

Impact of fishing losses of males on the reproductive output of the large protogynous fish, *Choerodon schoenleinii*

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ABSTRACT: Protogynous fishes are major components of commercial fisheries worldwide. They can compensate for fishing losses of males through socially controlled sex change. If their resiliency, however, is insufficient to compensate for male loss, their reproductive output can decline. Understanding the effects of fishing losses of males on reproductive output of stocks requires a detailed understanding of species-specific physiological constraints on sex change and gametogenesis. The potential impact of fishing losses of males on the reproductive output of the large protogynous species *Choerodon schoenleinii* was investigated by assessing the seasonality of male fishing pressure using market research. In addition, laboratory experiments imitating male removal from a social group through fishing were used to assess (1) whether females change sex during the spawning season, (2) the period after change that is required for an individual to become a functionally mature male, (3) whether individuals have sufficient capacity to fertilize batches of spawned eggs just after sex change, and (4) the impact of fishing losses of males on reproductive output of a social group. Following removal of males from 8 artificial social groups, only one female changed sex. The inability of most females to change sex during the spawning season and the slow rate of sex change may be closely related to the low natural mortality and long lifespan of this species. The female that changed sex showed low fertilization rates for at least 1 mo after sex change. Males are fished from stocks mainly before and during the spawning season, which will result in a large reduction in reproductive output due to male limitation and lower fertilization rates.

KEY WORDS: Sex change · Protogynous fish · Fishing losses of males · Reproductive output · Fertilization rate · *Choerodon schoenleinii*

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INTRODUCTION

A number of species from a wide range of fish families, e.g. Labridae, Serranidae, Scaridae, and Lethrinidae, are sequentially hermaphroditic (Sadovy de Mitcheson & Liu 2008). These species are major components of important commercial fisheries around the world (Sadovy de Mitcheson & Liu 2008). Social and/or endogenous mechanisms regulate sex in sequentially hermaphroditic fishes (Robertson 1972, Shapiro 1979, Buxton 1993). For example, protogynous life histories are frequently observed in coral reef fishes with harem social systems (Ross 1990). The adaptive significance of the protogynous life history has been explained successfully by the size-advantage model (Ghiselin 1969, Warner et al. 1975). The removal of a male from a social group of protogynous fish stimulates a female, usually the largest dominant female, to change sex (Ross 1990, Warner 1988).

Most hermaphroditic fishes are managed by the same methods as those which are generally applied to non-hermaphroditic species (Provost & Jensen 2015). Fisheries often target the largest individuals in a population, most of which are males in protogynous fishes (Fenberg & Roy 2008). Excessive fishing pressure can limit male and sperm availability (Sato 2012), especially in species with an endogenous developmental schedule in which size or age at sex change is fixed (Alonzo & Mangel 2004, Heppell et al. 2006). Most protogynous fishes, however, have compensatory mechanisms such as socially controlled sex change, making their stocks resilient to fishing (Alonzo & Mangel 2005). This resiliency can compensate for the disproportional loss of males by sex change at smaller size or earlier age (e.g. Alonzo & Mangel 2004).

However, if resiliency through sex change is insufficient to compensate for disproportional male loss, the reproductive output of these populations will sharply decrease (Vincent & Sadovy 1998). If dominant males are fished at high frequency from social groups, the resiliency to fishing will depend on whether the turnover time for females to replace dominant males is fast enough to maintain the sex ratio and reproductive output (fertilization rate). Evaluating the impact of fishing on the reproductive output of stocks requires a detailed understanding of species-specific physiological constraints on sex change and gametogenesis. We must determine the species-specific turnover time for females to replace the dominant male, i.e. the period required to become a functionally mature male. It is also important to determine whether females can change sex at any time, irrespective of the spawning

season, because many protogynous fishes commonly change sex during the non-spawning season (e.g. Candi et al. 2004, Muncaster et al. 2013). Furthermore, it is necessary to determine the ability of these fishes to produce sperm just after sex change. Small harem protogynous fishes have shown a positive relationship between time after sex change and the number of ejaculated sperm or fertilization rate (Muñoz & Warner 2003).

Most empirical studies of the reproductive and physiological biology of hermaphroditic fishes have focused on small-sized species, non-fishery species, and species with low mobility, because they are the most tractable for research. Sex-change experiments in larger hermaphroditic fishes are extremely problematic, primarily because these fishes are difficult to maintain in captivity or to manipulate in the wild. Time-series observations of spawning harems in the field are also difficult, especially for deep water species. Therefore, not only the physiological constraints on sex change and gametogenesis, but the impact of fishing losses of males on reproductive output of large-sized protogynous fishery species remain unclear, despite many of these species being in danger from heavy exploitation. Understanding the physiological constraints on sex change and gametogenesis would allow mathematical models to better predict the impact of fishing on these species.

Choerodon schoenleinii is a large labrid species reaching 1 m in total length (TL) and an age of at least 17 yr. It is distributed throughout the tropical western Pacific region, in waters up to 60 m in depth (Araga 1997, Westneat 2001, Ebisawa et al. 2010). *C. schoenleinii* is monoandric and exhibits harem protogynous hermaphroditism, sexual size dimorphism with males being larger, and sexual dichromatism (Ebisawa et al. 1995, Fairclough 2005, Sato et al. 2018). Sex change of *C. schoenleinii* is socially controlled (Sato et al. 2018). Although very important commercially in Okinawa, southwest Japan, the species is considered overexploited and its stock has been reduced (Akita et al. 2015). Currently, *C. schoenleinii* is listed on the International Union for Conservation of Nature Red List as a Near-Threatened species and almost meets the criteria for Vulnerable. Several regions, including Okinawa, regulate size limits for *C. schoenleinii* fishing (Fairclough & Nakazono 2004). The species is fished mainly by spear fishing at night (Kanashiro et al. 1990). To date, the physiological constraints on sex change and gametogenesis in this species remain undetermined, and little is known about the impact of fishing losses of males on their reproductive output.

This study evaluated the potential impact of fishing losses of males on the reproductive output of *C. schoenleinii*, using market research and large-scale laboratory experiments. Market research was used to investigate the seasonality of male fishing pressure. In the laboratory experiments, artificial social groups were allowed to form in very large tanks and the removal of a dominant male from a social group mimicked fishing before and during the spawning season. Laboratory experiments were used to assess (1) whether females change sex during the spawning season if dominant males are removed from their social groups before and during the spawning season, (2) the period required after sex change to become a functionally mature male with the ability to fertilize eggs, (3) whether individuals just after sex change have sufficient capacity to fertilize batches of spawned eggs, and (4) the impact of fishing losses of males before or during the spawning season on the reproductive output of an artificial social group. In this paper, 'functionally mature male' is a male with the ability to fertilize eggs, and 'completion of sex change' means the individual has acquired the ability to fertilize eggs.

MATERIALS AND METHODS

Materials

This study analyzed *Choerodon schoenleinii* living around the Yaeyama Islands, located southwest of Okinawa, Japan. At this location, most individuals larger than 55 cm TL are males (Akita et al. 2017). The spawning season in the Yaeyama Islands starts between January and February (Hirai et al. 2015), and in laboratory tanks spawning occurs at dusk from late January through late May (T. Sato unpubl. data). *C. schoenleinii* is considered a harem species histologically (Fairclough & Nakazono 2004), with field observations showing that a single male has the same territory as 2 to 4 females in their natural habitat (pers. comms. with local diver fishermen). Sex change usually occurs during the non-spawning season (Ebisawa et al. 1995) and is deemed to be regulated by male-to-female tactile contact (Sato et al. 2018). Removal of tactile contact during the non-spawning season can result in sex change even in females smaller than 50 cm TL (Sato et al. 2018). Sex changers require several months to become functionally mature males (Sato et al. 2018). The sexual dichromatism of *C. schoenleinii* almost completely corresponds to sex transition (Ebisawa et

al. 1995). Females are greenish-yellow in color, whereas males are blue; during sex change, females pass through a phase in which they are greenish-yellow tinted with blue (Sato et al. 2018). Plasma concentrations of 11-ketotestosterone (11-KT) differ between sexes, enabling the sex of an individual to be identified (Sato et al. 2018). Plasma 11-KT levels increase at the onset of sex change, become more pronounced during sex change, and decrease prior to first spermiation and completion of sex change (Sato et al. 2018).

Market research

To determine the seasonal trend of fishing pressure on males around the Yaeyama Islands, market research was conducted at the Yaeyama fish market on Ishigaki Island (24°21'N, 124°08'E) and at the Tomari fish market on Okinawa Island (26°13'N, 127°41'E), because most *C. schoenleinii* fished around the Yaeyama Islands are traded at these markets. From January 2011 to March 2014, landed *C. schoenleinii* males from the Yaeyama Islands were counted at these markets twice per week. The site of landing could be determined from the names of the fishermen involved. The sex of an individual was determined by body color.

Collection and maintenance

Specimens of *C. schoenleinii* were collected in 2011 to 2013 from the Sekisei Lagoon, an area between the Yaeyama Islands. Individual fish were captured by purse seine fishing and transported in oxygenated holding tanks to the Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute (24°4'N, 124°21'E), where all laboratory experiments were performed. Juvenile fish were excluded by collecting only adult fish larger than 35 cm TL (Hirai et al. 2015). Each adult fish was tagged with a passive integrated transponder tag for individual identification. The sex of each fish was determined by body color.

Laboratory experiment

The fish were placed in 130 kl tanks (8 × 8 × 2 m deep) filled with aerated filtered seawater (up to 1.9 m in depth) at a flow rate of 90 l min⁻¹. Twelve social groups were formed (Table 1, Table S1 in

Table 1. Total length (cm; mean \pm SD and range) of *Choerodon schoenleinii* individuals (1 male, 3 females [F1–F3] per tank) in each treatment group in the laboratory experiment in early January

Fish	Control treatment (n = 4)		Before treatment (n = 4)		During treatment (n = 4)	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Male	63.7 \pm 3.7	60.4–67.2	64.4 \pm 4.2	60.7–68.0	64.9 \pm 1.5	63.3–66.8
F1	55.0 \pm 2.1	51.9–56.8	56.2 \pm 1.5	54.6–58.1	55.9 \pm 0.8	54.8–56.6
F2	48.3 \pm 1.9	46.6–50.7	47.7 \pm 0.8	47.0–48.7	46.9 \pm 1.6	45.3–49.0
F3	40.8 \pm 1.9	38.2–42.7	41.5 \pm 2.0	38.6–43.0	41.7 \pm 2.7	37.8–43.8

Supplement 1 at www.int-res.com/articles/suppl/m602p225_supp.pdf), each consisting of a large male and 3 females of different TLs (F1, F2, and F3) and unfamiliar with one another, a scenario not likely in their natural habitats. Because little is known about the effects of familiarity on social group dynamics in this species, the groups were formed by early October, at least 3 mo before the beginning of the spawning season, to allow the development of social hierarchies. Protective habitats were simulated by placing into each tank 4 plastic pipes (40 cm in diameter \times 90 cm in length) and 2 box-shaped structures (1.5 m in length \times 1 m in width \times 0.7 m in height) composed of plastic net (mesh size, 25 mm) largely opened on 3 sides.

Timing of male removal from the social groups was based on results of market research, in which males were fished mainly before and during the spawning season, from November to March (see 'Results'). The 12 social groups were subdivided (Table 1, Table S1). In 4 groups, the male was removed from the tank in early January, before the spawning season (Before treatment, Groups B1–B4). In another 4 groups, the male was removed in mid-March, during the spawning season, extending from late January to late May (During treatment, Groups D1–D4). In the remaining 4 groups, the largest female (F1) was removed in early January, before the spawning season, instead of male (Control treatment, Groups C1–C4). The 12 groups were monitored from October (2011 to 2013) to May of the following year (Table S1). Fish were fed Japanese littleneck *Ruditapes philippinarum* (5% of body weight) twice per week, and cuts of double-lined fusilier *Pterocaesio digramma* and squid *Todarodes pacificus* (5% of body weight) with an added vitamin supplement (Healthy mix-2, Dainippon Sumitomo Pharma) 3 times per week. The hours of light and dark matched natural local conditions, and the water temperature during the 3 study periods, from 2011 to 2014, ranged from 20.2 to 28.4°C, 20.0 to 27.8°C, and 19.9 to 28.1°C, respectively.

To determine the temporal dynamics of sex change, body color and behaviors, interactions between individuals were observed from October until May. The TL and body weight of all reared fish were measured every month by catching them with a net in a tank with a lowered water level. Caught fish were anesthetized for 3 min in 200 l of seawater containing 40 ml of phenoxyethanol (WAKO). Because spawning is observed from late January to late May and vitellogenin (Vtg) induction can be detected from October in *C. schoenleinii* reared in tanks (Sato et al. 2018), spermiation by each individual was confirmed by hand stripping every month from October to December and twice per month from January to May. Spermiation by individuals was considered a marker of gonadal sex change completion.

Plasma concentrations of 11-KT and estradiol-17 β (E2) were measured every month during the experimental period. After measuring TL and body weight, blood was collected from the artery under the spinal cord using a disposable needle (20 G, 70 mm, Terumo) attached to a 2.5 ml heparin-coated polypropylene syringe. From this, 2 ml samples were placed in microtubes and centrifuged at 1890 $\times g$ for 15 min at 4°C. Blood plasma was stored at –80°C until analysis with commercial ELISA kits (Cayman Chemical Company), as described by the manufacturer (Supplement 2). At the start of experiment, the sex of each individual was determined based on body color and on plasma 11-KT concentrations, and these were in complete agreement. Vtg was also analyzed every month by Ouchterlony double immunodiffusion (Ouchterlony & Nilsson 1973, Supplement 2).

To examine the impact of male removal on the reproductive output of the artificial social groups and fertilization ability of individuals that changed sex, spawning in each tank was assessed every morning from January to May in the years 2012 to 2014. If spawning had occurred, the spawned eggs were collected using an egg collection net (mesh size: 250 μ m) placed in each tank under the overflow pipe. Collected eggs were placed in a container containing

10 l seawater, and the total number of eggs spawned was estimated by counting the number of eggs in a 0.1 l subsample. Fertilization rate was determined by calculating the proportion of dividing eggs with more than 32 cells in the subsample.

Data analysis

All statistical analyses were performed using R v.3.3.2 software (R Development Core Team 2016). Seasonal fluctuations in monthly fishing pressure on males were determined using a generalized additive mixed model (GAMM) with a Poisson error distribution and log function. In the GAMM, the monthly number of landed males during the years 2011 to 2014 was regarded as the response variable, and the month was regarded as the explanatory variable. Year was treated as a random effect. The mgcv package (Wood 2006) was used for GAMM. A cyclic cubic regression spline that forces the response to have the same start and endpoints was used to smooth the month predictor. The number of knots of a smooth term for month was set at 4, and the gamma parameter, which penalizes models of increasing complexity, was set at 1.4 to avoid overfitting and to obtain ecologically relevant responses (Wood 2006). The unbiased risk estimator (UBRE, i.e. the prediction error criterion when the scale parameter is known; Wood 2016) and the deviance explained (%) were calculated.

To determine the effects of male removal before and during the spawning season on the total number of fertilized eggs per spawning season of artificial social groups, bootstrap samples and bootstrap confidence intervals of total number of fertilized eggs actually observed in 0.1 l subsamples per spawning season were determined for each treatment using the simpleboot package (Peng 2008) because of the overdispersion and non-normality of the data (Supplement 3). Confidence intervals were calculated according to the bootstrap bias-corrected and accelerated interval method with 10 000 bootstrap data samples.

RESULTS

Market research

A total of 249 *Choerodon schoenleinii* males, of 58.6 ± 5.9 cm TL (mean \pm SD; range 39–75 cm; Fig. S1 in Supplement 1), were landed during the study period

(Fig. 1). The GAMM showed that the number of fished males could be explained by month of the year, and that month had a significant nonlinear effect on the number of landed males (estimated degrees of freedom [edf] = 1.92, df = 2, n = 39, $\chi^2 = 51.74$, $p < 0.01$, UBRE = 1.46, deviance explained = 39.9%, Fig. 2). The number of landed males was high from November to March, i.e. before and during the spawning season, and low from May to September.

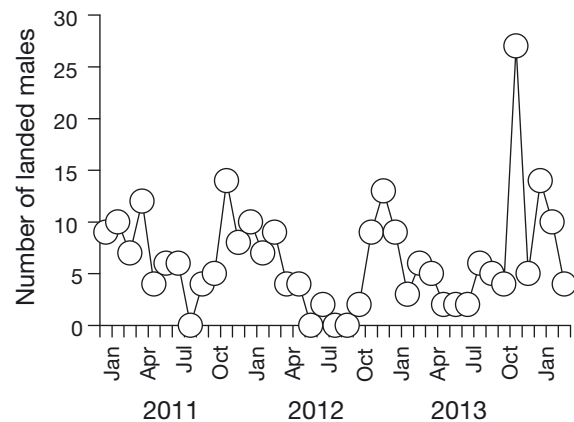


Fig. 1. Monthly number of *Choerodon schoenleinii* males landed from January 2011 to March 2014 at Sekisei Lagoon, an area between the Yaeyama Islands, Japan

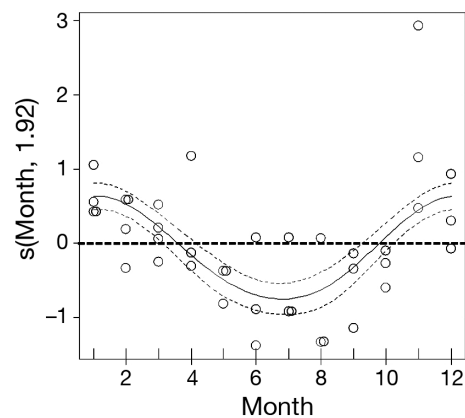


Fig. 2. Estimated smoothing curve for the generalized additive mixed model (GAMM) of *Choerodon schoenleinii*, showing the relationship between month and total number of fished males during each month (solid line). The y-axis represents the normalized effect of the variable, with 0 corresponding to no effect of the covariate on the estimated response, values larger than 0 indicating a positive correlation, and values smaller than 0 indicating a negative correlation. The dashed horizontal line indicates zero effect of month on the total number of landed males in each month. The y-axis is labeled s(covariate name, estimated degrees of freedom), indicating that the curve has been smoothed. Thin dashed lines indicate 95% confidence intervals

Laboratory experiments

Agonistic rushes by males towards females were frequently observed, and males were the dominant individuals in all social groups. Rushes by the largest females (F1) towards subordinates (F2 and F3) were also observed occasionally. Spawning started in January and spawned eggs were collected until May (from late January until late May in 2012 and 2013; and from mid-January to late May in 2014) (Fig. 3). All females started to have plasma Vtg between November and February. They retained plasma Vtg until the end of the spawning season, except for 1 F1 that changed sex following removal of the dominant male (Group B1).

Although we did not measure the activities of individuals quantitatively, qualitative observations suggest that after removal of males before and during the spawning period, most remaining females in each social group hid and were inactive for a few days. Female activity increased gradually over several weeks after male removal. Although some fe-

males spawned eggs in the absence of males, the number of days when spawned eggs were collected was small after male removal (Fig. 3a). In contrast, F1 removal from the Control Groups C1 to C4 had no effect on the behaviors of the remaining individuals. Our qualitative observations suggest that the remaining females in each social group did not hide and become inactive after F1 removal. Plasma concentrations of 11-KT were higher and plasma E2 concentrations were lower in males than in females throughout the study period (Figs. 4a–d & 5a–d), with males maintaining dominance in each social group.

In 7 of the 8 groups, 3 of those from which males were removed before the spawning period (Groups B2–B4) and all 4 from which males were removed during the spawning period (Groups D1–D4), no female changed sex. In the eighth group (Group B1), the F1 changed sex and matured functionally as a male. From 21 d after the male removal, the body color of the F1 changed to a greenish-yellow tinted with blue at dusk (early February), and eventually from greenish-yellow to a terminal blue phase. In this group, unfertilized eggs were first collected 36 d after male removal (mid-February), with the F1 first successfully fertilizing eggs 49 d after male removal (early March). The estimated fertilization rate of the collected eggs was 11.1%, with rates increasing gradually over time (Fig. S2 in Supplement 1). For at least 1 mo after sex change, the estimated fertilization rate of the F1 in Group B1 was lower than that of the 2 control groups (Groups C1 and C2) formed the same year (Fig. 6). Although Vtg was present in the plasma of the sex changer from December to February, no Vtg was detected after March. Plasma 11-KT concentration in this fish increased markedly in February, 1 mo after male removal, and decreased in March, when spermiation and fertilized eggs were first observed (Fig. 4e). Plasma E2 concentrations began to decline 1 mo after male removal (Fig. 5e).

The F1s in Groups B2 and D4 showed a sharp rise in plasma 11-KT concentration and a decline in plasma E2 concentration in May (Figs. 4f,l & 5f,l). In these F1s, Vtg was detected throughout the spawning season, and no spermiation was observed. Beginning in late April, both fish showed a change in body color, to greenish-yellow tinted with blue during dusk, but only non-fertilized eggs were observed after male removal from these 2 groups.

None of the F1s in the other 5 groups (Groups B3, B4, and D1–D3) showed conspicuous responses to male removal. Vtg was detected in these fish throughout the spawning season, and none showed a change

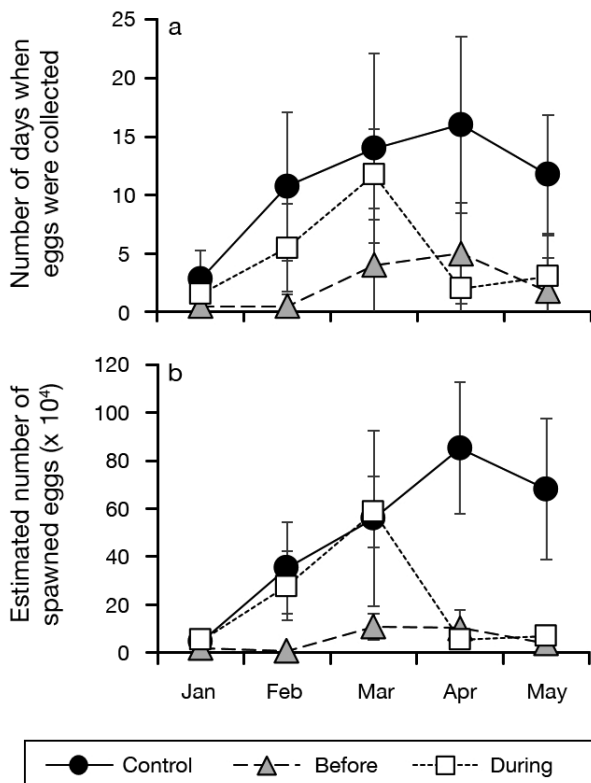


Fig. 3. Results of spawning of *Choerodon schoenleinii* in the laboratory. (a) Mean total number of days when eggs were collected and (b) mean estimated number of spawned eggs in each month in the Control, Before, and During groups. Error bars indicate standard deviations (SD)

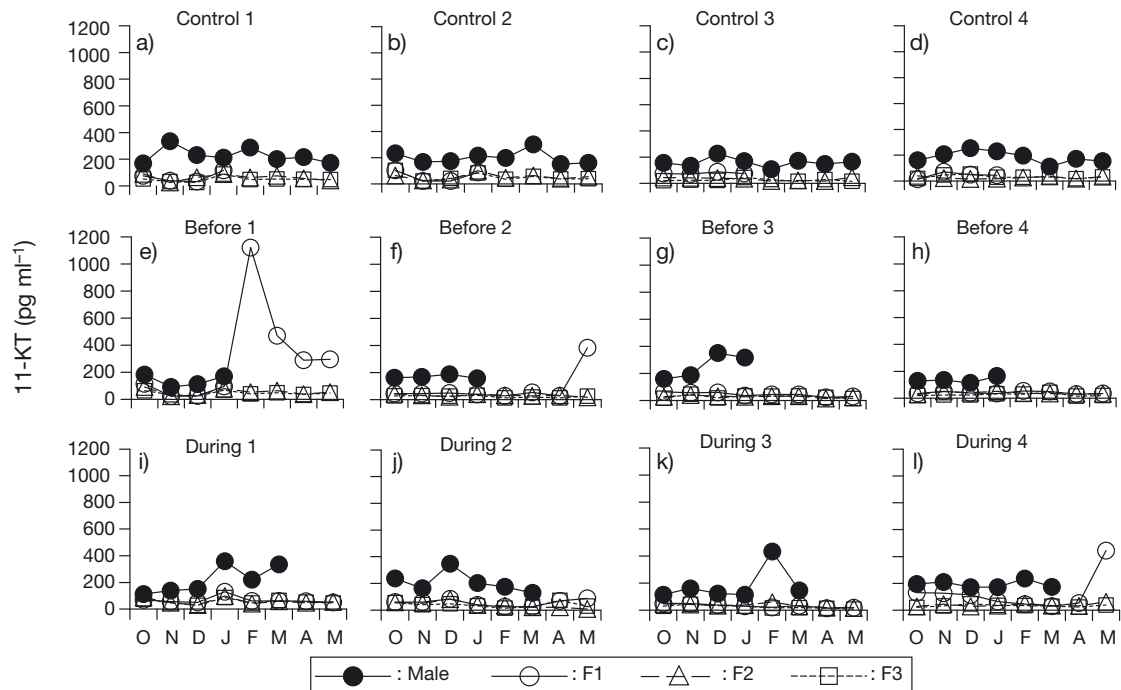


Fig. 4. Plasma concentrations of 11-ketotestosterone (11-KT) of *Choerodon schoenleinii* in each of the (a–d) Control, (e–h) Before, and (i–l) During treatments. Each group consisted of a large male and 3 females of different total lengths (F1, F2, and F3) at the start of the experiment. Males were removed from groups in early January in Before and in mid-March in During treatments, respectively. In Control treatments, the largest female (F1) was removed in early January, instead of the male

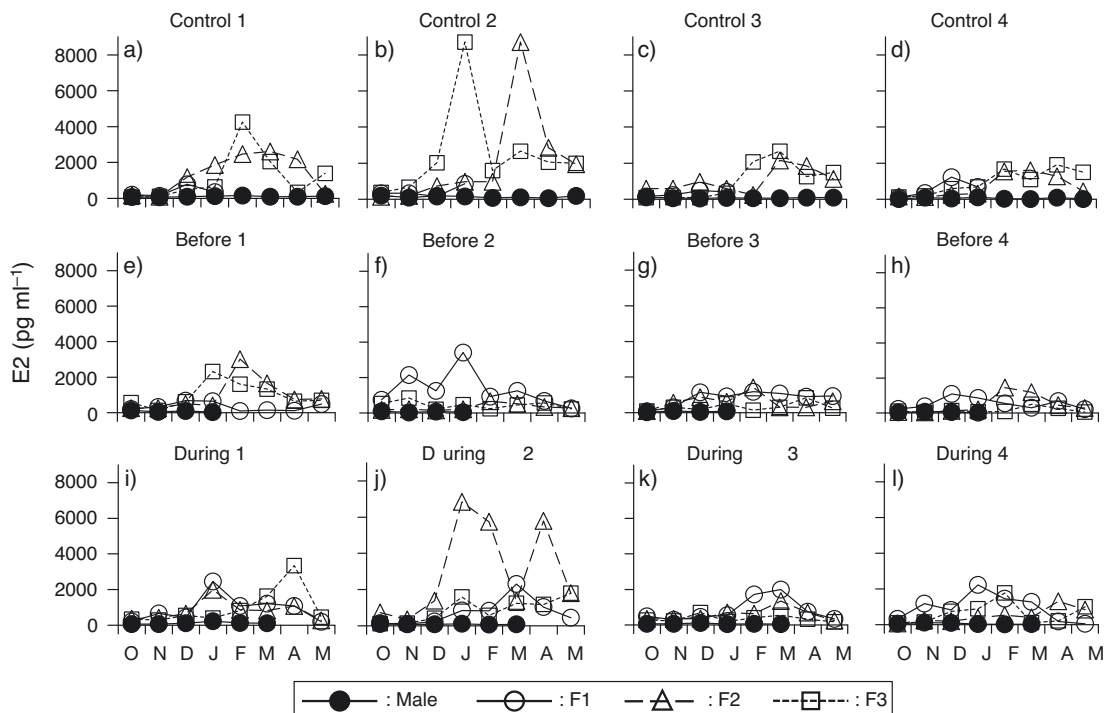


Fig. 5. Plasma concentrations of estradiol-17 β (E2) of *Choerodon schoenleinii* in each of the (a–d) Control, (e–h) Before, and (i–l) During treatments. Each group consisted of a large male and 3 females of different total lengths (F1, F2, and F3) at the start of the experiment. Males were removed from groups in early January in Before and in mid-March in During treatments, respectively. In Control treatments, the largest female (F1) was removed in early January, instead of the male

in body color, spermiation, or any conspicuous change in plasma 11-KT and E2 concentrations (Figs. 4g–k & 5g–k).

The total number of eggs observed in subsamples obtained during the spawning season differed depending upon whether males were removed before or during the spawning season. The mean total number of eggs in the 4 Control groups ($17\,502.3 \pm 2911.2$) was 20-fold and 4-fold higher than the mean number in groups from which males were removed before (855.5 ± 1711.0) and during (4087.0 ± 3203.1) the spawning season, respectively (Fig. 7).

DISCUSSION

Limited ability to change sex before and during the spawning season

Most, but not all, *Choerodon schoenleinii* females may be unable to change sex in response to the disappearance of the dominant male immediately before or during the spawning season. Sex change in protogynous fishes involves the changing of ovaries into functional testes. Vitellogenic oocytes degenerate, accompanied by a rapid decline in plasma E2 levels. Furthermore, plasma 11-KT levels increase parallel to the increase in testicular tissues (e.g. Nakamura et al. 1989, Muncaster et al. 2013), with increases in 11-KT inducing a change in body color (Grober et al. 1991). Only one F1 in Group B1 showed these 4 signs of sex change onset: (1) change in body color, (2) disappearance of Vtg from the plasma, (3) a sharp increase in plasma 11-KT concentration, and (4) a decline in plasma E2 concentration. The F1s in Groups B3, B4 and D1 to D3, however, showed none of these signs after male removal. Histologic examination of the gonads of these females at the end of the laboratory experiment (early June) showed no evidence of spermatocytes and spermatids (Figs. S3a,b,c & S4a in Supplement 1), indicating that these females did not start to change sex during the spawning season.

Although the F1s in Groups B2 and D4 exhibited several signs of sex change onset (body color change, increased plasma 11-KT, and decreased plasma E2) in May, just before the end of the spawning season (4 and 2 mo after male removal, respectively), neither showed spermiation or fertilized eggs, indicating that these fish initiated but did not complete sex change by the end of the spawning season. Histologic examination of their gonads showed that these F1s had previtellogenic oocytes, yellow-brown bodies, sper-

matocytes and spermatids (Figs. S3d,e,f & S4b). Yellow-brown bodies may derive from degenerating ovarian tissue (Nakamura et al. 1989). Although spawned eggs were collected 5 and 8 times from Groups B2 and D4, respectively, no fertilized eggs were collected. These findings suggest that the F1s started to change sex just before the end of the spawning season, in preparation for becoming the dominant male during the next, not the present, spawning season. Similar to other species of protogynous fishes (e.g. Candi et al. 2004, Muncaster et al. 2013), *C. schoenleinii* females in their natural habitat usually change sex during non-spawning seasons (Ebisawa et al. 1995), including under rearing conditions (Sato et al. 2018). Sex change in response to the disappearance of the male during the spawning season would be rather unusual in *C. schoenleinii*.

Potential reasons for the limited ability to change sex

Generally, the largest dominant females of protogynous fishes exhibit male sexual behaviors immediately after male removal and then become functionally male (e.g. Sakai et al. 2002). However, the dominant females do not always change sex, even after the disappearance of dominant males. In species with strong male–male competition and strong female mate choice for larger-sized males, relatively small dominant females are less likely to change sex (Sakai et al. 2002). Moreover, if the combined fecundity of other smaller females is less than the current fecundity of the dominant female or if sperm competition is intense, the dominant female can decline to change sex, with one of the smaller females becoming male (Muñoz & Warner 2003, 2004). The body sizes of the F1s in groups from which the males had been removed were comparable to the body sizes of males fished from natural habitats (Fig. S1). In addition, the F2 and F3 fishes in these groups did not change sex. The reason that 7 of 8 F1s did not change sex remains unclear. It was not due to the nutritional condition of the fish, as indicated by Fulton's condition factor, *K* (Htun-Han 1978) (Table S2 in Supplement 1).

The lack of sex change of most *C. schoenleinii* females in response to the disappearance of dominant males may be related to interspecific differences in natural mortality rates. The natural mortality rates of fish species and other marine organisms are closely related to their body size (body weight) (McGurk 1986). Differences in natural mortality rates

may determine the frequency of collapse of the hierarchy of social groups during the spawning season resulting from the disappearance of dominant males, e.g. due to predation. The frequency of the disappearance of dominant males would be higher in small protogynous species than in large protogynous species. Selective pressure in small protogynous species would likely favor females that can respond urgently to the disappearance of males during the spawning season. In contrast, selective pressure in large protogynous species such as *C. schoenleinii* would likely be weaker in favoring such females. Between-species differences in longevity may also affect the ability of females to change sex during the spawning season. If expected reproductive success during the next spawning season is negligible due to a short lifespan, a response to male disappearance by changing sex within the present spawning season would be indispensable for increasing their reproductive success. Natural mortality and longevity would therefore be closely related to the ability to respond immediately to the disappearance of the dominant male by changing sex during the spawning season.

The slow rate of sex change in *C. schoenleinii* may also be due to their low natural mortality rates and longer lifespan. The one *C. schoenleinii* F1 that changed sex required 49 d after male removal to function as a male. The time required to complete sex change varies widely among fish species, with the frequency of hierarchy collapse of social groups likely related to the rate of sex change in harem protogynous fishes (Ross 1990). Females in several small protogynous wrasses were found to complete gonadal sex change within a few weeks (reviewed by Sakai et al. 2003). Although manipulative experiments have not been performed to date in large protogynous wrasses, sex-changing female Ballan wrasse *Labrus bergylta*, of maximum 60 cm TL and maximum age 25 yr (Muncaster et al. 2013), and hog fish *Lachnolaimus maximus*, of maximum 82 cm TL and maximum age 21 yr (McBride & Johnson 2007), require several months to gain male functionality. Female *C. schoenleinii* that change sex during non-spawning seasons require several months to gain male functionality (Sato et al. 2018). The slow rate of sex change in larger protogynous fishes such as *C. schoenleinii* may be due to the stability of their social groups. However, the decrease in mass-specific metabolic rate with increasing body mass (Schmidt-Nielsen K 1984, Oikawa et al. 1992) may also be related to the slow rate of sex change.

Fertilization ability

To date, the fertilization ability of individual fishes just after sex change has not often been examined (Muñoz & Warner 2003). Compared with males in 2 of the Control groups (C1 and C2), the F1 that changed sex in Group B1 showed a low fertilization rate for at least 1 mo after sex change (Fig. 6, Fig. S2). Histological examination of the gonads of F1s in Groups B2 and D4, which initiated but did not complete sex change by the end of the spawning season, about 1 mo after the onset of sex change indicated that the percentages of testicular tissues in sections of their gonads, especially the anterior and middle parts (Fig. S3d,e,f), were much lower than that in sections of gonads of control males (Fig. S3g,h,i). Testicular tissues were observed only in the peripheral areas of the gonads of the F1s from Groups B2 and D4 (Fig. S3d,e) but occupied all sections of the gonads of males (Fig. S3g,h,i). A similar difference would also be expected between the gonads of the F1 in Group B1 soon after sex change and the males in Groups C1 and 2, which would cause low sperm production and the observed low fertilization rates of the F1 in Group B1 soon after sex change.

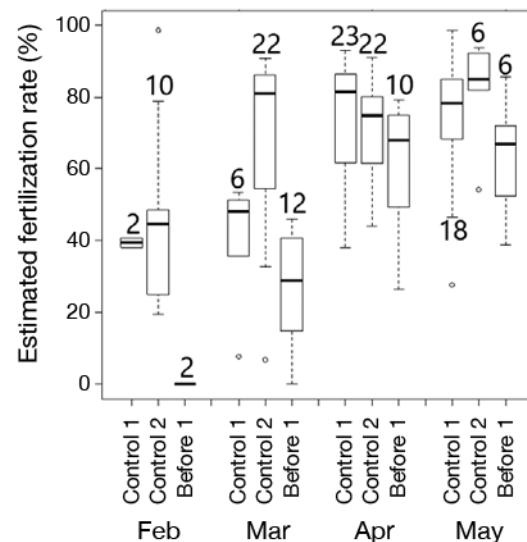


Fig. 6. Estimated fertilization rates in the Control Groups C1 and C2, and Before Group B1 of *Choerodon schoenleinii* from February 2012 to May 2012. Each box shows the median, quartiles, and minimum and maximum values during each month. Open circles: outliers; i.e. values more than 1.5 box lengths from the upper or lower edge of the box. The box length is the interquartile range. Each value indicates sample size

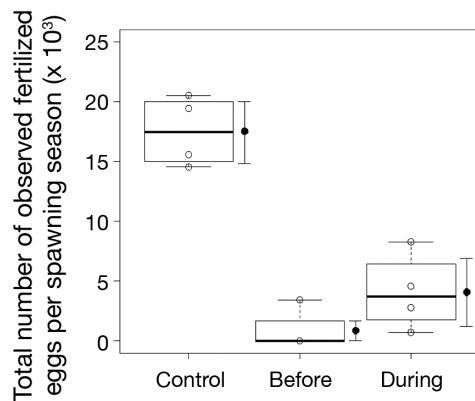


Fig. 7. *Choerodon schoenleinii*. Total numbers of observed fertilized eggs in subsamples per spawning season in the Control, Before, and During groups. Each box shows the median, quartiles, and minimum and maximum values during each month. The box length presents the interquartile range. Solid circles and lines: means and bootstrap confidence intervals of the responsible variable (total number of eggs actually observed in subsamples per spawning season). Open circles: raw data

Prejudicial impacts of fishing losses of males

Socially controlled sex change can enhance the resiliency of *C. schoenleinii* stocks to male removal (Sato et al. 2018). Our findings, however, suggest that fishing losses of males before and during the spawning season has serious impacts on the reproductive output of this species through male limitation and lower fertilization rates. This negative impact of male removal results from (1) the low frequency of sex change during the spawning season, (2) the slow rate of sex change, and (3) the insufficient ability to fertilize all spawned eggs for a period of time after sex change. To understand the resilience of large protogynous fishes to fishing losses of males, investigations into the species-specific physiological constraints on sex change and gametogenesis are crucial.

The results of this study suggest a need to reevaluate the present size limit for *C. schoenleinii* in the area around the Yaeyama Islands. Current regulations allow the fishing of all *C. schoenleinii* individuals larger than 30 cm TL around the Yaeyama Islands without sex and seasonal limitations. The minimum legal size is considerably smaller than that of terminal males in this area (Fig. S1). This means that dominant males are repeatedly removed from social groups. Compensating for fishing losses of males by females changing sex at a smaller size will succeed only if they live long enough to become large enough to change sex. Raising the minimum legal size is needed

to protect and increase the percentage of females that can change sex. However, raising the minimum size would only have a limited effect on reducing the potential impact of male removal. Fishing of terminal males should be completely prohibited before and during the spawning season. Sex- and season-selective fishing regulations are also needed for large protogynous fishes, such as *C. schoenleinii*, with physiological constraints on sex change and gametogenesis during the spawning season.

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