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

著者	福田 和也, Hisaya Manabe, Makoto Sakurai, Shin-ichi Dewa, Akihiko Shinomiya, 須之部 友基
journal or publication title	Journal of Ethology
volume	35
number	1
page range	121-130
year	2017-01
科学研究費研究課題	魚類における配偶システムの系統進化と脳内神経葉ホルモン遺伝子に関する研究
研究課題番号	16K07507
URL	http://id.nii.ac.jp/1342/00001581/

doi: 10.1007/s10164-016-0499-z

1 Monogamous mating system and sexuality in the gobiid fish, *Trimma marinae*

2 (Actinopterygii: Gobiidae)

3

4 Kazuya Fukuda · Hisaya Manabe · Makoto Sakurai · Shin-ichi Dewa · Akihiko

5 Shinomiya · Tomoki Sunobe

6

7 Text: 36 pages

8 Tables: 1

9 Figures: 7

10

11 Kazuya Fukuda (✉) · Tomoki Sunobe

12 Laboratory of Fish Behavioral Ecology, Tateyama Station, Field Science Center, Tokyo

13 University of Marine Science and Technology, 670 Banda, Tateyama 294-0308, Japan

14 E-mail: kazuya.f47@gmail.com; Tel: +81-470-29-1144; Fax: +81-470-29-1145

15

16 Hisaya Manabe

17 Education Center, Kagoshima University, 1-21-30 Korimoto, Kagoshima 890-0065,

18 Japan

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
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 19 Makoto Sakurai
- 20 Kagoshima Immaculate Heart College, 4-22-1 Toso, Kagoshima 890-8525, Japan
- 21
- 22 Shin-ichi Dewa
- 23 Diving Service Umi-Annai, 7-7 Masagohonmachi, Kagoshima 890-0067, Japan
- 24
- 25 Akihiko Shinomiya
- 26 Faculty of Fisheries, Kagoshima University, Kagoshima 890-0056, Japan
- 27

Abstract

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

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

Mating system

44 Introduction

45
46 Mating systems are generally determined by the distribution of resources (e.g., food,
47 mating sites, hiding place, and/or mates). There may be little potential for polygamy
48 under conditions of evenly distributed resources, resulting in monogamy (Emlen and
49 Oring 1977; Davies et al. 2012). Barlow (1988) defined a pair as monogamous if 1) the
50 male and female confined most of their spawning to the same partner or 2) they
51 remained partners after fertilization until the young no longer required care. They
52 concluded that the necessity for biparental care may be the main driving force for the
53 evolution of monogamy. Whiteman and Côté (2004) reviewed reports on monogamous
54 marine fish and found that the evolutionary factors driving monogamy are not limited to
55 biparental care. They proposed six hypotheses for the evolution of monogamy: 1)
56 biparental care; 2) habitat limitations; 3) low population density/low mate
57 availability/low mobility; 4) increased reproductive efficiency; 5) territorial defense;
58 and 6) net benefit of sequestering a single mate.

59 The family Gobiidae contains a large number of species among teleosts (Nelson et al.
60 2016), and some monogamous species belong to this taxon (e.g., Kuwamura et al. 1993;
61 Nakashima et al. 1996; Sunobe and Nakazono 1999; Munday et al. 2002). Among them,

hermaphroditic species have been reported in the genera *Gobiodon*, *Paragobiodon*,
Priorepis (Sadovy de Mitcheson and Liu 2008), and *Bryaninops* (Munday et al. 2002).
It has been suggested that these species reproduce monogamously because of low
mobility, low mate availability, or intra-specific competition related to resources in their
specific habitats (Kuwamura et al. 1993; Nakashima et al. 1996; Sunobe and Nakazono
1999; Munday et al. 2002; Whiteman and Côté 2004; Wong et al. 2008). These
ecological and social characteristics have also been considered to facilitate the evolution
of sex change in these species (Nakashima et al. 1995; Munday et al. 1998; Munday
2002; Manabe et al. 2013). Therefore, revealing the mechanisms leading to various
mating systems is important when examining the evolution of hermaphroditism.

Sex change or sequential hermaphroditism among teleosts, such as protogyny
(female-to-male sex change) and protandry (male-to-female sex change), has been well
documented. The size-advantage (SA) model predicts evolution of protogyny when
species are polygynous because large males obtain remarkably greater benefit than
small males through intense mating competition and mate choice by females.

Meanwhile, the evolution of protandry is favored when males reproduce and are
reproductively successful regardless of their body size (Ghiselin 1969; Warner 1975;
Warner 1984; Kuwamura and Nakashima 1998; Munday et al. 2006).

1
2
3 80 In addition to these types of sex change mechanisms, bidirectional sex change has
4
5
6 81 been reported in Gobiidae, Serranidae, Pseudochromidae, Pomacanthidae, Cirrhitidae,
7
8
9 82 Labridae, and Pomacentridae (Munday et al. 2010; Kuwamura et al. 2016). In particular,
10
11
12 83 the gobiid genus *Trimma* has been studied to clarify the adaptive significance of
13
14
15 84 bidirectional sex change (Sunobe and Nakazono 1993; Manabe et al. 2007; Manabe et al.
16
17
18 85 2008; Sakurai et al. 2009). This genus contains 92 valid species distributed on rocky
19
20
21 86 ledges and coral reefs in temperate to tropical waters of the Indo-Pacific Ocean (Suzuki
22
23
24 87 et al. 2012; Winterbottom et al. 2015). Gonadal histological observations of *T.*
25
26
27 88 *grammistes*, *T. kudo*, *T. okinawae*, *T. unisquamis*, and *T. yanagitai* show that ovarian
28
29
30 89 and testicular tissues are present simultaneously (Cole 1990; Manabe et al. 2007;
31
32
33 90 Sunobe and Nakazono 1993; Sakurai et al. 2009; Shiobara 2000). Bidirectional sex
34
35
36 91 change has been confirmed in these species by field observations or rearing experiments,
37
38
39 92 except in *T. unisquamis*, and these species may be polygynous (Manabe et al. 2007;
40
41
42 93 Munday et al. 2010; Sakurai et al. 2009; Shiobara 2000). Except in *T. kudo*, the largest
43
44
45 94 female changes to male when the dominant male disappears, and the smaller individual
46
47
48 95 of a male–male pair changes sex to female (Sunobe and Nakazono 1993; Manabe et al.
49
50
51 96 2007; Manabe et al. 2008; Sakurai et al. 2009; Shiobara 2000).
52
53
54
55
56
57
58
59
60
61
62
63
64
65

The present study species, *T. marinae*, reaches a total length (TL) of 25 mm and is distributed in the western Pacific Ocean where it forms aggregations on the coral-reef slopes of enclosed bays at depths of 5–35 m (Shibukawa 2004). In this study, we show the monogamous mating system and gonochorism of this species in rearing experiments and perform a gonadal histological examination. Then, we discuss the adaptive aspects of the mating system and how gonochorism evolves among species with bidirectional sex change.

Materials and Methods

Specimen collection

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

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

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

Observations of reproductive behavior

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

in length) were cut in half and placed on the substratum as a spawning nest. We set a waterproof sheet inside the pipes to count the number of spawned eggs. We removed the sheet when the fish had spawned and counted the number of eggs using a microscope.

Fish were fed formula food and live *Artemia* nauplii twice daily.

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

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

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

Estimates of reproductive success

1
2
3 151 To examine the effects of body size on the reproductive success of females, we used a
4
5
6 152 generalized linear mixed-effects model (GLMM; R package lme4). We included clutch
7
8
9 153 size and TL of the female as the response variable and fixed effect, respectively. As
10
11
12 154 clutches were sampled several times from an individual, we set individual identity as a
13
14
15 155 random effect term in the GLMM. The response variable was modelled with a Poisson
16
17
18 156 distribution and the log link function.

19
20
21
22 157 We investigated the relationship between female body size and spawning frequency
23
24
25 158 to examine the size-dependent advantage related to reproduction in females from 23
26
27
28 159 May to 28 June 2014. We recorded the individual identity of females that spawned and
29
30
31 160 counted spawning frequency and the intervals.

32
33
34
35 161 We also examined the relationship between body size and reproductive success in
36
37
38 162 males. We recorded the spawning frequency of each male and the individual identity of
39
40
41 163 spawned females. Clutch size of spawned females was estimated from the above model
42
43
44 164 using their TL. Then, we derived the expected number of fertilized eggs for each male
45
46
47 165 to define reproductive success throughout the study period.

48
49
50
51 166
52
53
54 167 Rearing experiment to assess sex change

55
56
57 168

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

Gonadal histology

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

Results

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
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

187

188 Size distribution of the sexes and the sex ratio

189

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

191 and females (25.8 ± 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

196

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.

201

202 Mating system

203

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 ≥ 2 days, regardless of whether they had spawned,
 221 we regarded it as maintaining a pair bond. No association was observed between male

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

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

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

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

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

Aggressive behavior

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

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

Gonadal structure

The testes in males were filled with spermatozoa, and a developed accessory gonadal
structure (AGS; Cole 1990) was attached to the testis (Fig. 7a), indicating that the
individual was a functional male. The ovaries in females contained oocytes at various
stages of development, but most were vitellogenic, indicating that the individual was a
functional female. No females had a precursor AGS (p-AGS) (Fig. 7b). No bisexual
gonads, as observed in other *Trimma* species (e.g., Sunobe and Nakazono 1993; Manabe
et al. 2008), were observed in either sex.

The mean TL of the nine juveniles was 12.6 ± 1.7 mm (range: 10.0–15.5 mm). The
gonad of the smallest juvenile consisted of gonial germ cells, indicating no sexual
differentiation (Fig. 7c). Meanwhile, the largest juvenile had only ovarian tissue
containing primary growth oocytes, indicating that this individual had differentiated into
a female (Fig. 7d). Both ovarian and testicular tissues were detected in the other seven

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.
 295
 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

1
2
3 310 first definition of Barlow (1988) and suggests that *T. marinae* has a monogamous
4
5
6 311 mating system.
7
8

9 312 As males established a spawning territory and females formed aggregations, there
10
11
12 313 may be a high possibility for polygyny. Why is the mating system of this species
13
14
15 314 monogamous? The present observations show that females were aggressive toward
16
17
18 315 bachelor females when the bachelor females approached their partners (Fig. 6). Paternal
19
20
21 316 care in *T. marinae* might be limited because egg decomposition and cannibalism were
22
23
24 317 observed. These results indicate that females suffer a cost from polygyny by sharing in
25
26
27 318 parental care. As *T. marinae* form an aggregation in the wild, there is high potential for
28
29
30 319 polygyny. Therefore, females may compete to defend their mate (Fig. 6). Male pipefish,
31
32
33 320 *Corythoichthys haematopterus*, can only accept one clutch in the brood pouch. A female
34
35
36 321 guards the male as females compete because of a female-biased sex ratio, resulting in
37
38
39 322 repeated spawning with the same partner (Matsumoto and Yanagisawa 2001). It has also
40
41
42 323 been reported that the coral goby, *Paragobiodon xanthosomus*, has a monogamous
43
44
45 324 mating system as a result of female mate guarding and limited care of the eggs by the
46
47
48 325 male (Wong et al. 2008). Wong et al. (2008) suggested that the monogamous mating
49
50
51 326 system in group-living fish results from intra-sexual competition for resources among
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 327 females. Present observation suggested that the monogamous mating system of *T.*

4
5
6 328 *marinae* may have evolved for a similar reason.

7
8
9 329 Males were near their nest throughout the day and only attacked other males and both

10
11
12 330 sexes, except their mate, when the nest was without and with eggs, respectively (Fig. 6).

13
14
15 331 This observation indicates that males only guard their nest against the same sex and

16
17
18 332 guard their eggs against both sexes except their mate. Kuwamura (1985) suggested that

19
20
21 333 egg predation by members of an *Apogon notatus* aggregation and excluding

22
23
24 334 conspecifics from the reproductive pair are associated with forming a continuous

25
26
27 335 reproductive pair in this species. We also observed that eggs of *T. marinae* were eaten

28
29
30 336 by bachelor females that did not spawn. This result shows that members of an

31
32
33 337 aggregation may be potential egg predators. Therefore, this aggressive behavior may

34
35
36 338 also contribute to formation of a monogamous mating system because the male drives

37
38
39 339 away almost all conspecifics except the mate while egg guarding.

40
41
42 340 However, we observed that some males accepted two females on the same day (Table

43
44
45 341 1), suggesting temporal polygyny. The mating system of the monogamous species

46
47
48 342 *Oxymonacanthus longirostris* converts to polygyny when the sex ratio becomes slightly

49
50
51 343 female-biased (Kokita and Nakazono 1998; Kokita 2002). Approximately two times

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

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

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

1
2
3 361 If reproductive success is equal between males and females of each size class,
4
5
6 362 gonochorism may be favored according to the SA model (Warner 1984). Some
7
8
9 363 group-spawning groupers are gonochoristic because reproductive success increases
10
11
12 364 similarly in males and females as size increases (Erisman et al. 2009). The present
13
14
15 365 results show that the reproductive success of females was positively correlated with
16
17
18 366 their body size, whereas no difference in reproductive success was observed among
19
20
21 367 males of different sizes (Fig. 4) because females may not choose their mate based on
22
23
24 368 body size (Fig. 3). These results reveal the conditions for the evolution of protandry
25
26
27 369 according to the SA model. Several explanations may explain why *T. marinae* is
28
29
30 370 gonochoristic. First, the easy accessibility of new mates may be why this species is
31
32
33 371 gonochoristic. The frequency of sex change in the hermaphroditic damselfish, *Dascyllus*
34
35
36 372 *aruanus*, is higher in small isolated groups than that in a large aggregation (Asoh 2003;
37
38
39 373 Erisman et al. 2013). This difference between groups may be affected by finding a new
40
41
42 374 mate in both sexes. If individuals can easily form a new reproductive pair, they will not
43
44
45 375 suffer from the cost of changing sex. Males in a monogamous *T. marinae* pair that
46
47
48 376 breaks up can easily mate with a new female, as this species forms aggregations. Then,
49
50
51 377 gonochorism may be favored as described above. Second, *T. marinae* may suffer some
52
53
54 378 cost for sex change. Charnov (1982, 1986) suggested that sex change is not always

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

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

Acknowledgments

We are grateful to H. Nogiwa, T. Shitamitu, M. Kasai, and other members of the Laboratory of Fish Behavioral Ecology, Tokyo University of Marine Science and Technology for their advice and support during our study. Critical reading by S. Awata

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
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

415 and two anonymous reviewers led to significant improvements in the manuscript.

416 Thanks are also due to M. Masuko, S. Yokoyama, and T. Momii for their assistance

417 during the study. This study was supported by JSPS KAKENHI Grants to T.S. (no.

418 19570016 and 24370006) from the Japan Society for the Promotion of Science.

419

420

References

- Asoh K (2003) Gonadal development and infrequent sex change in a population of the humbug damselfish, *Dascyllus aruanus*, in continuous coral-cover habitat. Mar Biol 142:1207-1218
- Asoh K, Shapiro DY (1997) Bisexual juvenile gonad and gonochorism in the fairy basslet, *Gramma loreto*. Copeia 1997:22-31
- Barlow GW (1988) Monogamy in relation to resource. In: Slobodchikoff CN (ed.) The ecology of social behavior. Academic Press, New York, pp55-79
- Charnov EL (1982) The theory of sex allocation. Princeton University Press, Princeton
- Charnov EL (1986) Size advantage may not always favor sex change. J Theor Biol 119: 283-285
- Cole KS (1988) Predicting the potential for sex-change on the basis of ovarian structure in gobiid fishes. Copeia 1988: 1082-1086
- Cole KS (1990) Patterns of gonad structure in hermaphroditic gobies (Teleostei: Gobiidae). Environ Biol Fish 28:125-142

- 1
2
3 437 Cole KS (2010) Gonad morphology in hermaphroditic gobies. In: Cole KS (ed.)
4
5
6 438 Reproduction and sexuality in marine fishes: Patterns and processes. University of
7
8
9 439 California Press, Berkley and Los Angeles, California, pp 117-162
10
11
12 440 Cole KS, Robertson DR, Cedeno AA (1994) Does gonad structure reflect sexual pattern
13
14
15 441 in all gobiid fishes? Environ Biol Fish 41: 301-309
16
17
18 442 Davies NB, Krebs JR, West SA (2012) Mating systems. In: Davies NB, Krebs JR, West
19
20
21 443 SA (eds.) An introduction to behavioural ecology. Fourth edition. Wiley-Blackwell,
22
23
24 444 pp254-281
25
26
27 445 Devlin RH, Nagahama Y (2002) Sex determination and differentiation in fish: an
28
29
30 446 overview of genetic, physiological, and environmental influences. Aquaculture
31
32
33 447 208:191-364
34
35
36 448 Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating
37
38
39 449 system. Science 197:215-223
40
41
42 450 Erisman BE, Craig MT, Hastings PA (2009) A phylogenetic test of the size-advantage
43
44
45 451 model: evolutionary changes in mating behavior influence the loss of sex change in
46
47
48 452 a fish lineage. Am Nat 174:83-99
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 453 Erisman BE, Petersen CW, Hasting PA, Warner RR (2013) Phylogenetic perspectives on
4
5
6 454 the evolution of functional hermaphroditism in teleost fishes. Integr Comp Biol
7
8
9 455 53:736-754
10
11
12 456 Erisman BE, Rosales-Casián JA, Hastings PA (2008) Evidence of gonochorism in a
13
14
15 457 grouper, *Mycteroperca rosacea*, from the Gulf of California, Mexico. Environ Biol
16
17
18 458 Fish 82:23-33
19
20
21
22 459 Ghiselin MT (1969) The evolution of hermaphroditism among animals. Q Rev Biol
23
24
25 460 44:189-208
26
27
28 461 Iwasa Y (1991) Sex change evolution and cost of reproduction. Behav Ecol 2: 56-68
29
30
31 462 Kokita T (2002) The role of female behaviour in maintaining monogamy of a coral reef
32
33
34 463 filefish. Ethology 108: 157-168
35
36
37 464 Kokita T, Nakazono A (1998) Plasticity in the Mating System of the Longnose Filefish,
38
39
40 465 *Oxymonacanthus longirostris*, in Relation to Mate Availability. J Ethol 16:81-89
41
42
43 466 Kuwamura T (1985) Social and reproductive behavior of three mouthbrooding
44
45
46 467 cardinalfishes, *Apogon doederleini*, *A. niger* and *A. notatus*. Environ Biol Fish 13:
47
48
49 468 17-24
50
51
52 469 Kuwamura T, Nakashima Y (1998) New aspects of sex change among reef fishes: recent
53
54
55 470 studies in Japan. Environ Biol Fish 52:125-135
56
57
58

- 1
2
3 471 Kuwamura T, Suzuki S, Kadota T (2016) Male-to-female sex change in widowed males
4
5
6 472 of the protogynous damselfish *Dascyllus aruanus*. J Ethol 34: 85-88
7
8
9 473 Kuwamura T, Yogo Y, Nakashima Y (1993) Size-assortative monogamy and paternal
10
11
12
13 474 egg care in a coral goby *Paragobiodon echinocephalus*. Ethology 95:65-75
14
15
16 475 Lawrence C, Ebersole JP, Kesseli RV (2008) Rapid growth and out-crossing promote
17
18
19 476 female development in zebrafish (*Danio rerio*). Environ Biol Fish 81:239-246
20
21
22 477 Manabe H, Ishimura M, Shinomiya A, Sunobe T (2007) Field evidence for
23
24
25 478 bi-directional sex change in the polygynous gobiid fish *Trimma okinawae*. J Fish
26
27
28
29 479 Biol 70:600-609
30
31
32 480 Manabe H, Matsuoka M, Goto K, Dewa S, Shinomiya A, Sakurai M, Sunobe T (2008)
33
34
35 481 Bi-directional sex change in the gobiid fish *Trimma* sp.: does size-advantage exist?
36
37
38 482 Behaviour 145:99-113
39
40
41 483 Manabe H, Toyoda K, Nagamoto K, Dewa S, Sakurai M, Hagiwara K, Shinomiya A,
42
43
44 484 Sunobe T (2013) Bidirectional sex change in seven species of *Priolepis*
45
46
47
48 485 (Actinopterygii: Gobiidae). Bulletin of Marine Science 89:635-642
49
50
51 486 Matsumoto K, Yanagisawa Y (2001) Monogamy and sex role reversal in the pipefish
52
53
54 487 *Corythoichthys Haematopterus*. Anim Behav 61:163-170
55
56
57
58
59
60
61
62
63
64
65

- 1
2
3 488 Munday PL (2002) Bi-directional sex change: testing the growth-rate advantage model.
4
5
6 489 Behav Ecol Sociobiol 52: 247-254
7
8
9 490 Munday PL, Caley MJ, Jones GP (1998) Bi-directional sex change in a coral-dwelling
10
11
12 491 goby. Behav Ecol Sociobiol 43:371-377
13
14
15 492 Munday PL, Buston PM, Warner RR (2006) Diversity and flexibility of sex-change
16
17
18 493 strategies in animals. Trends in Ecol Evol 21:89-95
19
20
21 494 Munday PL, Kuwamura T, Kroon FJ (2010) Bidirectional sex change in marine fishes.
22
23
24 495 In: Cole KS (ed.) Reproduction and sexuality in marine fishes: Patterns and
25
26
27 496 processes. University of California Press, Berkley and Los Angeles, California,
28
29
30 497 pp241-271
31
32
33 498 Munday PL, Pierce SJ, Jones GP, Larson (2002) Habitat use, social organization and
34
35
36 499 reproductive biology of the seawhip goby, *Bryaninops yongei*. Marine and
37
38
39 500 Freshwater Research 53: 769-775
40
41
42 501 Nakashima Y, Kuwamura T, Yogo Y (1995) Why be a both-ways sex changer. Ethology
43
44
45 502 101:301-307
46
47
48 503 Nelson JS, Grande TC, Wilson MV (2016) Fishes of the World. John Willey & Sons,
49
50
51 504 New Jersey
52
53
54
55
56
57
58
59
60
61
62
63
64
65

- 1
- 2
- 3 505 Sadovy Y, Shapiro, DY (1987) Criteria for the diagnosis of hermaphroditism in fishes.
- 4
- 5
- 6 506 Copeia 1987:136-156
- 7
- 8
- 9
- 10 507 Sadovy de Mitcheson Y, Liu M (2008) Functional hermaphroditism in teleosts. Fish
- 11
- 12
- 13 508 (Oxf) 9:1-43
- 14
- 15
- 16 509 Sakurai M, Nakakoji S, Manabe H, Dewa S, Shinomiya A, Sunobe T (2009)
- 17
- 18
- 19 510 Bi-directional sex change and gonad structure in the gobiid fish *Trimma yanagitai*.
- 20
- 21
- 22 511 Ichthyol Res 56:82-86
- 23
- 24
- 25 512 Shiobara Y (2000) Reproduction and hermaphroditism of the gobiid fish, *Trimma*
- 26
- 27
- 28 513 *grammistes*, from Suruga Bay, central Japan. Sci Rep Mus Tokai Univ 2:19-30
- 29
- 30
- 31 514 Sunobe T, Nakazono A (1990) Polygynous mating system of *Trimma okinawae* (Pisces:
- 32
- 33
- 34 515 Gobiidae) at Kagoshima, Japan with a note on sex change. Ethology 84:133-143
- 36
- 37
- 38 516 Sunobe T, Nakazono A (1993) Sex change in both directions by alteration of social
- 39
- 40
- 41 517 dominance in *Trimma okinawae* (Pisces: Gobiidae). Ethology 94:339-345
- 43
- 44 518 Sunobe T, Nakazono A (1999) Mating system and hermaphroditism in the gobiid fish,
- 45
- 46 519 *Priolepis cincta*, at Kagoshima, Japan. Ichthyol Res 46:103-105
- 47
- 48
- 49
- 50 520 Shibukawa K (2004) Genus *Trimma*. In: Senou H (ed.) A photographic guide to the
- 51
- 52
- 53 521 gobiid fishes of Japan. Heibonsha, Tokyo, pp96-117
- 54
- 55
- 56
- 57
- 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65

- 1
- 2
- 3 522 Suzuki T, Sakaue J, Senou H (2012) Two new species of the gobiid fish genus *Trimma*
- 4
- 5
- 6 523 (Actinopterygii: Perciformes: Gobiidae) from Japan and Palau. Bull Natl Mus Nat
- 7
- 8
- 9 524 Sci, Ser A, Suppl 6:67-77
- 10
- 11
- 12 525 Takahashi H (1977) Juvenile hermaphroditism in the Zebrafish, *Brachydanio rerio*. Bull
- 13
- 14
- 15 526 Fac Fish Hokkaido Univ 28:57-65
- 16
- 17
- 18
- 19 527 Warner RR (1975) The adaptive significance of sequential hermaphroditism in animals.
- 20
- 21
- 22 528 Am Nat 109:61-82
- 23
- 24
- 25 529 Warner RR (1984) Mating behavior and hermaphroditism in coral reef fishes. Am Sci
- 26
- 27
- 28 530 72:128-136
- 29
- 30
- 31 531 Whiteman EA, Côté IM (2004) Monogamy in marine fishes. Biol Rev 79:351-375
- 32
- 33
- 34
- 35 532 Winterbottom R (2005) Two new species of the genus *Trimma tevegae* species group
- 36
- 37
- 38 533 from the Western Pacific (Percomorpha: Gobiidae). Aqua Journal of Ichthyology
- 39
- 40
- 41 534 and Aquatic Biology 10: 29-38
- 42
- 43
- 44 535 Winterbottom R, Southcott L (2008) Short lifespan and high mortality in the Western
- 45
- 46
- 47 536 Pacific coral reef goby *Trimma nasa*. Mar Ecol Prog Ser 366: 203-208
- 48
- 49
- 50
- 51 537 Winterbottom R, Alofs KM, Marseu A (2011) Life span, growth and mortality in the
- 52
- 53
- 54 538 Western Pacific goby *Trimma benjamini*, and comparisons with *T. nasa*. Environ
- 55
- 56
- 57 539 Biol Fish 91: 295-301
- 58

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
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

540 Winterbottom R, Erdmann MV, Caryani NKD (2015) New species of Trimma
541 (Actinopterygii, Gobiidae) from Indonesia, with comments on head papillae
542 nomenclature. Zootaxa 3973:201-226
543 Wong MYL, Munday PL, Buston PM, Jones GP (2008) Monogamy when there is
544 potential for polygyny: tests of multiple hypotheses in a group-living fish. Behav
545 Ecol 19: 353-361
546

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

29	ID	May							June																															
30		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		
31	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>	<u>F14</u>	<u>F14</u>	<u>F14</u>		
32		<u>F2</u>			<u>F1</u>																				<u>F8</u>				<u>F5</u>		<u>F1</u>	<u>F8</u>		<u>F12</u>						
33																																								
34	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	F11	<u>F11</u>	F11	F11	<u>F11</u>	F11	F11	F11	<u>F11</u>	F11	F11	<u>F11</u>
35			<u>F11</u>																													<u>F12</u>		<u>F12</u>						
36																																								
37	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	<u>F2</u>	F2	<u>F2</u>
38																			<u>F1</u>			<u>F1</u>				<u>F5</u>		<u>F5</u>	<u>F5</u>			<u>F5</u>								
39																																								
40	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	<u>F4</u>		<u>F15</u>					
41																			<u>F5</u>														<u>F8</u>							
42																																								
43	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>	F16	F16	<u>F16</u>	F16	F16	<u>F16</u>	
44			<u>F2</u>		<u>F14</u>														<u>F9</u>			<u>F9</u>		<u>F9</u>		<u>F9</u>				<u>F5</u>					<u>F5</u>					
45																																								
46	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>					
47					<u>F2</u>	<u>F13</u>				<u>F10</u>																									<u>F9</u>					
48																																								
49	M7		<u>F3</u>			<u>F17</u>	<u>F14</u>			<u>F6</u>	F6	F6	F6	<u>F6</u>	F6	F6	F6	F6	F6	F6		F7	<u>F7</u>	F7	F7	<u>F7</u>	F7	F7	F7	<u>F7</u>	F7	<u>F7</u>	F7	F7	<u>F7</u>	F7	<u>F7</u>	<u>F7</u>		
50														<u>F7</u>							<u>F14</u>																			
51																																								
52	M8	F16	F16	F16	<u>F16</u>	<u>F16</u>	<u>F16</u>		<u>F9</u>		<u>F3</u>	<u>F3</u>	F3	F3	F3	F3	F3	F3	F3	F3	<u>F3</u>	F3	F3	<u>F3</u>	F3	<u>F3</u>	F3	<u>F3</u>	F3	<u>F3</u>	F3	<u>F3</u>	<u>F3</u>	<u>F3</u>	<u>F3</u>	<u>F3</u>	<u>F3</u>	<u>F3</u>	F3	
53			<u>F12</u>									<u>F4</u>									<u>F4</u>																			
54																																								
55																																								

Figure legends

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

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

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

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

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

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

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