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# Monogamous mating system and sexuality in the gobiid fish, *Trimma marinae* (Actinopterygii: Gobiidae)

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28 **Abstract**

29 The mating system and sexuality of the gobiid fish *Trimma marinae* were investigated  
30 in aquaria and by gonadal histological examination. The male to female sex ratio in the  
31 study aggregation was female-biased (14:27), and females were larger than males. *T.*  
32 *marinae* were monogamous because they established continuous pairs and spawned  
33 repeatedly with the same individuals. Observations of aggressive behavior suggested  
34 that the monogamous mating system resulted from female mate guarding. We also  
35 performed a rearing experiment to test whether sex change occurs in this species. As a  
36 result, none of the males or females reared separately in aquaria for 63 days changed sex.  
37 Additionally, gonadal histology revealed that mature fish had unisexual gonads (testis or  
38 ovary). These results strongly suggest that *T. marinae* is gonochoristic. However,  
39 immature fish had a bisexual gonadal structure, indicating juvenile hermaphroditism.

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41 Key words: *Trimma marinae* · Gobiidae · Juvenile hermaphroditism · Monogamy ·

42 Mating system

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3 **44 Introduction**  
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10 46 Mating systems are generally determined by the distribution of resources (e.g., food,  
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12 47 mating sites, hiding place, and/or mates). There may be little potential for polygamy  
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16 48 under conditions of evenly distributed resources, resulting in monogamy (Emlen and  
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19 49 Oring 1977; Davies et al. 2012). Barlow (1988) defined a pair as monogamous if 1) the  
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22 50 male and female confined most of their spawning to the same partner or 2) they  
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25 51 remained partners after fertilization until the young no longer required care. They  
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29 52 concluded that the necessity for biparental care may be the main driving force for the  
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32 53 evolution of monogamy. Whiteman and Côté (2004) reviewed reports on monogamous  
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35 54 marine fish and found that the evolutionary factors driving monogamy are not limited to  
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38 55 biparental care. They proposed six hypotheses for the evolution of monogamy: 1)  
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41 56 biparental care; 2) habitat limitations; 3) low population density/low mate  
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44 57 availability/low mobility; 4) increased reproductive efficiency; 5) territorial defense;  
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48 58 and 6) net benefit of sequestering a single mate.  
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51 59 The family Gobiidae contains a large number of species among teleosts (Nelson et al.  
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54 60 2016), and some monogamous species belong to this taxon (e.g., Kuwamura et al. 1993;  
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57 61 Nakashima et al. 1996; Sunobe and Nakazono 1999; Munday et al. 2002). Among them,  
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3 62 hermaphroditic species have been reported in the genera *Gobiodon*, *Paragobiodon*,  
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6 63 *Priorepis* (Sadovy de Mitcheson and Liu 2008), and *Bryaninops* (Munday et al. 2002).  
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9 64 It has been suggested that these species reproduce monogamously because of low  
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12 65 mobility, low mate availability, or intra-specific competition related to resources in their  
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15 66 specific habitats (Kuwamura et al. 1993; Nakashima et al. 1996; Sunobe and Nakazono  
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18 67 1999; Munday et al. 2002; Whiteman and Côté 2004; Wong et al. 2008). These  
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22 68 ecological and social characteristics have also been considered to facilitate the evolution  
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25 69 of sex change in these species (Nakashima et al. 1995; Munday et al. 1998; Munday  
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28 70 2002; Manabe et al. 2013). Therefore, revealing the mechanisms leading to various  
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32 71 mating systems is important when examining the evolution of hermaphroditism.  
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35 72 Sex change or sequential hermaphroditism among teleosts, such as protogyny  
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38 73 (female-to-male sex change) and protandry (male-to-female sex change), has been well  
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41 74 documented. The size-advantage (SA) model predicts evolution of protogyny when  
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44 75 species are polygynous because large males obtain remarkably greater benefit than  
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47 76 small males through intense mating competition and mate choice by females.  
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51 77 Meanwhile, the evolution of protandry is favored when males reproduce and are  
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54 78 reproductively successful regardless of their body size (Ghiselin 1969; Warner 1975;  
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57 79 Warner 1984; Kuwamura and Nakashima 1998; Munday et al. 2006).  
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3 80 In addition to these types of sex change mechanisms, bidirectional sex change has  
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6 81 been reported in Gobiidae, Serranidae, Pseudochromidae, Pomacanthidae, Cirrhitidae,  
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9 82 Labridae, and Pomacentridae (Munday et al. 2010; Kuwamura et al. 2016). In particular,  
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12 83 the gobiid genus *Trimma* has been studied to clarify the adaptive significance of  
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15 84 bidirectional sex change (Sunobe and Nakazono 1993; Manabe et al. 2007; Manabe et al.  
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18 85 2008; Sakurai et al. 2009). This genus contains 92 valid species distributed on rocky  
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21 86 ledges and coral reefs in temperate to tropical waters of the Indo-Pacific Ocean (Suzuki  
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24 87 et al. 2012; Winterbottom et al. 2015). Gonadal histological observations of *T.*  
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27 88 *grammistes*, *T. kudo*, *T. okinawae*, *T. unisquamis*, and *T. yanagitai* show that ovarian  
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30 89 and testicular tissues are present simultaneously (Cole 1990; Manabe et al. 2007;  
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33 90 Sunobe and Nakazono 1993; Sakurai et al. 2009; Shiobara 2000). Bidirectional sex  
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36 91 change has been confirmed in these species by field observations or rearing experiments,  
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39 92 except in *T. unisquamis*, and these species may be polygynous (Manabe et al. 2007;  
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42 93 Munday et al. 2010; Sakurai et al. 2009; Shiobara 2000). Except in *T. kudo*, the largest  
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45 94 female changes to male when the dominant male disappears, and the smaller individual  
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48 95 of a male–male pair changes sex to female (Sunobe and Nakazono 1993; Manabe et al.  
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51 96 2007; Manabe et al. 2008; Sakurai et al. 2009; Shiobara 2000).  
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97 The present study species, *T. marinae*, reaches a total length (TL) of 25 mm and is  
98 distributed in the western Pacific Ocean where it forms aggregations on the coral-reef  
99 slopes of enclosed bays at depths of 5–35 m (Shibukawa 2004). In this study, we show  
100 the monogamous mating system and gonochorism of this species in rearing experiments  
101 and perform a gonadal histological examination. Then, we discuss the adaptive aspects  
102 of the mating system and how gonochorism evolves among species with bidirectional  
103 sex change.

104  
105 **Materials and Methods**

106  
107 **Specimen collection**

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109 Forty-one *T. marinae* in an aggregation were collected by hand net offshore of Amami  
110 Oshima, Kagoshima Prefecture, Japan, at a depth of 21 m on 15 April 2014 using  
111 SCUBA. As this species forms aggregations around isolated dead coral on muddy  
112 bottoms, we captured all fish in a single aggregation to clarify group structure. Of these  
113 specimens, 16 died during collection and were measured for TL to the nearest 0.5 mm  
114 and sexed from the shape of the urogenital papilla (Sunobe and Nakazono 1993;



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115 Manabe et al. 2008). Eleven specimens were preserved in 70% ethanol for a future  
116 experiment, and five were fixed in Bouin's solution for 24 h and then preserved in 70%  
117 ethanol. Twenty-five live fish were transported to the laboratory for the rearing  
118 experiment.

119 We collected nine juveniles by hand net at the same site on 30 July 2014 to examine  
120 sexuality. They were measured for TL and fixed using the methods described above.

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122 Observations of reproductive behavior

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124 To reveal the reproductive behavior, 25 live individuals (eight males; mean  $\pm$  SD = 25.0  
125  $\pm$  0.5 mm TL, range = 23.5–25.0 mm TL and 17 females; 26.0  $\pm$  1.0 mm TL, range =  
126 24.0–27.0 mm TL) were anesthetized with quinaldine, measured for TL, sexed, marked  
127 by subcutaneously injecting a visible implant Elastomer Tag (Northwest Marine  
128 Technology Inc., Shaw Island, WA, USA), and deposited in an aquarium (120  $\times$  45  $\times$  45  
129 cm) on 16 April 2014. The fish density in the tank was approximately 0.1 individuals  
130 per liter. The sex ratio in the aquarium was adjusted to that observed in the original  
131 study aggregation (Fisher's exact test,  $P = 1.0$ ) (see Results). Water was circulated,  
132 filtered, and maintained at 25°C. Ten opaque PVC pipes (5 cm inside diameter and 6 cm

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133 in length) were cut in half and placed on the substratum as a spawning nest. We set a  
134 waterproof sheet inside the pipes to count the number of spawned eggs. We removed the  
135 sheet when the fish had spawned and counted the number of eggs using a microscope.  
136 Fish were fed formula food and live *Artemia* nauplii twice daily.

137 The fish were acclimated to an aquarium from 17 to 23 April 2014. We recorded  
138 spawning bouts, their clock times, and clutch size from 0400 h to 1800 h from 24 April  
139 to 22 May 2014. We observed reproductive behavior from 23 May to 28 June 2014.

140 This species reproduces in a pair (see Results). Thus, we considered a male and a  
141 female that hovered close together near the spawning nest as a reproductive pair. If  
142 several females hovered near a single male, we considered the female that hovered  
143 nearest the male and was aggressive toward other females as the pair-forming female.

144 We recorded the individual identity of the participants involved in pairing, spawning,  
145 and aggressive behavior to clarify the social relationships among the fish. As most  
146 spawning occurred between 0500 h and 0900 h (see Results), we observed the fish  
147 between 0400 h and 1200 h.

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149 Estimates of reproductive success

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151 To examine the effects of body size on the reproductive success of females, we used a  
152 generalized linear mixed-effects model (GLMM; R package lme4). We included clutch  
153 size and TL of the female as the response variable and fixed effect, respectively. As  
154 clutches were sampled several times from an individual, we set individual identity as a  
155 random effect term in the GLMM. The response variable was modelled with a Poisson  
156 distribution and the log link function.

157 We investigated the relationship between female body size and spawning frequency  
158 to examine the size-dependent advantage related to reproduction in females from 23  
159 May to 28 June 2014. We recorded the individual identity of females that spawned and  
160 counted spawning frequency and the intervals.

161 We also examined the relationship between body size and reproductive success in  
162 males. We recorded the spawning frequency of each male and the individual identity of  
163 spawned females. Clutch size of spawned females was estimated from the above model  
164 using their TL. Then, we derived the expected number of fertilized eggs for each male  
165 to define reproductive success throughout the study period.

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167 Rearing experiment to assess sex change

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169 To confirm the ability to change sex from female to male and male to female, we reared  
170 16 females and eight males in two aquaria (90 × 45 × 45 cm and 120 × 45 × 45 cm)  
171 from 16 July to 16 September, respectively. Two opaque PVC pipes (5 cm inside  
172 diameter and 6 cm in length) were cut in half and placed on the substratum in each  
173 aquarium as a spawning nest. The aquaria were maintained under the same conditions as  
174 those used for the previous rearing experiment. After the rearing experiments, the  
175 participants were killed in iced seawater to conduct a histological examination to  
176 confirm their sex. The abdomen was fixed in Bouin's solution for 24 h and preserved in  
177 70% ethanol, and the other parts were preserved in 100% ethanol for future  
178 experiments.

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### 180 Gonadal histology

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182 Tissues fixed in Bouin's solution were used for the gonadal histological observations.  
183 The abdomens of each specimen were embedded in paraffin, sectioned at 7 μm, and  
184 stained with hematoxylin and eosin.

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### 186 **Results**

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188 Size distribution of the sexes and the sex ratio

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190 Of the 41 fish captured, 14 and 27 were identified as males ( $24.5 \pm 0.6$  mm TL,  $N = 14$ )

191 and females ( $25.8 \pm 1.0$  mm TL,  $N = 27$ ), respectively. Females were significantly larger

192 than males (Mann–Whitney  $U$  test,  $U = 50$ ,  $P < 0.01$ ) (Fig. 1). The sex ratio in this

193 group was biased towards females (chi-square test,  $\chi^2 = 4.12$ ,  $P < 0.05$ ).

194

195 Spawning time

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197 A total of 60 spawning events were observed from 24 April to 22 May 2014. No

198 spawning activity was observed at 0400 h, and fish spawned from 0500 h to 0900 h. The

199 spawning bouts decreased and ceased after 1000 h, except in sporadic cases (Fig. 2).

200 These results show that the spawning time of this species is between 0500 h and 1000 h.

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202 Mating system

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204 Spawning was observed 117 times from 23 May to 28 June 2014 (Table 1). The eight  
205 males maintained the spawning nests against others throughout the day by defending the  
206 areas in and above their nests. Meanwhile, the 17 females usually formed an  
207 aggregation in the water column. A female approached one of the nesting males at  
208 spawning time. If the male accepted the female, they remained in the nest or hovered  
209 close together around the nest. The male frequently exhibited a courtship display,  
210 swaying his body back and forth. If the female accepted and entered the nest, they  
211 began to spawn. Eggs were spawned on the inner surface of the nest in a single layer.  
212 After spawning, the spawned female remained near the nest and the nesting male until  
213 about 1000 h when spawning stopped. Then, the spawned female returned to the  
214 aggregation. The nesting male guarded the egg mass until hatching 3–4 days later.

215 A notable characteristic of the *T. marinae* mating system was continuous pair  
216 formation (Table 1). The female approached and paired with the same male they had  
217 spawned with the previous day. Pairing behavior without spawning was also observed.  
218 In such cases, although the male and female formed a pair and the male exhibited a  
219 courtship display, they did not spawn and separated when spawning time ended. If this  
220 pairing behavior was exhibited for  $\geq 2$  days, regardless of whether they had spawned,  
221 we regarded it as maintaining a pair bond. No association was observed between male

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222 and female size in continuous pairs (Spearman's rank correlation coefficient,  $r = -0.39$ ,  
223  $P > 0.05$ ,  $N = 21$ ) (Fig. 3). The spawning frequency of each male ( $15 \pm 4$  times, range =  
224 7–18,  $N = 8$ ) was not associated with the TL of the males (Pearson's correlation,  $r =$   
225  $-0.33$ ,  $P > 0.05$ ,  $N = 8$ ).

226 In the 21 pairs that persisted for  $\geq 2$  days, the duration of maintaining a pair bond was  
227 2–32 days (mean,  $11.6 \pm 9.0$  days). One ( $N = 4$  pairs), two ( $N = 7$ ), three ( $N = 4$ ), five ( $N$   
228  $= 2$ ), six ( $N = 1$ ), seven ( $N = 2$ ), and 10 ( $N = 1$ ) spawning events were observed in these  
229 continuous pairs. However, in 13 cases, the pair split after one spawning event on the  
230 day of pair formation. In addition to these spawning events, bachelor females, which  
231 had not paired with any male, spawned with paired males in 34 cases, demonstrating  
232 that males could spawn with two females in 1 day. These secondary females approached  
233 males when they spawned but did not establish a continuous pair (Table 1). The males  
234 and females in the 21 continuous reproductive pairs reproduced 70.3% (mean  $\pm$  SD =  
235  $70.3 \pm 4.0$ ,  $N = 21$ ) and 97.2% ( $97.2 \pm 1.8$ ,  $N = 21$ ) of the time within their continuous  
236 pair, respectively.

237 Ten (i.e., M1 on 24, 27 May, 22, 23, 27 June; M2 on 26 May; M6 on 27 May, 25  
238 June; M8 on 26 May, 24 June) and four (M1 on 24, 25 June; M5 on 26 May; M6 on 26  
239 June) cases of additional third and fourth spawning events were observed in males

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240 during the 1–3 days after spawning with a secondary female (Table 1). The males  
241 obtained egg masses in four cases but did not care for all of the eggs, and the outer parts  
242 decomposed as judged by a color change from transparent to white. In these four cases,  
243 the four egg masses were spawned by two or three different females. Meanwhile, a  
244 female that did not spawn cannibalized the eggs in the nests of M1 on 22 June and M6  
245 on 25 June, respectively. The female intruded and picked at the eggs while the nesting  
246 male that was caring for the egg mass was courting another female at a distance from  
247 his nest.

248

249 Reproductive success

250

251 Spawning frequency throughout the observation period (Pearson’s correlation,  $r = -0.25$ ,  
252  $P > 0.1$ ,  $N = 16$ ) and the length of the spawning interval during pair formation  
253 (Pearson’s correlation,  $r = 0.24$ ,  $P > 0.1$ ,  $N = 14$ ) were unrelated to female TL. The  
254 GLMM predicted that female clutch size increased with TL (coefficient = 0.21769, SE =  
255 0.07156,  $Z = 3.042$ ,  $P < 0.01$ ,  $y = \exp [0.21769x - 0.20623]$ ) (Fig. 4). A positive  
256 correlation was detected between the clutch size and TL of females (Pearson’s  
257 correlation,  $r = 0.56$ ,  $P < 0.05$ ,  $N = 16$ ), indicating that reproductive success of females



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258 increased with body size. In contrast, no association was found between TL of males  
259 and the expected number of fertilized eggs throughout the study period (Pearson's  
260 correlation,  $r = -0.51$ ,  $P > 0.05$ ,  $N = 8$ ) (Fig. 5), indicating that males are reproductively  
261 successful regardless of their TL.

263 Aggressive behavior

264  
265 Aggressive behavior by *T. marinae* was divided into the following phases: nest with and  
266 without eggs of a male alone, paired, or spawning. Although nesting males did not  
267 attack spawning females, these males and females exhibited aggressive behavior 178  
268 times against intruding males and bachelor females approaching their nests. In particular,  
269 females only attacked other females. The frequency of aggressive behavior differed  
270 among phases (Fig. 6). More aggressive behaviors were observed by nesting males  
271 against intruding males at nests without eggs than at nests with eggs ( $\chi^2 = 8.1$ ,  $P < 0.01$ ).  
272 Nesting males were more aggressive toward bachelor females at nests with eggs than at  
273 nests without eggs both when the male was alone ( $\chi^2 = 19$ ,  $P < 0.001$ ) and paired ( $\chi^2 =$   
274 47,  $P < 0.001$ ). Meanwhile, the frequency of aggressive behavior by spawned females  
275 against bachelor females did not differ between nests with and without eggs ( $\chi^2 = 3$ ,  $P >$

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276 0.05). A nesting male and spawning female that spawned frequently attacked

277 approaching individuals.

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279 Gonadal structure

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281 The testes in males were filled with spermatozoa, and a developed accessory gonadal

282 structure (AGS; Cole 1990) was attached to the testis (Fig. 7a), indicating that the

283 individual was a functional male. The ovaries in females contained oocytes at various

284 stages of development, but most were vitellogenic, indicating that the individual was a

285 functional female. No females had a precursor AGS (p-AGS) (Fig. 7b). No bisexual

286 gonads, as observed in other *Trimma* species (e.g., Sunobe and Nakazono 1993; Manabe

287 et al. 2008), were observed in either sex.

288 The mean TL of the nine juveniles was  $12.6 \pm 1.7$  mm (range: 10.0–15.5 mm). The

289 gonad of the smallest juvenile consisted of gonial germ cells, indicating no sexual

290 differentiation (Fig. 7c). Meanwhile, the largest juvenile had only ovarian tissue

291 containing primary growth oocytes, indicating that this individual had differentiated into

292 a female (Fig. 7d). Both ovarian and testicular tissues were detected in the other seven

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293 juveniles (range: 10.5–14.0 mm TL) (Fig. 7e, f). The ovarian zone contained only  
294 primary growth oocytes, and the testicular zone contained spermatogonium.

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296 Testing sex change ability

297

298 No spawning occurred during the sex change experiments in either males or females.

299 Histological observations revealed that the gonads of fish in the male and female groups  
300 consisted of only testicular tissue with AGS and oocytes, respectively. These results  
301 show that no sex change occurred.

302

303 **Discussion**

304

305 Barlow (1988) provided two definitions for monogamy: 1) the male and female confine  
306 most of their spawning to the same partner or 2) they remain partners after fertilization  
307 until the young no longer require care. The present observations show that *T. marinae*  
308 tended to establish a continuous reproductive pair and that most spawning occurred in  
309 these pairs (Table 1). This relationship between males and females corresponds to the

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310 first definition of Barlow (1988) and suggests that *T. marinae* has a monogamous  
311 mating system.

312 As males established a spawning territory and females formed aggregations, there  
313 may be a high possibility for polygyny. Why is the mating system of this species  
314 monogamous? The present observations show that females were aggressive toward  
315 bachelor females when the bachelor females approached their partners (Fig. 6). Paternal  
316 care in *T. marinae* might be limited because egg decomposition and cannibalism were  
317 observed. These results indicate that females suffer a cost from polygyny by sharing in  
318 parental care. As *T. marinae* form an aggregation in the wild, there is high potential for  
319 polygyny. Therefore, females may compete to defend their mate (Fig. 6). Male pipefish,  
320 *Corythoichthys haematopterus*, can only accept one clutch in the brood pouch. A female  
321 guards the male as females compete because of a female-biased sex ratio, resulting in  
322 repeated spawning with the same partner (Matsumoto and Yanagisawa 2001). It has also  
323 been reported that the coral goby, *Paragobiodon xanthosomus*, has a monogamous  
324 mating system as a result of female mate guarding and limited care of the eggs by the  
325 male (Wong et al. 2008). Wong et al. (2008) suggested that the monogamous mating  
326 system in group-living fish results from intra-sexual competition for resources among

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327 females. Present observation suggested that the monogamous mating system of *T.*  
328 *marinae* may have evolved for a similar reason.

329 Males were near their nest throughout the day and only attacked other males and both  
330 sexes, except their mate, when the nest was without and with eggs, respectively (Fig. 6).  
331 This observation indicates that males only guard their nest against the same sex and  
332 guard their eggs against both sexes except their mate. Kuwamura (1985) suggested that  
333 egg predation by members of an *Apogon notatus* aggregation and excluding  
334 conspecifics from the reproductive pair are associated with forming a continuous  
335 reproductive pair in this species. We also observed that eggs of *T. marinae* were eaten  
336 by bachelor females that did not spawn. This result shows that members of an  
337 aggregation may be potential egg predators. Therefore, this aggressive behavior may  
338 also contribute to formation of a monogamous mating system because the male drives  
339 away almost all conspecifics except the mate while egg guarding.

340 However, we observed that some males accepted two females on the same day (Table  
341 1), suggesting temporal polygyny. The mating system of the monogamous species  
342 *Oxymonacanthus longirostris* converts to polygyny when the sex ratio becomes slightly  
343 female-biased (Kokita and Nakazono 1998; Kokita 2002). Approximately two times

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344 more females were in our experimental group than males, and females that could not  
345 establish a pair attempted to intrude into nests and spawn.

346         However, we studied the mating system and sex ratio of only one wild aggregation,  
347 so a comparison with more aggregations would lend much stronger support to the  
348 present results.

349         The remarkable feature of the gonadal structures in *T. okinawae*, *T. grammistes*, *T.*  
350 *kudoii*, and *T. yanagitai* is that they are simultaneously composed of ovary, testis, and  
351 AGS in both sexes. Bidirectional sex change has been confirmed by rearing experiments  
352 of these species (Sunobe and Nakazono 1993; Shiobara 2000; Manabe et al. 2008;  
353 Sakurai et al. 2009). In contrast, male and female *T. marinae* had a unisexual gonadal  
354 structure composed of either testis with an AGS or an ovary, respectively (Fig. 7). The  
355 ovarian lumen, which is a typical characteristic of protogynous fish (Sadovy and  
356 Shapiro 1987), was not detected in testes. In addition, the p-AGS, a unique feature  
357 confirmed in protogynous gobiid fish (Cole 1988; Cole et al. 1994; Cole 2010), was not  
358 confirmed in female *T. marinae*. The 63-day rearing experiment showed that the *T.*  
359 *marinae* male–male and female–female groups did not change sex, indicating that this  
360 species is gonochoristic.

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361 If reproductive success is equal between males and females of each size class,  
362 gonochorism may be favored according to the SA model (Warner 1984). Some  
363 group-spawning groupers are gonochoristic because reproductive success increases  
364 similarly in males and females as size increases (Erisman et al. 2009). The present  
365 results show that the reproductive success of females was positively correlated with  
366 their body size, whereas no difference in reproductive success was observed among  
367 males of different sizes (Fig. 4) because females may not choose their mate based on  
368 body size (Fig. 3). These results reveal the conditions for the evolution of protandry  
369 according to the SA model. Several explanations may explain why *T. marinae* is  
370 gonochoristic. First, the easy accessibility of new mates may be why this species is  
371 gonochoristic. The frequency of sex change in the hermaphroditic damselfish, *Dascyllus*  
372 *aruanus*, is higher in small isolated groups than that in a large aggregation (Asoh 2003;  
373 Erisman et al. 2013). This difference between groups may be affected by finding a new  
374 mate in both sexes. If individuals can easily form a new reproductive pair, they will not  
375 suffer from the cost of changing sex. Males in a monogamous *T. marinae* pair that  
376 breaks up can easily mate with a new female, as this species forms aggregations. Then,  
377 gonochorism may be favored as described above. Second, *T. marinae* may suffer some  
378 cost for sex change. Charnov (1982, 1986) suggested that sex change is not always

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3 379 favored if it is too costly to change sex. The gobiid fish *T. nasa* is closely related to *T.*  
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6 380 *marinae* (Winterbottom 2005) and has an extremely short lifespan of 87.5 days  
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9 381 (Winterbottom and Southcott 2008), which is shorter than the 140-day lifespan of the  
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12 382 hermaphroditic species *T. benjamini* (Sunobe unpubl. data) (Winterbottom et al. 2011).  
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16 383 If *T. marinae* live as long as *T. nasa*, the time spent changing sex during reproductive  
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19 384 periods may be a large cost. Thus, gonochorism may be adaptive in this species. A  
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22 385 further comparative investigation of lifespan among hermaphroditic fishes will confirm  
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25 386 this hypothesis. Third, as *T. marinae* is sexually size dimorphic, there is a possibility  
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28 387 that the sex-specific growth rate or the sex determination mechanism may have led *T.*  
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31 388 *marinae* to be gonochoristic. *T. marinae* had a bisexual gonad only at the juvenile stage.  
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35 389 Some gonochoristic fishes differentiate into males or females during a bisexual juvenile  
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38 390 phase (e.g., Takahashi 1977; Asoh and Shapiro 1997; Devlin and Nagahama 2002;  
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41 391 Erisman et al. 2008). Low and high growth rates during early development of *Danio*  
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44 392 *rerio* induce an individual to become male and female, respectively, because a larger  
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47 393 body size may be more advantageous to fecundity of females than that of males  
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51 394 (Lawrence et al. 2008). A larger female and smaller male in a monogamous *T. marinae*  
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54 395 pair achieve greater reproductive success than vice versa. As females are larger than  
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57 396 males (Fig. 1), there is a possibility that the difference in growth rate determines sexual



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397 differentiation in this species. In contrast, a difference in the sex-specific growth rate  
398 after sex determination would also explain gonochorism and sexual size dimorphism in  
399 *T. marinae*. The SA model and mortality-advantage and growth-rate-advantage  
400 hypotheses explain the evolution of sex change (Ghiselin 1969; Warner 1975; Charnov  
401 1982; Iwasa 1991). These hypotheses suggest that sex change from the faster-growing  
402 to the slower-growing sex is favored if there is a difference in growth rate related to sex  
403 (Charnov 1986; Iwasa 1991). Therefore, *T. marinae* should favor the evolution of  
404 protogynous sex change because females grow faster than males. This effect and the  
405 size-advantage of reproduction in this species (which favors protandry) may work in  
406 opposite ways to cancel each other out. Nevertheless, this species is gonochoristic, and  
407 the present results of reproductive success predict the evolution of protandrous sex  
408 change.

409

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**Table 1** *Trimma marinae* spawning pairs during the rearing experiment. *M*, individual identity of males; *F*, individual identity of females; *Underlined*

*individual identity*, spawned female; *Surrounded individual identity*, females that mated temporary with males and did not establish a continuous pair. F6 died

on 11 June

ID	May								June																															
	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28			
M1	<u>F8</u>	<u>F8</u>	F8	<u>F8</u>	<u>F8</u>	F8	F8	F8	F8	F8	F8	F8	F8	<u>F8</u>	F8	F8	F8	F8	F16	F16	F16	F16	F16	F16	<u>F16</u>		F14	F14	F14	F14	<u>F14</u>	<u>F14</u>	<u>F14</u>	<u>F14</u>	F14	F14	F14	F14		
	<u>F2</u>			<u>F1</u>																					<u>F8</u>			<u>F5</u>		<u>F1</u>	<u>F8</u>		<u>F12</u>							
M2		<u>F5</u>	F5	<u>F5</u>	<u>F5</u>	F5		F16	F16	<u>F16</u>	F16	F11	<u>F11</u>	F11	F11	F11	F11	F11	F11	<u>F11</u>	F11	F11	F11	F11	<u>F11</u>	F11	F11	<u>F11</u>	F11	F11	F11	<u>F11</u>	F11	F11	F11	F11	F11	F11		
		<u>F11</u>																													<u>F12</u>		<u>F12</u>							
M3	F3	<u>F3</u>	F3	<u>F3</u>	<u>F3</u>	F2	F2	F2	F2	<u>F2</u>	F2	F2	F2	<u>F2</u>	F2	F2	F2	F2	F2	F2	F2	<u>F2</u>	F2	F2	<u>F2</u>	F2	F2	F2	F2	F2	F2	F2	<u>F2</u>	F2	F2	F2	F2	F2	F2	
																	<u>F1</u>			<u>F1</u>						<u>F5</u>		<u>F5</u>	<u>F5</u>		<u>F5</u>									
M4		F12	F12	F12	<u>F12</u>	F12	F12	F12	F12	F12	F12	F12	F12	F12	F12	F12	<u>F12</u>				<u>F12</u>						F4	F4	F4	F4	F4	F4	F4	<u>F4</u>			<u>F15</u>			
																	<u>F5</u>															<u>F8</u>								
M5		<u>F9</u>	F9	<u>F9</u>		<u>F6</u>		F17	F17	F17	F17	F17	F17	F17	F17	F17	<u>F17</u>	F17	F17	F17	<u>F17</u>	F17	F17	F17	<u>F17</u>	F17	F17	F17	F17	F17	F17	<u>F17</u>	F17	F16	F16	<u>F16</u>	F16	F16	F16	
		<u>F2</u>		<u>F14</u>													<u>F9</u>			<u>F9</u>				<u>F9</u>						<u>F5</u>								<u>F5</u>		
M6	<u>F7</u>	F7	F7	<u>F7</u>	<u>F7</u>	F7	F7	F7	F7	F7			<u>F9</u>				<u>F15</u>			<u>F8</u>	<u>F15</u>	F15	F15	<u>F15</u>		F10	<u>F10</u>		<u>F15</u>	<u>F9</u>		<u>F10</u>	<u>F10</u>	<u>F4</u>						
				<u>F2</u>	<u>F13</u>				<u>F10</u>																								<u>F9</u>							
M7		<u>F3</u>			<u>F17</u>	<u>F14</u>			<u>F6</u>	F6	F6	F6	F6	<u>F6</u>	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	F6	
														<u>F7</u>												<u>F7</u>	F7	F7	F7	<u>F7</u>	F7	F7	F7	F7	F7	F7	F7	F7	F7	F7
M8	F16	<u>F16</u>	<u>F16</u>	<u>F16</u>	<u>F16</u>	<u>F16</u>		<u>F9</u>		<u>F3</u>	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	F3	
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551 **Figure legends**

552

553 **Fig. 1** Size distributions of male and female *Trimma marinae* in the study group ( $N =$   
554 41)

555

556 **Fig. 2** Frequency of spawning and spawning time of *Trimma marinae* from 24 April to  
557 22 May 2014 ( $N = 60$ )

558

559 **Fig. 3** Relationship between sizes of males and females in all continuous pairs of  
560 *Trimma marinae* ( $N = 21$ ). *Symbol size* represents the sample size

561

562 **Fig. 4** Relationship between clutch size and total length (TL) of female *Trimma marinae*  
563 ( $N = 16$ ). *Solid curve* obtained with the generalized linear mixed-effects model

564

565 **Fig. 5** Estimate of *Trimma marinae* male mating success ( $N = 8$ )

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567 **Fig. 6** Frequency of aggressive behavior by *Trimma marinae* when the male and female  
568 were paired or the male remained alone in their nest. **a** Frequency that nesting males

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569 attacked an intruding male. **b** Frequency that nesting males attacked bachelor females. **c**  
570 Frequency that mated females attacked bachelor females. Chi-square test for deviation  
571 from equality:  $**P < 0.01$ ;  $***P < 0.001$   
572  
573 **Fig. 7** *Trimma marinae* gonadal cross-sections. **a** Gonad of mature male. (*Bar* = 0.1  
574 mm). **b** Gonad of mature female. (*Bar* = 0.1 mm). **c** Gonad of the smallest immature  
575 individual (*Bar* = 0.03 mm). **d** Gonad of the largest immature individual (*Bar* = 0.03  
576 mm). **e** Intersexual gonad of juvenile. (*Bar* = 0.03 mm). **f** Enlarged picture of Fig. 5e  
577 (*Bar* = 0.03 mm). *T*, testicular tissues; *O*, ovarian tissues; *AGS*, accessory gonadal  
578 structure; *G*, gonial germ cells  
579

Fig. 1

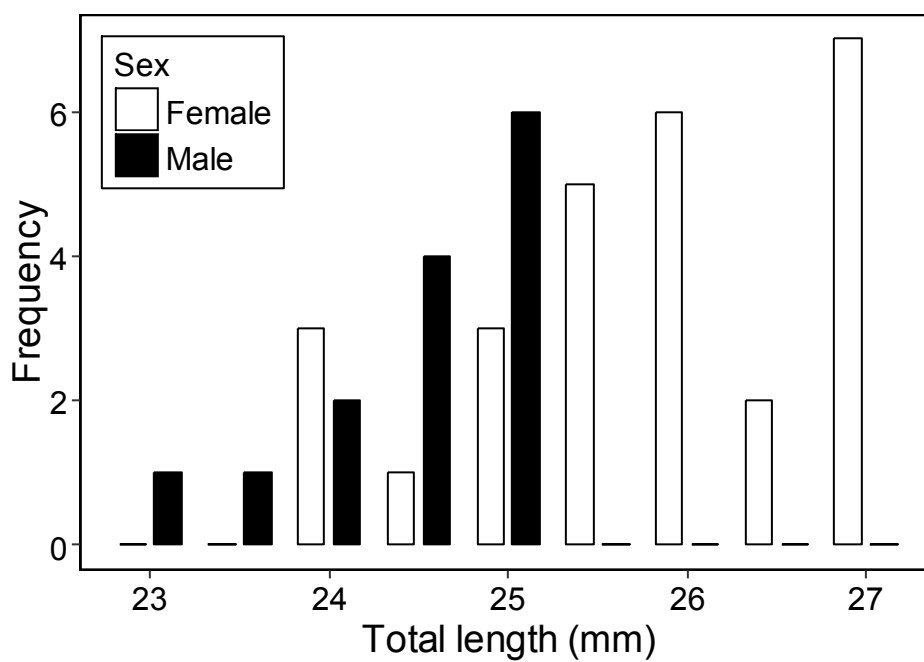


Fig. 2

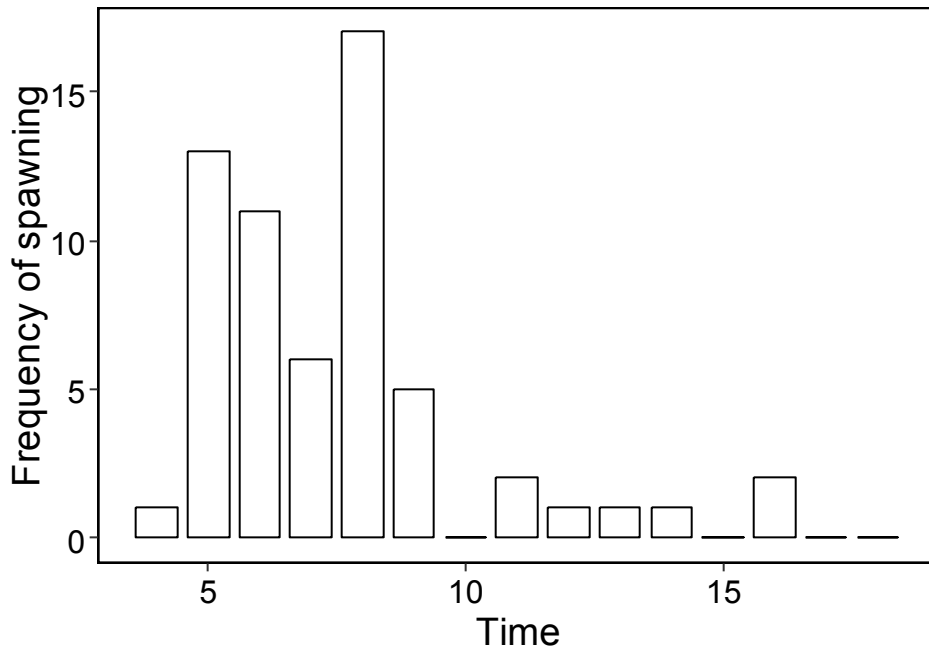


Fig. 3

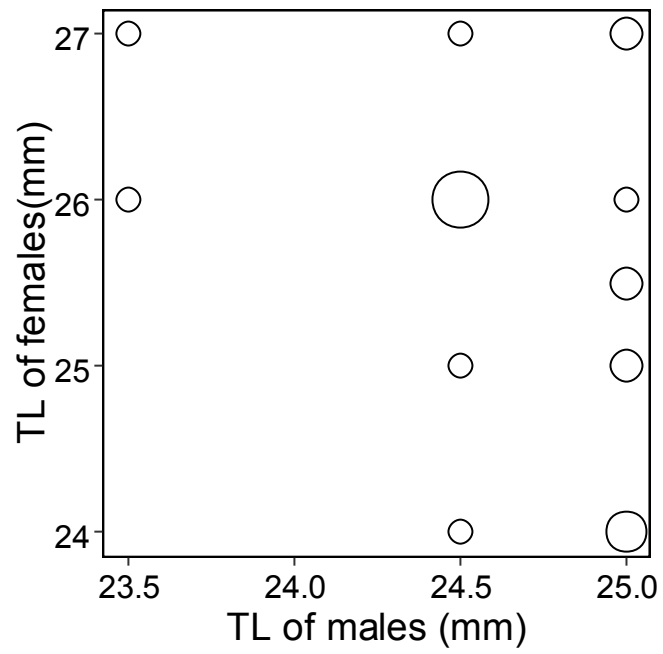




Fig. 4

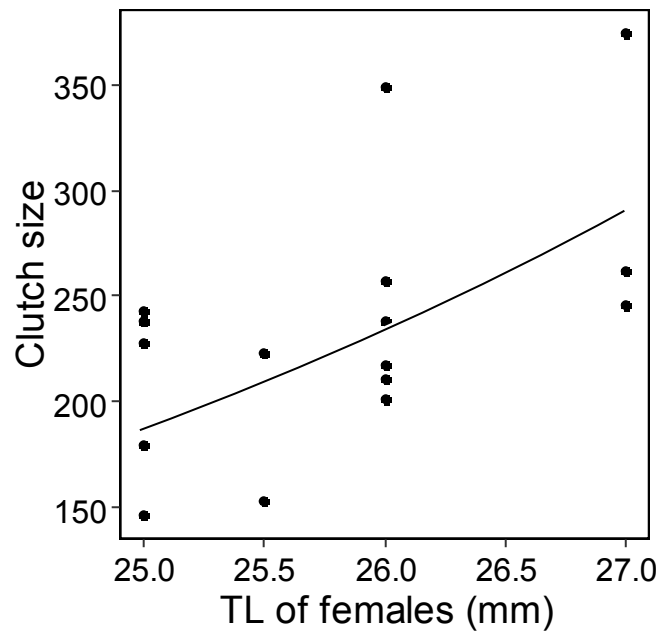


Fig. 5

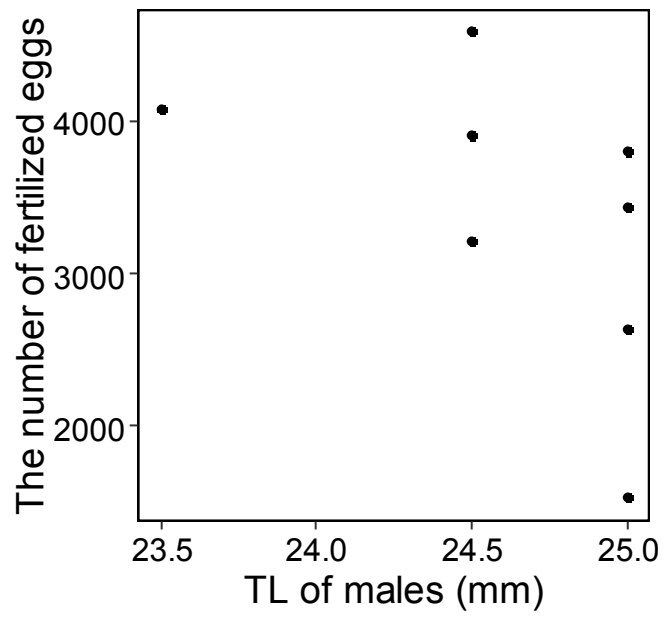


Fig. 6



Fig. 7

