The following supplement accompanies the article

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

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Marine Ecology Progress Series 602: 225–235 (2018)

Supplement 1.

Table S1. Total length (TL) of *Choerodon schoenleinii* individuals used in the laboratory experiment just before removal of male, before (early January) and during (mid-March) the spawning season.

Treatment	Study period	TL (cm) (early January and mid-March)			
		Male	Female 1 (F1)	Female 2 (F2)	Female 3 (F3)
Group C1	October 2011-May 2012	60.6, 61.0	55.7, n.d.	48.7, 49.4	41.4, 42.4
Group C2	October 2011-May 2012	67.2, 67.4	55.6, n.d.	47.0, 48.2	42.7, 43.3
Group C3	October 2012-May 2013	66.6, 66.8	56.8, n.d.	46.6, 47.2	41.0, 41.8
Group C4	October 2013-May 2014	60.4, 61.5	51.9, n.d.	50.7, 51.1	38.2, 39.1
Group B1	October 2011-May 2012	60.8, n.d.	54.6, 55.2	47.0, 47.6	41.5, 41.9
Group B2	October 2012-May 2013	68.0, n.d.	56.3, 56.6	47.8, 48.6	43.0, 43.6
Group B3	October 2013-May 2014	60.7, n.d.	55.8, 56.1	47.1, 47.6	42.8, 43.3
Group B4	October 2013-May 2014	67.9, n.d.	58.1, 59.7	48.7, 49.0	38.6, 39.6
Group D1	October 2011-May 2012	65.2, 65.3	56.4, 56.8	47.1, 48.1	41.8, 42.2
Group D2	October 2012-May 2013	64.4, 64.7	54.8, 55.4	46.3, 47.2	43.8, 44.1
Group D3	October 2012-May 2013	63.3, 63.7	56.6, 56.9	49.0, 49.8	43.2, 43.5
Group D4	October 2013-May 2014	66.8, 66.9	55.8, 56.2	45.3, 46.2	37.8, 38.8

Table S2. Fulton's condition factors (*K*) of *Choerodon schoenleinii* individuals used in the laboratory experiment just before removal of males, before (early January) and during (mid-March) the spawning season.

Group	Fulton's condition factors (early January and mid-March)			
	Male	Female 1 (F1)	Female 2 (F2)	Female 3 (F3)
C1	22.1, 22.9	21.4, n.d.	21.4, 22.5	21.3, 21.4
C2	23.2, 24.1	21.8, n.d.	20.9, 20.8	20.9, 21.3
C3	22.0, 22.9	22.7, n.d.	22.5, 21.8	19.7, 21.0
C4	23.0, 21.8	22.1, n.d.	21.1, 22.9	20.3, 21.2
B1	22.5, n.d.	22.6, 22.9	22.2, 22.5	21.7, 22.4
B2	24.3, n.d.	23.0, 23.0	21.5, 21.4	20.2, 21.5
B3	21.6, n.d.	23.3, 24.0	22.1, 21.6	20.8, 22.7
B4	25.3, n.d.	23.2, 23.0	23.8, 24.2	21.1, 21.0
D1	22.3, 22.6	22.7, 22.9	22.4, 22.4	21.1, 22.1
D2	22.2, 22.5	22.5, 22.9	23.2, 23.7	21.0, 21.5
D3	23.0, 23.7	21.8, 21.6	21.4, 22.1	21.0, 20.8
D4	24.7, 24.2	22.8, 23.5	23.3, 23.6	22.6, 23.0

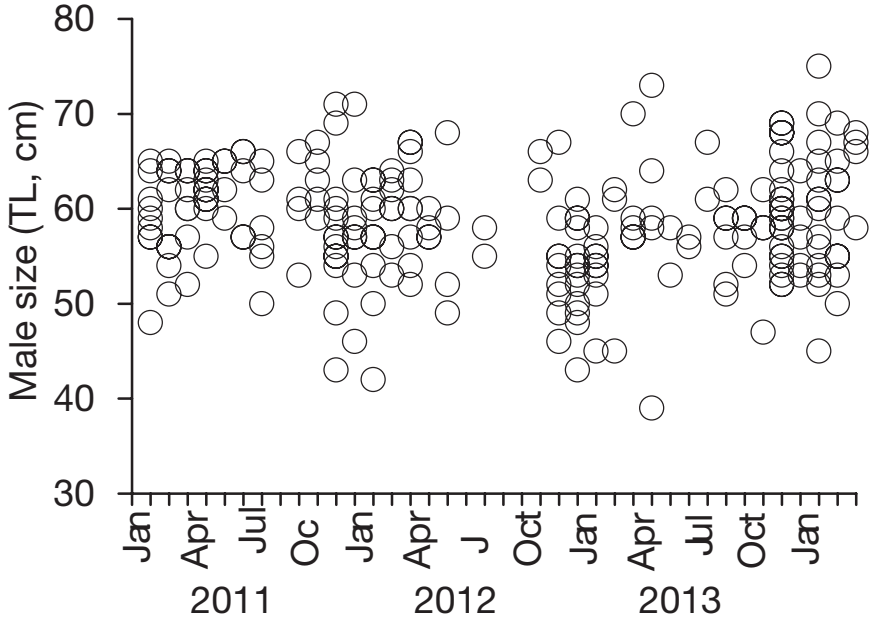

Figure S1. Total length (TL, cm) of *Choerodon schoenleinii* males fished from January 2011 to March 2014 at Sekisei Lagoon, an area between the Yaeyama Islands, Japan (n = 249; mean± SD, 58.6 ± 5.9 cm TL; range, 39-75 cm TL).

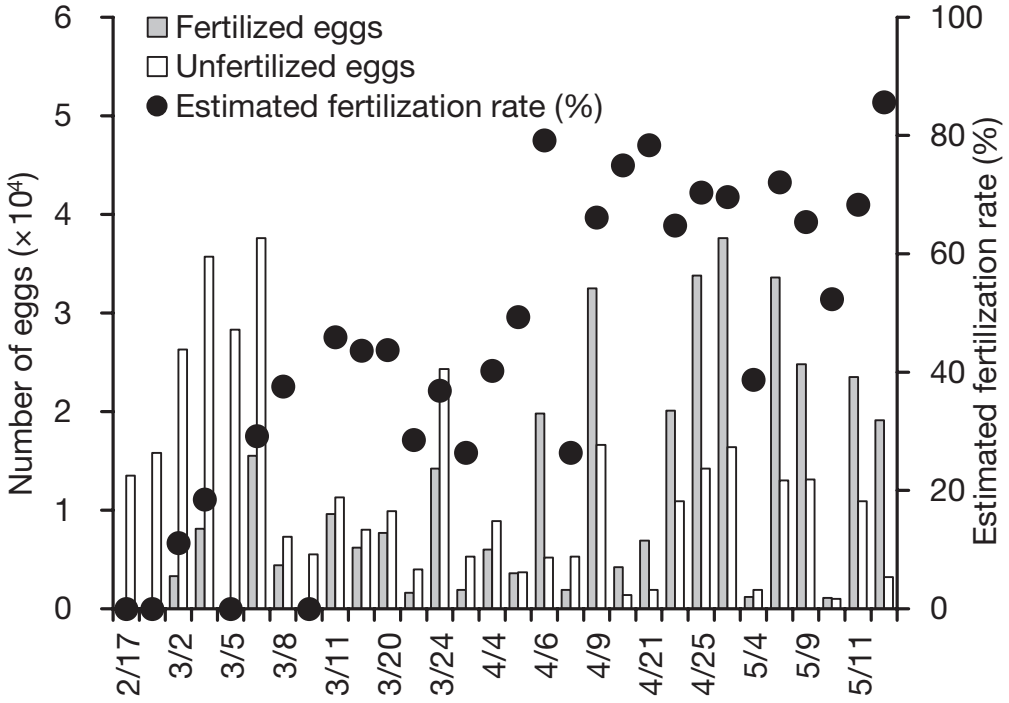

Figure S2. *Choerodon schoenleinii*. Daily numbers of fertilized eggs and unfertilized eggs and estimated fertilization rates (%) in Before 1 group after male removal.

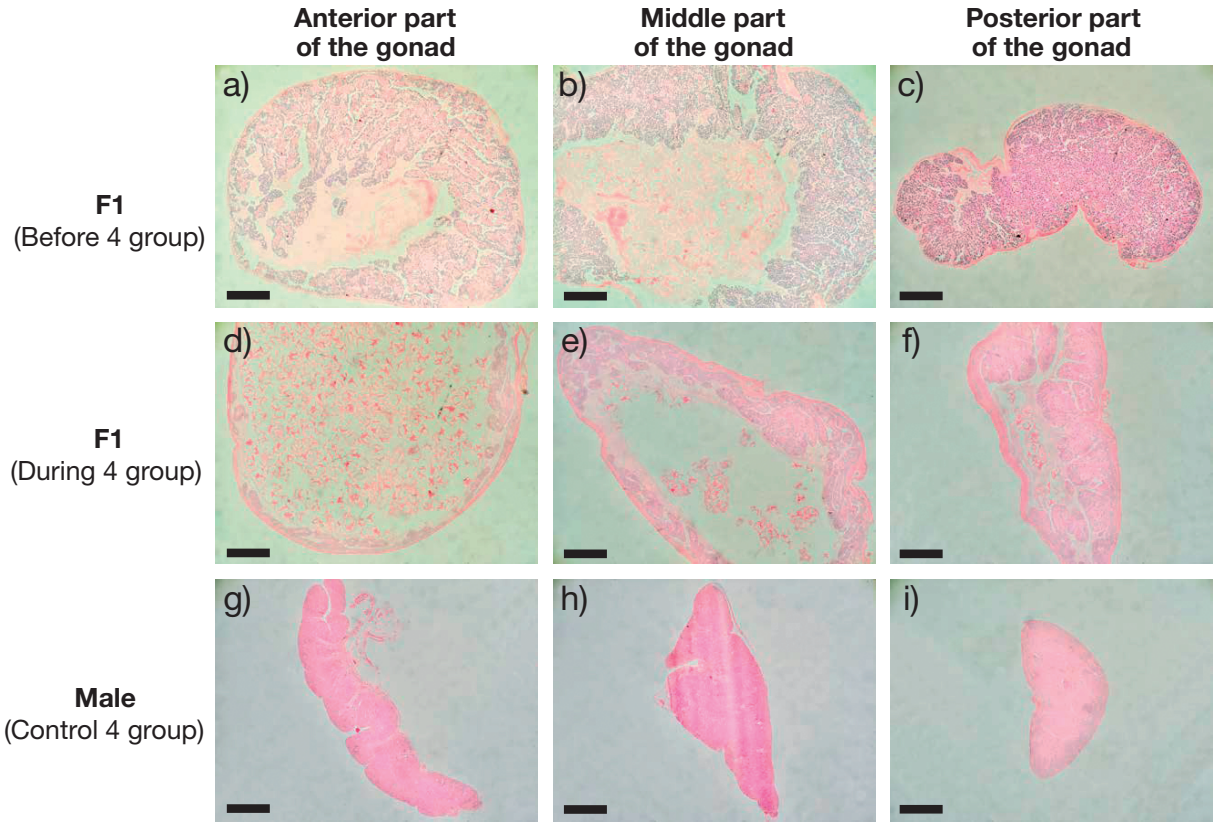

Figure S3. Representative cross sections of gonadal tissue of *Choerodon schoenleinii* stained with hematoxylin and eosin of a-c) largest female (F1) that exhibited no signs of sex change in Before 4 group, d-f) largest female (F1) that exhibited several signs of sex change (Body color change, increase in plasma 11-ketotestosterone concentration, and decline in plasma E2 concentration) in During 4 group, and g-i) dominant male in Control 4 group at the end of laboratory experiment (early June). Scale bars indicate 1 mm.

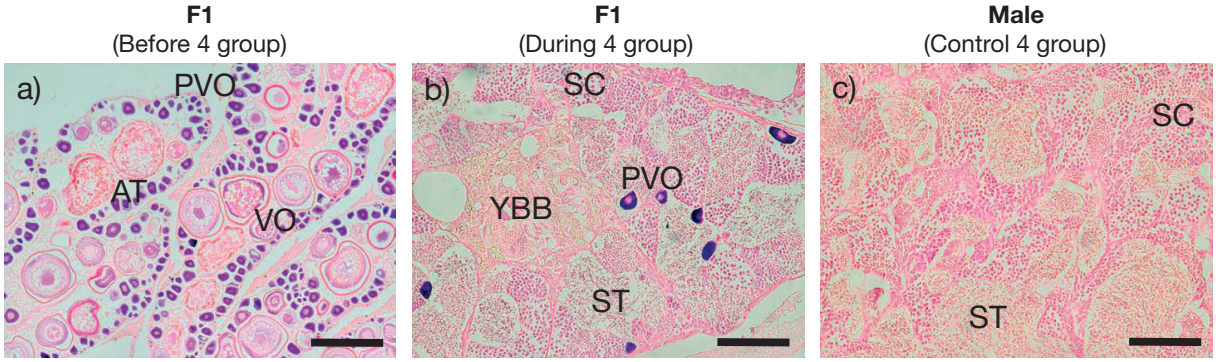

Figure S4. Representative cross sections of anterior part of the gonadal tissue of *Choerodon schoenleinii* stained with hematoxylin and eosin of a) largest female (F1) that exhibited no signs of sex change in Before 4 group, b) largest female (F1) that exhibited several signs of sex change (Body color change, increase in plasma 11-ketotestosterone concentration, and decline in plasma E2 concentration) in During 4 group, and c) dominant male in Control 4 group at the end of laboratory experiment (early June). AT, atretic oocyte; PVO, previtellogenic oocyte; SC, spermatocyte; ST, spermatids; VO, vitellogenic oocyte; YBB, yellow brown body. Scale bars indicate 100 μm.

Supplement 2.

Analyses of 11-KT, E2, and Vtg

To extract steroids, plasma of *Choerodon schoenleinii* (20 µL in the case of 11-KT or 100 µL in the case of E2) was placed in 5 ml silicon coated glass tubes and diluted in EIA buffer of the kit to 200 µL. A 1 mL aliquot of diethyl ether was added to each sample which was then mixed in a vortex mixer for 30 sec. The aqueous layer was frozen in chilled methanol (-20 °C), and the upper, diethyl ether layer was decanted into a new 5 mL silicon-coated glass tube. The extraction procedure was repeated twice for each sample. Each sample was subsequently dried at 40 °C for at least 2 hours and the residue dissolved in 200 µL EIA buffer by vortexing for 30 sec.

Vtg was analyzed by Ouchterlony double immunodiffusion (Ouchterlony and Nilsson 1973). Vtg of *C. schoenleinii* (CsVtg) was purified from the serum of an estradiol-17beta-treated female and anti-CsVtg antiserum was prepared as described (Sawaguchi et al. 2005). Double immunodiffusion was performed using 10 µL of plasma and 10 µL of anti-CsVtg antiserum on 1.8% agarose gel plates (2.5×2.5 cm, thickness: approximately 1 mm). The gel plates were incubated for 24 h at room temperature (20–25 °C) in a sealed plastic petri dish to prevent gel desiccation. Vtg was detected visually in the presence of immunoprecipitate.

LITERATURE CITED

Ouchterlony O, Nilsson LA (1973) Immunodiffusion and immunoelectrophoresis. In: Weir DM (ed) Handbook of experimental immunology. Blackwell Scientific Publications, Oxford, p 19.1–19.34

Sawaguchi S, Koya Y, Yoshizaki N, Ohkubo N, Andoh T, Hiramatsu N, Sullivan CV, Hara A, Matsubara T (2005) Multiple vitellogenins (Vgs) in mosquitofish (*Gambusia affinis*): identification and characterization of three functional Vg genes and their circulating and yolk protein products. Biol Repro 72:1045-1060

Supplement 3.

Overdispersion and non-normality of the data

At first, we tried to analyze the effect of male removal before or during the spawning season on the total number of fertilized eggs per spawning season of artificial social groups of *Choerodon schoenleinii* using generalized linear mixed-effects model. In the model, the total number of fertilized eggs observed in 0.1 L subsample per spawning season was regarded as the response variable, the total volume of subsamples checked per season was regarded as the offset term, and treatment (removal of individuals from social groups) was regarded as explanatory variables. Individual fish identity was treated as a random effect. However, overdispersion of the data for the total number of fertilized eggs observed was detected (dispersion parameter, $\varphi = 13.4$), which had been probably due to unique variances and the small sample size in each treatment group. Eventually, we could not correct the overdispersion by any method. Furthermore, data sets were homoscedastic (Levene's test, $F_{2, 9} = 1.47$, $p = 0.28$) but do not have a normality (Shapiro-Wilk normality test; Control: $W = 0.89$, $p = 0.39$; Before: $W = 0.63$, $p < 0.01$; During: $W = 0.98$, $p = 0.89$). Thus, bootstrap samples and bootstrap confidence intervals of the responsible variable (total number of fertilized eggs observed in 0.1 L subsamples per spawning season) were obtained in each treatment group using the simpleboot packages described in this paper.