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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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### 44 Introduction

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, 4

62	hermaphroditic species have been reported in the genera Gobiodon, Paragobiodon,
63	Priorepis (Sadovy de Mitcheson and Liu 2008), and Bryaninops (Munday et al. 2002).
64	It has been suggested that these species reproduce monogamously because of low
65	mobility, low mate availability, or intra-specific competition related to resources in their
66	specific habitats (Kuwamura et al. 1993; Nakashima et al. 1996; Sunobe and Nakazono
67	1999; Munday et al. 2002; Whiteman and Côté 2004; Wong et al. 2008). These
68	ecological and social characteristics have also been considered to facilitate the evolution
69	of sex change in these species (Nakashima et al. 1995; Munday et al. 1998; Munday
70	2002; Manabe et al. 2013). Therefore, revealing the mechanisms leading to various
71	mating systems is important when examining the evolution of hermaphroditism.
72	Sex change or sequential hermaphroditism among teleosts, such as protogyny
73	(female-to-male sex change) and protandry (male-to-female sex change), has been well
74	documented. The size-advantage (SA) model predicts evolution of protogyny when
75	species are polygynous because large males obtain remarkably greater benefit than
76	small males through intense mating competition and mate choice by females.
77	Meanwhile, the evolution of protandry is favored when males reproduce and are
78	reproductively successful regardless of their body size (Ghiselin 1969; Warner 1975;
79	Warner 1984; Kuwamura and Nakashima 1998; Munday et al. 2006). 5

80	In addition to these types of sex change mechanisms, bidirectional sex change has
81	been reported in Gobiidae, Serranidae, Pseudochromidae, Pomacanthidae, Cirrhitidae,
82	Labridae, and Pomacentridae (Munday et al. 2010; Kuwamura et al. 2016). In particular,
83	the gobiid genus Trimma has been studied to clarify the adaptive significance of
84	bidirectional sex change (Sunobe and Nakazono 1993; Manabe et al. 2007; Manabe et al.
85	2008; Sakurai et al. 2009). This genus contains 92 valid species distributed on rocky
86	ledges and coral reefs in temperate to tropical waters of the Indo-Pacific Ocean (Suzuki
87	et al. 2012; Winterbottom et al. 2015). Gonadal histological observations of T.
88	grammistes, T. kudoi, T. okinawae, T. unisquamis, and T. yanagitai show that ovarian
89	and testicular tissues are present simultaneously (Cole 1990; Manabe et al. 2007;
90	Sunobe and Nakazono 1993; Sakurai et al. 2009; Shiobara 2000). Bidirectional sex
91	change has been confirmed in these species by field observations or rearing experiments,
92	except in <i>T. unisquamis</i> , and these species may be polygynous (Manabe et al. 2007;
93	Munday et al. 2010; Sakurai et al. 2009; Shiobara 2000). Except in T. kudoi, the largest
94	female changes to male when the dominant male disappears, and the smaller individual
95	of a male-male pair changes sex to female (Sunobe and Nakazono 1993; Manabe et al.
96	2007; Manabe et al. 2008; Sakurai et al. 2009; Shiobara 2000).

97	The present study species, <i>T. marinae</i> , 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 <i>T. marinae</i> 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;

116experiment, and five were fixed in Bouin's solution for 24 h and then preserved in 70%117ethanol. Twenty-five live fish were transported to the laboratory for the rearing118experiment.119We collected nine juveniles by hand net at the same site on 30 July 2014 to examine120sexuality. They were measured for TL and fixed using the methods described above.121Observations of reproductive behavior122Observations of reproductive behavior.123To reveal the reproductive behavior, 25 live individuals (eight males; mean $\pm$ SD = 25.0125 $\pm$ 0.5 mm TL, range = 23.5–25.0 mm TL and 17 females; 26.0 $\pm$ 1.0 mm TL, range =12624.0–27.0 mm TL) were anesthetized with quinaldine, measured for TL, sexed, marked127by subcutaneously injecting a visible implant Elastomer Tag (Northwest Marine128Technology Inc., Shaw Island, WA, USA), and deposited in an aquarium (120 × 45 × 45129em) on 16 April 2014. The fish density in the tank was approximately 0.1 individuals130per liter. The sex ratio in the aquarium was adjusted to that observed in the original131study aggregation (Fisher's exact test, $P = 1.0$ ) (see Results). Water was circulated,132filtered, and maintained at 25°C. Ten opaque PVC pipes (5 cm inside diameter and 6 cm1328	115	Manabe et al. 2008). Eleven specimens were preserved in 70% ethanol for a future
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	132	filtered, and maintained at 25°C. Ten opaque PVC pipes (5 cm inside diameter and 6 cm 8

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 

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 $\times$ 45 $\times$ 45 cm and 120 $\times$ 45 $\times$ 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.
179	
180	Gonadal histology
181	
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 $\mu m,$ and
184	stained with hematoxylin and eosin.
185	
186	Results
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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 $(24.5 \pm 0.6 \text{ mm TL}, N = 14)$
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
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.
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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 13

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 ( $15 \pm 4$  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  $\geq 2$  days, the duration of maintaining a pair bond was 2-32 days (mean,  $11.6 \pm 9.0$  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 =  $70.3 \pm 4.0, N = 21$ ) and  $97.2\% (97.2 \pm 1.8, N = 21)$  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 

238 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 15

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.
262	
263	Aggressive behavior
264	
265	Aggressive behavior by <i>T. marinae</i> 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 ( $\chi^2 = 3$ , $P > 16$

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  $12.6 \pm 1.7$  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 

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

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
	18

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

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

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

Males were near their nest throughout the day and only attacked other males and both sexes, except their mate, when the nest was without and with eggs, respectively (Fig. 6). This observation indicates that males only guard their nest against the same sex and guard their eggs against both sexes except their mate. Kuwamura (1985) suggested that egg predation by members of an *Apogon notatus* aggregation and excluding conspecifics from the reproductive pair are associated with forming a continuous reproductive pair in this species. We also observed that eggs of *T. marinae* were eaten by bachelor females that did not spawn. This result shows that members of an aggregation may be potential egg predators. Therefore, this aggressive behavior may also contribute to formation of a monogamous mating system because the male drives away almost all conspecifics except the mate while egg guarding. However, we observed that some males accepted two females on the same day (Table 1), suggesting temporal polygyny. The mating system of the monogamous species Oxymonacanthus longirostris converts to polygyny when the sex ratio becomes slightly 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. kudoi, 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.

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 22

379	favored if it is too costly to change sex. The gobiid fish <i>T. nasa</i> is closely related to <i>T</i> .
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 <i>T. benjamini</i> (Sunobe unpubl. data) (Winterbottom et al. 2011).
383	If <i>T. marinae</i> live as long as <i>T. nasa</i> , 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 <i>T. marinae</i> 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 <i>T. marinae</i>
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 23

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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411	
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37 38 39	M3	F3	<u>F3</u> I	F3 <u>F3</u>	<u>B</u> <u>F3</u>	F2	F2	F2	F2 <u>F2</u>	<u>2</u> F2	F2	F2	<u>F2</u>	F2 F	2 F.	2 F2 F1	F2	F2	F2 F1	<u>F2</u> F2	2 F2	2 <u>F2</u>	F2 F5	F2	F2 F5	F2 I F5	F2 ]	<u>F2</u> F2 F2	2 F2	<u>F2</u> F2	F2 <u>F2</u>	
40 41 42	M4		F12 F	12 F1	2 <u>F12</u>	F12	F12 I	F12 F	F12 F1	2 F12	F12	F12	F12 1	F12 F	12 <u>F1</u> F	<u>2</u> 5	_	[	F12				F4	F4	F4	F4 I	F4 ]	F4 <u>F4</u>	<u>4</u> 8	<u>F15</u>	]	
43 44 45 46	M5		<u>F9</u> I <u>F2</u>	F9 <u>F9</u> F1	<u>)</u> 4	<u>F6</u>	]	F17 F	F17 F1	7 F17	F17	F17	F17 ]	F17 F	17 <u>F1</u>	<u>7</u> F17 <u>F9</u>	F17	F17	<u>F17</u> I	F17 F1 <sup>*</sup> F9	7 F11	7 F17 <u>F9</u>	F17	F17	F17	F17 F	F <u>5</u>	<u>F17</u> F1	6 F16	<u>F16</u> F16	F16 <u>F16</u> <u>F5</u>	<u>)</u>
47 48 49	M6	<u>F7</u>	F7 I	F7 <u>F7</u> F2	<u>7 F7</u> 2 F13	F7	F7	F7	F7 F' F1	7 0		[	<u>F9</u>			<u>F15</u>	]	[	<u>F8</u> <u>F</u>	<u>F15</u> F1:	5 F1:	5 <u>F15</u>		F10	<u>F10</u>	F	15	<u>F9</u>	<u>F10</u>	<u>F10 F4</u> <u>F9</u>	]	
50 51 52	M7		<u>F3</u>		<u>F17</u>	<u>F14</u>			<u>F</u>	<u>6</u> F6	F6	F6	<u>F6</u> <u>F7</u>	F6 F	6 F	6 F6	F6		F7	F7 F7 F <u>14</u>	7 F7	<u>F7</u>	F7	F7	F7	<u>F7</u> I	F <b>7</b> ]	<u>F7</u> F1	7 F7	<u>F7</u> F7	<u>F7</u> <u>F7</u>	
53 54 550 <sup>55</sup>	M8	F16	F16 <u>F</u> F12	<u>16</u> F1	<u>6</u> F16	<u>F16</u>		<u>F9</u>	<u>F</u> .	B F3 F4	F3	F3	F3	F3 F	3 F	3 F3	F3 <u>F4</u>	F3	F3	F3 <u>F3</u>	<u>3</u> F3	<u>F3</u>	F3	<u>F3</u>	F3	<u>F3</u> I	F3 <u>]</u>	<u>F3 F3</u>	<u>3 F3</u>	F3 <u>F3</u>	<u>F3</u> F3	_
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551	Figure legends
552	
553	<b>Fig. 1</b> Size distributions of male and female <i>Trimma marinae</i> in the study group ( $N =$
554	41)
555	
556	Fig. 2 Frequency of spawning and spawning time of <i>Trimma marinae</i> from 24 April to
557	22 May 2014 ( <i>N</i> = 60)
558	
559	Fig. 3 Relationship between sizes of males and females in all continuous pairs of
560	<i>Trimma marinae</i> ( $N = 21$ ). <i>Symbol size</i> 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	<b>Fig. 5</b> Estimate of <i>Trimma marinae</i> male mating success $(N = 8)$
566	
567	Fig. 6 Frequency of aggressive behavior by <i>Trimma marinae</i> when the male and female
568	were paired or the male remained alone in their nest. <b>a</b> Frequency that nesting males $35$

569	attacked an intruding male. <b>b</b> Frequency that nesting males attacked bachelor females. <b>c</b>
570	Frequency that mated females attacked bachelor females. Chi-square test for deviation
571	from equality: ** <i>P</i> < 0.01; *** <i>P</i> < 0.001
572	
573	<b>Fig. 7</b> <i>Trimma marinae</i> gonadal cross-sections. <b>a</b> Gonad of mature male. ( $Bar = 0.1$
574	mm). <b>b</b> Gonad of mature female. ( <i>Bar</i> = $0.1$ mm). <b>c</b> Gonad of the smallest immature
575	individual ( $Bar = 0.03$ mm). <b>d</b> Gonad of the largest immature individual ( $Bar = 0.03$
576	mm). <b>e</b> Intersexual gonad of juvenile. ( $Bar = 0.03$ mm). <b>f</b> 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. 6



## Fig. 7

