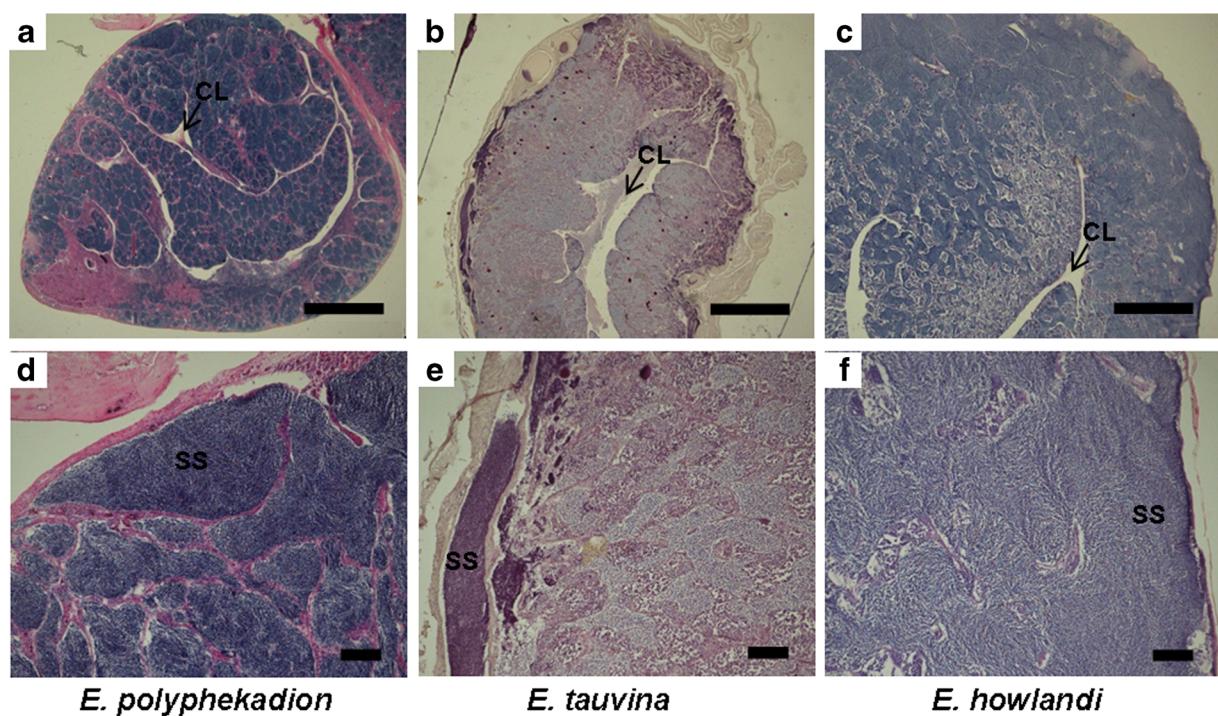


Fig. 7 Transverse sections of testis at functional maturation stage: *E. polyphekadion* (left) [(a, d) 420 mm TL], *E. tauvina* (center) [(b, e) 429 mm TL], *E. howlandi* (right) [(c, f) 442 mm TL]. Showing

central lumen (CL), sperm sinus (SS) filled with spermatozoa. Scale bars = 1 mm (a, b, c) or = 0.1 mm (d, f, g)

three species of groupers in the Yaeyama Islands showed monandric protogynous hermaphroditism.

The sex ratios (proportion of mature females to males) were 5.7 for *E. polyphekadion* and 1.8 for *E. tauvina* and significantly skewed to female (Table 4). On the other hand, the sex ratio of *E. howlandi* was 1.0 and equivalent between the sexes (Table 4).

Reproductive seasonality and relative testis size of male

The reproductive seasonality was evident and was similar among species with active vitellogenesis occurring from March to May. Specifically, ovarian development was similar between *E. polyphekadion* and *E. howlandi*. Of these two species, the occurrence of hydrated oocytes indicating the spawning period concentrated between April and May for *E. polyphekadion*, and in May for *E. howlandi*, with high values of GSI (Fig. 11). On the other hand, the seasonal development of ovaries in *E. tauvina* was somewhat different from that of the other two species. Hydrated oocytes were found in each month from March to June with high values of GSI, indicating that the spawning occurred through the period from March to June (Fig. 11).

The actively spawning males of each species occurred around each spawning period, but longer than the spawning periods based on the gonadal development, occurring from March to May for *E. polyphekadion* (but no male samples in February and from June to July), from March to July for *E. tauvina*, and from February to July for *E. howlandi*. The mean values (\pm SD) of GSI of actively spawning males were quite low with 0.2 ± 0.1 for *E. tauvina* and moderately low with 0.6 ± 0.3 for *E. howlandi* (Fig. 12). However, the value of male GSI for *E. polyphekadion* showed considerable variation among individuals, with the mean value 1.4 ± 1.8 (Fig. 12). The value of one male of the species was attained to 4.3 (Fig. 12).

Discussion

This study demonstrated the age-based demography and sexual patterns of three *Epinephelus* groupers, *E. polyphekadion*, *E. tauvina*, and *E. howlandi*, in the Yaeyama Islands. This is the first known research on the latter two species. Age assessment in this study confirmed that opaque zones on sectioned otoliths were

Table 4 Number of samples and statistics to summarize reproduction and demography for three *Epinephelus* groupers

Data	<i>E. polyphekadion</i>		<i>E. tauvina</i>		<i>E. howlandi</i>	
	Female	Male	Female	Male	Female	Male
Size analysis						
N ₁ (sex determined)	131	12	107	32	90	60
N ₂ (maturity determined)	120	12	107	32	87	60
Minimum (mm in TL)	207	379	211	330	210	341
Maximum (mm in TL)	618	556	555	521	432	455
Mean ± SD (mm in TL)	370 ± 71	474 ± 53	376 ± 72	414 ± 43	343 ± 39	400 ± 22
Kolmogorov-Smirnov test D	0.71 (<i>P</i> < 0.001)		0.35 (<i>P</i> < 0.01)		0.76 (<i>P</i> < 0.001)	
First maturity (mm in TL)	307	379	331	330	311	341
50% maturity (mm in TL)	358		371		327	
Age analysis						
N ₁ (sex determined)	125	11	100	30	81	53
N ₂ (maturity determined)	115	11	99	30	78	53
Minimum (yr)	3	7	2	6	2	5
Maximum (yr)	26	15	21	23	12	17
Mean ± SD (yr)	6.9 ± 3.2	11.2 ± 3.0	7.8 ± 4.1	9.3 ± 3.5	5.0 ± 2.0	9.0 ± 3.0
Kolmogorov-Smirnov test D	0.61 (<i>P</i> < 0.01)		0.33 (<i>P</i> < 0.05)		0.58 (<i>P</i> < 0.001)	
First maturity (yr)	5	7	5	6	3	5
50% maturity (yr)	6.0		6.7		4.1	
Sex ratio						
Number of mature individuals	68	12	56	32	58	60
Female: Male	5.7	1	1.8	1	1.0	1
Chi-square test χ^2	39.2 (<i>P</i> < 0.001)		6.55 (<i>P</i> < 0.05)		0.03 (<i>P</i> = 0.85)	

annuli in all three species, thus reflected effective growth functions. The maximum ages for these species showed moderate longevity in comparison to other *Epinephelus* groupers (Matheson and Huntsman 1984; Manooch 1987; Sadovy et al. 1992; Grandcourt, 2005; Fennessy 2006; Pears et al. 2006; Williams et al. 2009; Rhodes et al. 2011).

For *E. polyphekadion*, the growth functions and longevity were similar among the Yaeyama Islands, the Seychelles, and the GBR, but the longevity varied among these locations as follows: 26 year in the Yaeyama Islands, 31 year in the Seychelles, and 44 year in the GBR (Table 3) (Grandcourt, 2005; Mapleston et al. 2009). This fact indicated that the growth characteristics are identical among these localities with a potential longevity of over 40 year in the species. On the other hand, the growth curve of the Pohnpei population was considerably different from those of the other three locations, with a smaller L_{∞} and maximum age (22 year) (Table 3) (Rhodes et al. 2011). The regional difference

of growth parameters and lifespan within a species are often found in reef fishes. A previous study reported that a negative relationship between lifespan and mean annual sea temperature in some acanthurids (Choat and Robertson 2002) and blackspot snapper *Lutjanus fulviflammus* (Shimose and Nanami 2015), but results of the present study is not the case.

The present study, based on an age-based analysis of sex distribution with detailed histological observation, provided strong evidence of sexual patterns (Sadovy and Shapiro 1987). All three species, *E. polyphekadion*, *E. tauvina*, and *E. howlandi*, in the Yaeyama Islands exhibited several convincing characteristics indicative of protogynous hermaphroditism (Sadovy and Shapiro 1987; Sadovy de Mitcheson and Liu 2008). Furthermore, the fact that the size/age of most males was beyond the first maturity of females suggested monandric protogynous hermaphroditism (Fennessy 2006; Pears et al. 2006; Williams et al. 2009; Ohta and Ebisawa 2016).

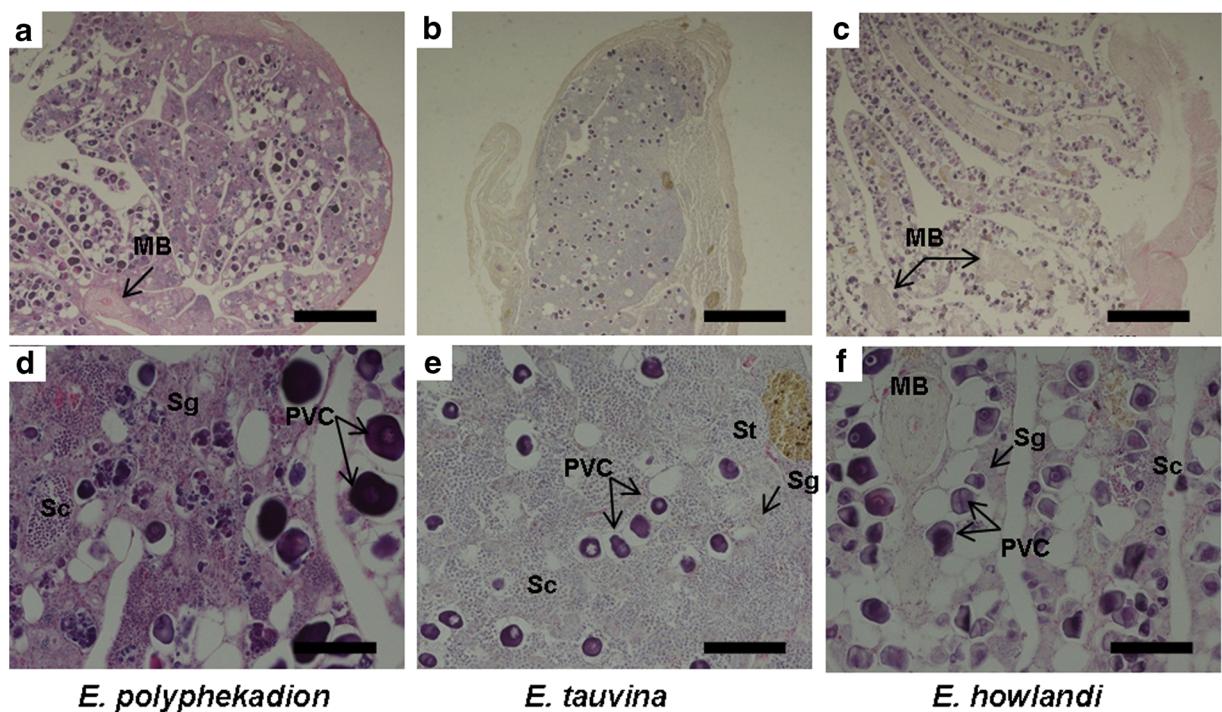


Fig. 8 Transverse sections of transitional gonads: *E. polyphekadion* (left) [(a, d) 426 mm TL], *E. tauvina* (center) [(b, e) 387 mm TL], *E. howlandi* (right) [(c, f) 390 mm TL]. Showing previtellogenic

oocytes (PVO), muscle bundle (MB), spermatogonium (Sg), spermatocyte (Sc), spermatid (St). Scale bars = 0.5 mm (a, b, c) or = 0.1 mm (d, f, g)

For *E. polyphekadion*, the sexual pattern found in the population of the Yaeyama Islands showed protogyny as in the population of Tuamoto, French Polynesia (Bruslé-Sicard et al. 1992) and that of Palau (Johannes et al. 1999), but differed from that of the population in Pohnpei, Micronesia (Rhodes et al. 2011). A previous study in Pohnpei concluded that the Pohnpei population of *E. polyphekadion* is functionally gonochoristic, with the potential for protogynous sexual transition; however, the factors contributing to the regional variation observed in the sexual pattern are unknown (Rhodes et al. 2011). Regional variations of the sexual pattern such as functional hermaphroditism or functional gonochorism have also been reported in other hermaphrodite reef fishes, such as some emperor fishes (Ebisawa 2006), the green humphead parrotfish, *Bolbometopon muricatum* (Hamilton et al. 2008), and the monogrammed monocle bream, *Scolopsis monogramma* (Akita and Tachihara 2014). Some studies have suggested that the regional differences in sexual patterns in protogyny are related to population density (Warner and Hoffman 1980; Ebisawa 2006) and to be historical fishing effort (Hamilton et al. 2008).

Selection of protogyny occurs in mating systems in which large males monopolize and pair spawn with

females (Warner 1984). Therefore, the general trend in protogynous hermaphrodites is characteristically biased in favor of females (Shapiro 1987; Sadovy and Shapiro 1987; Molloy et al. 2007), as observed in *E. polyphekadion* and *E. tauvina* in the present study. The relative testes sizes of mature males of *E. tauvina* were quite low with <0.5% of their body weight, suggesting a mating system with little or no sperm competition, such as pair spawning in single male/multi-female cluster (Sadovy et al. 1993; Stockley et al. 1997; Molloy et al. 2007; Erisman et al. 2009). In contrast, some males of *E. polyphekadion* had relatively large testes, as observed in the Pohnpei population that is functionally gonochoristic (Rhodes and Sadovy 2002b; Rhodes et al. 2011), indicating a high communal spawning in multi-male cluster with sperm competition (Sadovy et al. 1993; Stockley et al. 1997; Erisman et al. 2009). This is comparable to some gonochoric groupers, *Epinephelus striatus* and *Myctropica rosacea* that have relatively large testes and spawn in large group (Sadovy and Ekulund, 1999; Erisman et al. 2007; Erisman et al. 2009). A previous study observed the spawning behavior of *E. polyphekadion* in aquaria indicated that the basic mating system was pair spawning by a dominant male,

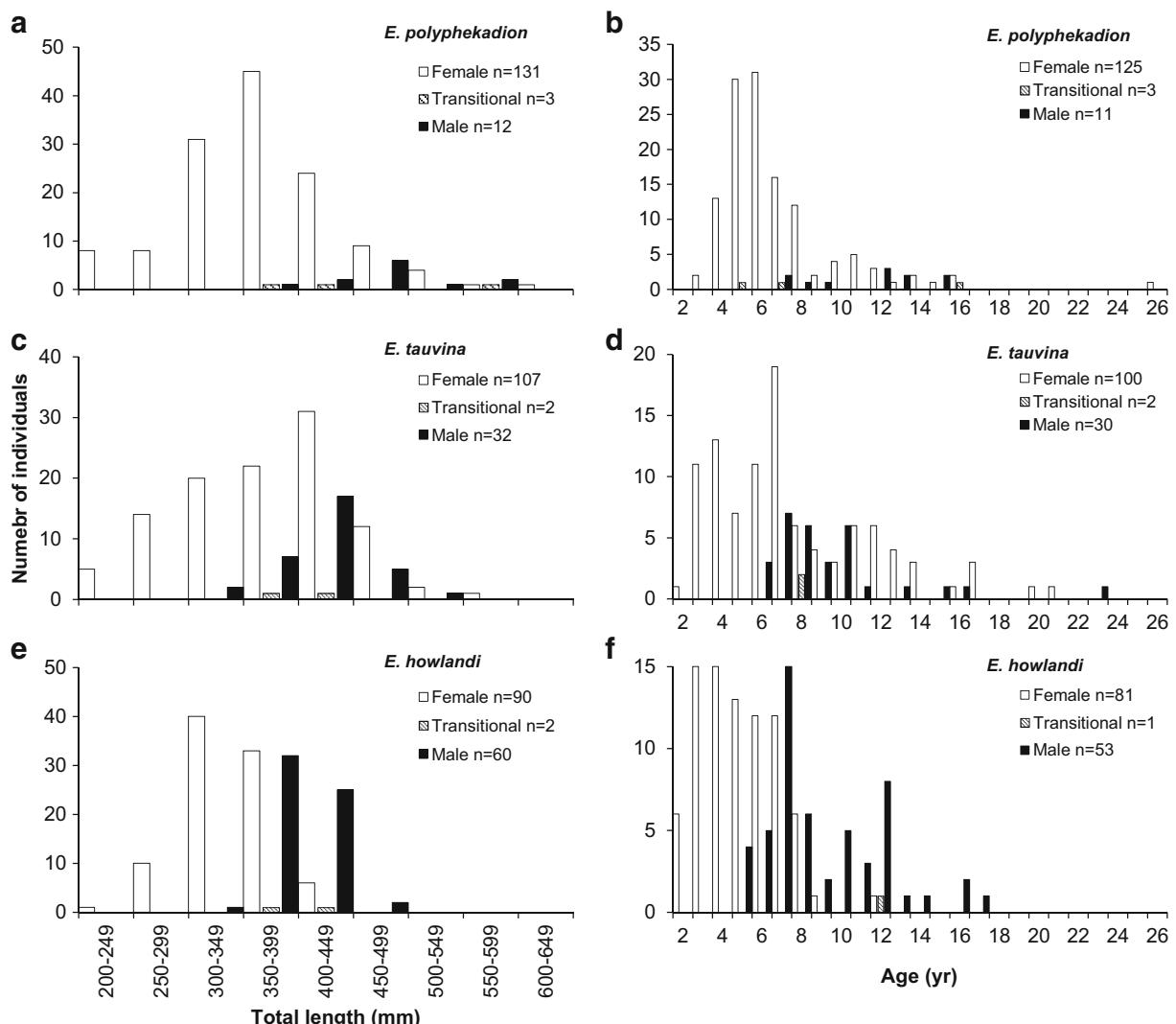


Fig. 9 Sex-specific length frequency and age frequency distributions of (a, b) *E. polyphekadion*, (c, d) *E. tauvina*, and (e, f) *E. howlandi* for the samples examined

but with sneaker by non-dominant males (Teruya et al. 2008). The sexual patterns of *E. polyphekadion* vary among locations mentioned above and may be closely related to the demography and mating system.

On the other hand, *E. howlandi* showed that the sex ratio was equivalent between female and male, relative testes sizes of mature males were moderately low with <1% of their body weight. These facts are similar to the case of the white-streaked grouper *E. ongus* in the Yaeyama Islands. The sex ratio of mature individuals of *E. ongus* was equivalent between sexes (Ohta and Ebisawa 2016). Their spawning behaviors (pair spawning with sneaker) were directly observed in their spawning aggregations (Nanami et al. 2013), and

consistent with the implication based on GSI of ripe males that were moderately low (1%) (Ohta and Ebisawa 2015). Consequently, *E. howlandi* may have a mating system with low or moderate sperm competition, such as pair spawning with sneaker, perhaps in spawning aggregation (Erisman et al. 2009). Furthermore, the use of a fishery in which larger individuals are selected may result in a male-biased sex ratio in local landings, especially for these smaller (<450 mm TL) species (Ohta and Ebisawa 2016).

The present study determined the spawning season for *E. polyphekadion*, *E. tauvina*, and *E. howlandi*, in the Yaeyama Islands. Active vitellogenesis of the three species from March to May coincides with the rise of the

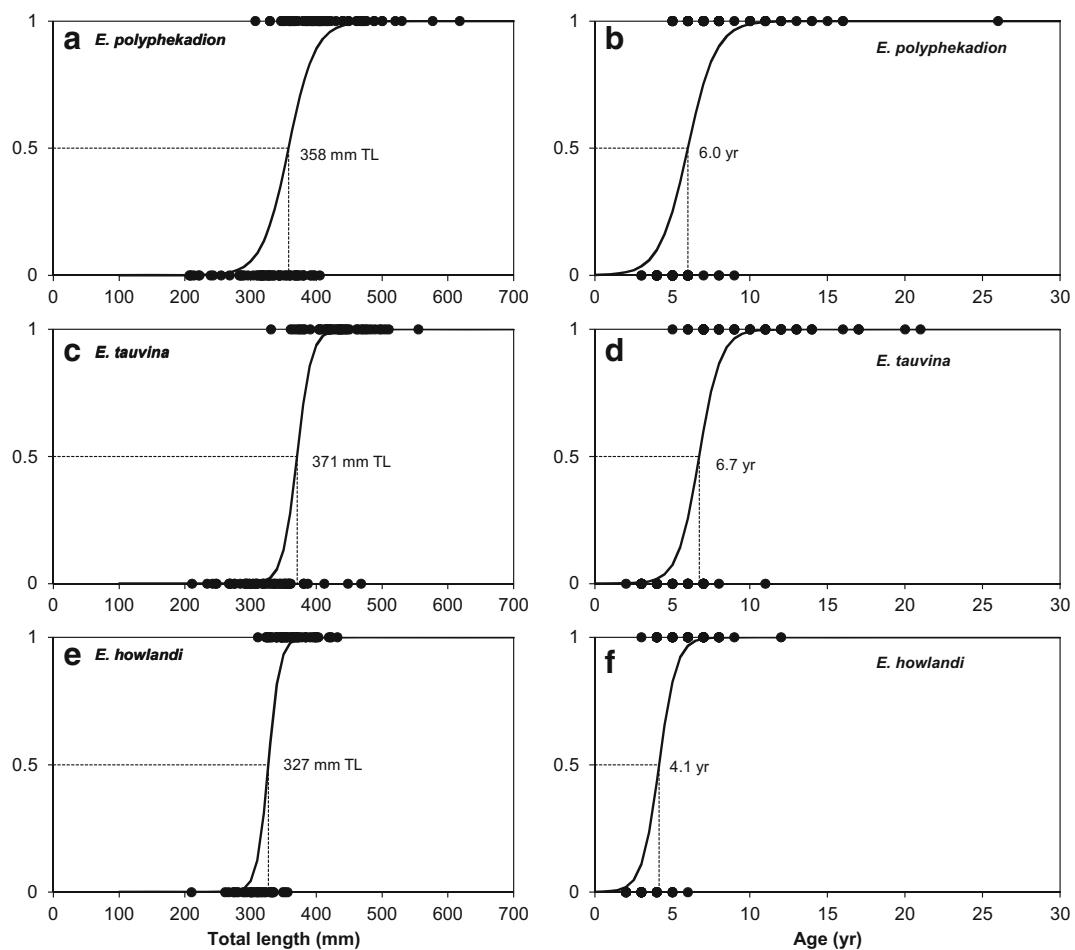


Fig. 10 Probability of mature females of (a, b) *E. polyphekadion*, (c, d) *E. tauvina*, and (e, f) *E. howlandi* by size (a, c, e) and age (b, d, f). Solid line indicates logistic equation fitted to the data. Dashed line indicates 50% maturity estimate by size and age

water temperature and the reproductive season of numerous reef fishes in Okinawa, such as groupers (Lee et al. 2002; Teruya et al. 2008; Ebisawa 2013; Ohta and Ebisawa 2015), emperors (Ebisawa 1999, 2006), and snappers (Nanami et al. 2010a, b; Shimose and Nanami 2014, 2015). Likewise, the spawning season of *E. polyphekadion* among some localities roughly correspond to early summer in Pohnpei between February and April (Rhodes and Sadovy 2002b), in Palau between June and August (Johannes et al. 1999), in the Seychelles between November and February (Robinson et al. 2008), and in the GBR, at least, between October and December (Mapleston et al. 2009).

However, the spawning season of the groupers often vary from year to year by inter-annual difference of the onset and/or frequency of spawning aggregations. *E. polyphekadion* is known to form spawning

aggregations at specific times and places in the Yaeyama Islands (Ohta 2008) and various locations throughout its distribution range, associated with the lunar cycle (Johannes et al. 1999; Rhodes and Sadovy 2002a, b; Russell et al., 2006; Robinson et al. 2008). The spawning aggregations of some grouper species often show inter-annual variations in the timing and frequency, and form once or a few times in the consecutive lunar months, due to a mutual interaction between seasonality (ex. sea water temperature) and lunar cycle (Johannes et al. 1999; Rhodes and Sadovy 2002a; Teruya et al. 2008; Ohta and Ebisawa 2015, 2017). Therefore, it is very important if the species forms spawning aggregation and their reproduction are associated with the lunar cycle for the understanding the general spawning season. However, there is no information that *E. tauvina* and *E. howlandi* form spawning aggregation, except for

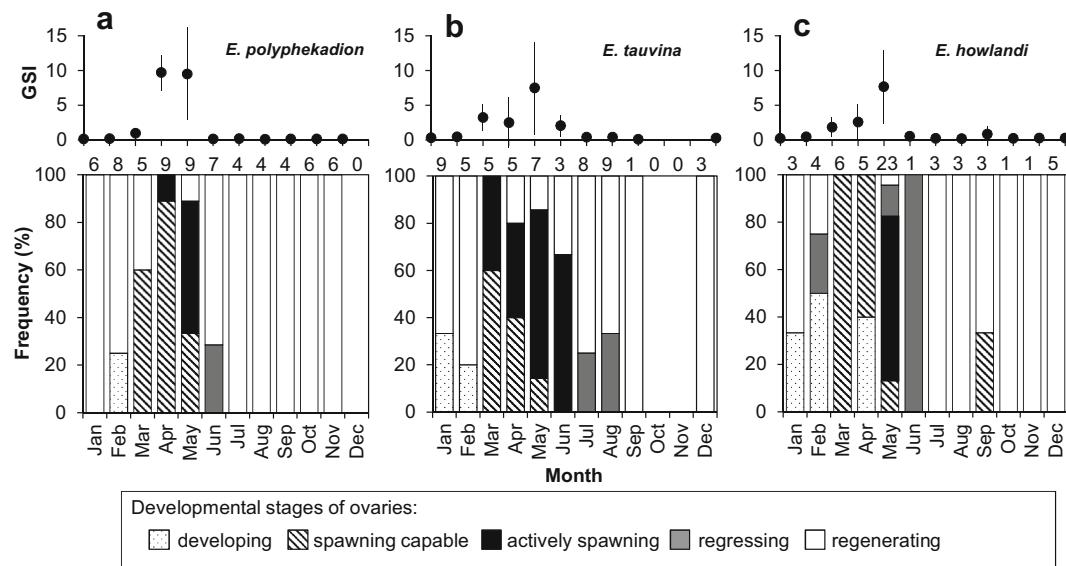


Fig. 11 Monthly changes of mean gonadosomatic index (GSI; \pm SD) and proportional frequency of developmental stages of gonads for (a) *E. polyphekadion*, (b) *E. tauvina*, and (c) *E. howlandi* females. Numbers indicate sample size of mature females

the case of *E. tauvina* in Kenya that indicated a possible spawning aggregation based on the empirical fisheries information and orientation behavior of the species associated with the lunar phases (Kaunda-Arara and Rose 2004). This study could not examine if the reproduction of the three groupers are associated with lunar cycle, because of a lack of samples to examine these issues. Further research is needed to examine the spawning aggregations and lunar cycle of the reproduction, especially for *E. tauvina* and *E. howlandi*.

The present study discussed about the similarity, diversity, and flexibility in demography and reproductive biology of three *Epinephelus* groupers, *E. polyphekadion*, *E.*

tauvina, and *E. howlandi*. The findings in present study can also be applied for fisheries management of each species. The size at maturity is available for the determination of the lower size limit as a basic management option protecting immature fishes and preventing growth and recruitment overfishing. Additionally, the upper size limit to conserve larger females and males may be needed, especially for *E. polyphekadion*, because of a paucity of males in the population of the Yaeyama Islands. Larger females are highly fecund and make important reproductive contributions (Pears et al. 2006). A paucity of males may cause sperm limitation and population collapse even at relatively low fishing mortality in protogynous species (Huntsman and Schaaf 1994; Coleman et al. 2000; Armsworth 2001; Alonso and Mangel 2004; Erisman et al. 2009). The marine protected area is considered one of the most useful options for the species such as *E. polyphekadion* that form spawning aggregations (Coleman et al. 2000; Rhodes et al. 2011; Sadovy de Mitcheson 2016). Closed season and sales ban during their reproductive season are also effective and more practical for the species that information if they form spawning aggregation is not available, such as *E. tauvina* and *E. howlandi*. The information of reproductive biology of some target species have contributed to consensus-building among local fishers regarding the coral reef fish fishing and the basis of the establishment of temporary closure of the several spawning aggregation sites in the

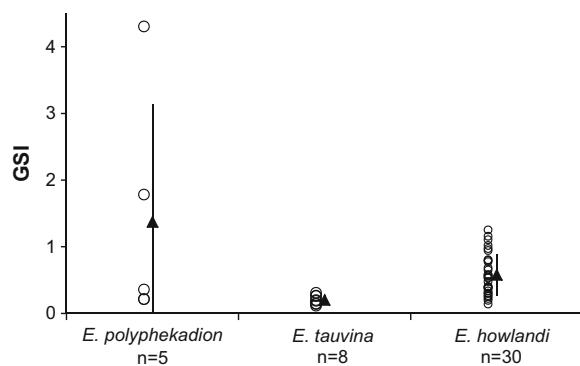


Fig. 12 Gonadosomatic index (GSI) of actively spawning males for the three *Epinephelus* species. Open circles indicate each value of GSI. Closed triangles and bars indicate the mean of GSI \pm SD

Yaeyama Islands (Ebisawa 1999; Akita and Yamauchi 2012; Ohta and Ebisawa 2017). Further research and management action are needed for sustainable fisheries of the species.

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