

First Description of Reproductive Behavior and Alternative Reproductive Strategy in the Deep-sea Fish, *Careproctus pellucidus*.

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Abstract

The reproductive biology of deep-sea fishes is largely unknown because of the difficulty of behavioral observations in this environment. In this study, the reproductive behavior of the deep-sea snailfish, *Careproctus pellucidus*, which lives at depths > 200 m, was observed in an aquarium. To the best of our knowledge, this is the first report to observe and describe the reproductive behavior of deep-sea fish under free-living conditions. Fishes were collected at a depth of approximately 300 m (36°70' N; 141°00' E) on July and August 2018 and kept for observation until March 2019. We observed that almost all spawnings took place between one male and one female. Males defended a spawning substrate against other males and exhibited body-wiggling behavior for both courtship and aggressive display. Females visited the male's territory and spawned a demersal adhesive egg mass on the substrate. The territorial male then sought the spawned eggs using filamentous rays in the lower pectoral-fin lobe and released sperm when he located the eggs. Males remained near the spawning substrate after spawning but did not perform any parental care. Notably, sneaking behavior by a non-territorial male was observed in one case; this is the first report of this alternative reproductive strategy (or tactic) in a deep-sea fish. Our study reveals the unique reproductive biology of the deep-sea fish, *C. pellucidus*, which does not depend on visual information and uses other sensory modalities.

Introduction

Knowledge of the reproductive biology of fishes is essential for understanding their life history and evolution. Since the development of SCUBA technology, a lot of studies of the reproductive biology of coral reef fishes using underwater observation have been reported (e.g., Kuwamura 1985; Warner and Lejeune 1985; Branconi et al. 2020). By contrast, the reproductive behavior of deep-sea fishes (fish found at approximately > 200 m depth) remains largely unknown because several factors make it difficult to observe them *in situ* or collect them alive for rearing (*ex situ*) observation. A recent video of deep-sea fish mating, captured at a depth of 800 m, became popular because of its rarity (Science 2018). Fish that are adapted to the unique deep-sea environmental conditions (e.g., high water pressure, low water temperature, low oxygen concentration, and absence of light) appear to have evolved quite different morphological traits, behaviors, and reproductive strategies to those of coral reef fish. For example, simultaneous hermaphroditism, i.e., producing eggs and sperm simultaneously, has evolved in many deep-sea aulopiform fishes (Davis and Fielitz 2010), despite its rarity in coral reef fishes. Because the population density of fish may be relatively low in the deep-sea, this feature is considered to increase reproductive opportunities because individuals can mate with any mature conspecifics that are encountered (Ghiselin 1969; Warner 1984). In some species of deep-sea anglerfish (Lophiiformes: Ceratioidei), dwarf males become attached by tissue fusion to larger females, either temporarily or permanently, and deliver sperm to the female (Pietsch 2005; Pietsch and Orr 2007). This reproductive strategy is also considered an adaptation to the deep-sea environment that increases reproductive opportunities (Pietsch 2005). However, some of these strategies and their significance are still hypothetical because our knowledge of the reproductive biology of deep-sea fishes is limited to

speculation based on morphological and histological observation of fixed specimens. The lack of behavioral observations restricts our understanding of the life history and evolution of the reproductive strategies of deep-sea fishes. Liparid fishes, investigated in this study, are one of the taxa containing deep-sea dwelling species whose reproductive behavior remains speculative.

The family Liparidae, commonly known as snailfish, comprises at least 32 valid (57 available) genera and 430 valid (503 available) species (Chernova et al. 2004; Nelson et al. 2016; Fricke et al. 2021). Snailfishes have an extraordinarily wide geographic range: from the northern to southern hemisphere (Stein 2012; Orr et al. 2015; Nelson et al. 2016), and from the tidal zone (Able and Musick 1976; Kido 1988; Nelson et al. 2016) to the hadal zone (there are records of some species at > 8000 m depth) (Fujii et al. 2010; Linley et al. 2016; JAMSTEC, 2017). Despite inhabiting such a broad range of habitats, the reproductive (spawning) behavior of snailfishes has only been reported in two species that inhabit shallow areas: *Liparis atlanticus* (Detwyler 1963) and *L. inquilinus* (Able and Musick 1976). Observations of fixed specimens indicate that some deep-sea snailfishes have unique reproductive strategies. Nakazawa (1915) reported that putative Cyclopteridae (or Liparidae) species eggs were laid in the gill cavity of *Paralithodes camtschaticus*, and Poltev and Mukhametov (2009) found egg masses deposited in the gill cavities of 9.9% of captured golden king crabs, *Lithodes aequispinus*, captured at depths of 209–716 m. Subsequently, Gardner et al. (2016) showed that eggs deposited in *Lithodes aequispinus* and *Lithodes couesi*, were from *Careproctus melanurus*, *C. colletti*, *C. furcellus*, and *C. simus*, on the basis of mitochondrial DNA sequences. Several other studies have reported similar egg deposition in the gill cavity of crabs by snailfishes (Gardner et al. 2016), indicating that this may be one of the widespread reproductive strategy in this family. The octocoral *Primnoa* sp., found at a depth of 397 m (Busby et al. 2006), the cavity of glass sponge *Schaudinnia rosea*, found at a depth of 320 m (Chernova 2014a), and some artificial structures found at depths of > 2000 m (Overdick et al. 2014) also appear to have been used as spawning substrate by snailfishes. These studies show that snailfishes for which eggs have been found deposited adhesive demersal eggs on the substrate, and some of these have a special preference for spawning substrate utilization. However, the process, procedure, and prior behaviors of reproduction (e.g., territoriality, intra- and intersexual selection, courtship, and spawning behavior), which are key to understanding the adaptive significance and evolutionary background of reproductive strategies in the deep-sea environment, have not been observed. From another point of view, it is expected that the dispersion of spawning substrate utilization makes the species with similar spawning niches less difficult to live sympatrically because it reduces interspecific competition (Reichard et al. 2007; Awata et al. 2019). There is a possibility that the unique preference for the spawning substrate in snailfishes has a similar function to the above. For this reason, shedding light on the reproductive biology of deep-sea snailfish is also significant for understanding their distribution patterns and inter- or intraspecific relationship in deep-sea.

In this study, we observed the reproductive behavior of a deep-sea snailfish, *C. pellucidus* (Gilbert and Burke 1912), which is distributed in the North Pacific Ocean region at depths > 200 m, in an aquarium. *Careproctus* is one of the most diverse genera among the Liparidae, comprising at least 140 known species (Orr et al. 2019, 2020). Most *Careproctus* species are found in the deeper zones of the sea, and

new species continue to be described (Chernova 2005; Orr 2012; Chernova 2014a, b; Orr 2016; Kai and Matsuzaki 2019; Matsuzaki et al.2020). Behavioral observation of this species, which belongs to a diverse group, provides new insight into the reproductive biology of fishes adapted to the deep-sea environment. For example, since the visual information is limited in the deep-sea compared with shallow areas by dimming downwelling daylight, some deep-sea fishes do not really depend on visual information for a living (Munk 1965). The deep-sea snailfish *Careproctus rastrinus*, belonging to the same genus as *C. pellucidus*, seek food using taste buds in the filamentous rays in the lower pectoral-fin lobe s (Sakurai and Kido 1992). This behavior makes us infer that this species, and related *C. pellucidus*, does not depend on the visual sense in their ecology. Thus, it is expected that the reproductive behavior of *C. pellucidus* is adapted to the deep-sea environment and distinctive compared to that of species inhabiting in shallow areas.

Materials And Methods

Specimen collection

We used 69 *C. pellucidus* individuals collected by trap fisheries off the coast of Takahagi City, Ibaraki Prefecture, Japan (36°70' N; 141°00' E) at a depth of approximately 300 m (Fig. 1) between July and August 2018. Species identification followed Gilbert and Burke (1912) and Orr et al. (2015). Live fish were transported to the Marine Science Museum, Fukushima Prefecture (Fig. 1) for observation. When individuals died during rearing, the standard length (SL) and body weight (BW) of the individuals were immediately measured to the nearest 0.1 mm and 0.1 g, respectively. We also observed the shape of the urogenital papilla and the structure of the gonad under a stereoscopic microscope for sexing. All individuals died by March 2019, and two specimens were deposited in the Marine Science Museum, Fukushima (AMF2-11-04-00-0100, AMF2-11-04-00-0101).

Observation of reproductive behavior

To observe reproductive behavior, 69 live individuals (34 males, 32 females, 3 unknown sex, see Results) were deposited in a tank (5 × 2.55 × 0.7 m, 9000 L), and reared from July 2018 to March 2019. Water was circulated, filtered, and maintained at 6°C. The bottom of the tank was covered with sand (0.77 ± 0.03 mm particle diameter, range = 0.60–1.16 mm, $n = 20$) to a thickness of approximately 10 mm. The rearing tank was an exhibition tank at the Marine Science Museum, which was observable by the public, and also contained 25 *Chionoecetes opilio* individuals. The tank was maintained under an 11:13-h light:dark photoperiod (lights on 07:00; lights off 18:00). Lights were kept on throughout the day on observation days to capture video (see following section).

We conducted behavioral observations from 19:00 to 07:00 on 17 days (non-sequential) between September 2018 and December 2018. During the observation time, we placed an artificial spawning substrate (Fig. 2) on the bottom of the tank and captured video footage around it using a video camera (HDR-CX590V, SONY). The artificial spawning substrate was removed from the tank after the observation period each day. We recorded and described the male and female reproductive behaviors and their

interactions (e.g., courtship, spawning, and male–male competition) on the basis of video footage, if the behaviors were wholly captured. Preliminary observations showed that the behavioral patterns of males and females differed: males exhibited territorial behaviors and/or body wiggling, whereas females did not show those behaviors (see Results). Therefore, we recognized an individual's sex based on their behavior during the observations. To reveal the significance of the body wiggling of males, we compared the frequency of this behavior in territorial males (T-males) in three situations: T-male alone, T-male with a non-territorial male (NT-male) (NT-male approached T-male's territory), and T-male with a female (female approached the T-male's territory). Because males wiggled their bodies several times quickly, we counted a series of these events as one bout, and recorded the number of these bouts in a 5-minute period (frequency). We observed the above three situations for 5 minutes in 21 cases, and analyzed these data. We collected five fertilized egg masses at random, counted the clutch size, and measured the diameter using a microscope.

Assessment of the existence of female mate choice

To assess whether there was mate choice (intersexual selection) by females, we recorded the reproductive success of males in the following situations. We placed two artificial spawning substrates 500–1000 mm apart in the tank for 6 of the 17 observation days. Because two males established a territory around each substrate and spawned with females, we counted the spawning bouts of each male.

Statistical analyses

Values are presented as means \pm standard error of the mean. All statistical analyses were conducted using R version 3.6.3 (R Core Team 2020). For all tests, the significance level used was $\alpha = 0.05$. We assessed the normality of datasets based on the Shapiro–Wilk test before each statistical test. We also assessed the homogeneity of compared datasets using F test, Bartlett test, or Levene's test. The sexual dimorphism in SL and BW was examined using the Wilcoxon rank sum test and Welch's two-sample t -test followed by calculation of the effect size index r (Cohen 1988) and Hedges' g (Rosenthal 1994), respectively. The frequency of body wiggling of T-males in the three situations was compared using the Tukey–Kramer test followed by calculation of Hedges' g . Male–male competition was observed between large and small males, and T-males and NT-males. Thus, we examined the deviation from equality of the winning rate in each situation using the Chi-square test and reported phi coefficients as the effect size. The relationship between the frequency of body wiggling and outcome of competitions (win or loss) was examined using the Wilcoxon rank sum test. The effect size index r was reported.

Results

Sexual difference in morphological characteristics

Of the 69 individuals captured, 34 were identified as male (160.1 ± 2 mm SL, range = 120.4–181.6; 89.7 ± 3.7 g BW, range = 35.7–123.0) and 32 as female (158.2 ± 1.7 mm SL, range = 138.7–175.7; 87.3 ± 4.2 g BW, range = 53.4–151.6) (Fig. 3). We could not measure the SL and BW or distinguish the sex of three

individuals because the extremities of their bodies had been bitten off by *Chionoecetes opilio* when we found them. There were no significant differences in SL ($W = 625$, $P = 0.3$, $r = 0.13$, $n = 66$) or BW ($t = 0.41$, $P = 0.68$, $g = -0.1$, $n = 66$) between males and females. Males and females had light pinkish bodies and there was no apparent difference in body color between the sexes. Individuals with mature testicular tissue (mature males) had an elongated (approximately 5 mm) urogenital papilla, located ventral to the anus, with a conical shape that tapered posteriorly (Fig. 4b). Individuals with mature ovarian tissue (mature females) had a urogenital papilla with a rounded tip with several processes (Fig. 4c) but it was not elongated.

Reproductive behavior

We observed two behavioral types in males: territorial males (T-male), which stayed near the artificial spawning substrate and frequently exhibited body-wiggling behavior (wiggling their body quickly several times; Fig. 5a) throughout the observation time; and non-territorial males (NT-male), which did not establish territories and wandered in the tank. When individuals (intruders) approached a T-male's territory, the T-male approached the individual and exhibited body wiggling toward them, regardless of their sex. If the intruder was male, the intruder responded by also performing body wiggling; they then fought by picking and biting the other individual's body and fins (Fig. 5d; Online Resource 1). This competition continued until the T- or NT-male left. If females approached a T-male's territory, females did not perform body wiggling, even when the T-male was exhibiting this behavior, and spawning occurred in some cases. There was a significant difference in the frequency of body wiggling of T-males between encounters with females ($n = 5$) and the other two situations [T-male alone ($n = 7$) and T-male with NT-male ($n = 9$)] (T-male alone vs T-male with female: $P < 0.01$, $g = -5.76$; T-male alone vs T-male with NT-male: $P = 0.85$, $g = -0.38$; T-male with female vs T-male with NT-male: $P < 0.001$, $g = 5.31$; Fig. 6). These results suggest that one purpose of the body-wiggling behavior by T-males is courtship.

Spawning was observed in 65 cases, and 64 (98%) of these took place in pairs (one male and one female). T-males usually exhibited body wiggling when alone within their territory (Fig. 5a). When a female approached the territory, the T-male also approached her and exhibited more frequent body wiggling than before (Fig. 5b; Online Resource 2). T-males exhibited body wiggling an average of 48.1 times (48.1 ± 7.3 times, range = 6–125, $n = 20$) for 115.2 s (115.2 ± 17.5 s, range = 21–359, $n = 22$). If the female accepted the T-male's courtship, the female laid a demersal adhesive egg mass with sticky ovarian cavity fluid on the wall of the artificial spawning substrate. The eggs formed an aggregate (egg mass) by adhering to each other, and the average clutch size was 222.2 eggs (222.2 ± 41.4 eggs, range = 102–357, $n = 5$). The female left the T-male's territory soon after spawning. The T-male swam around slowly with the filamentous rays of the lower pectoral-fin lobe extended vertically toward the artificial spawning substrate and touching its surface. When the filamentous rays touched the egg mass, the T-male immediately released sperm toward it (Online Resource 3). The time from spawning by the female to sperm release by the T-male was an average of 10.9 s (10.9 ± 3.1 s, range = 0–167, $n = 62$). Although the T-males remained and guarded their territory, they did not perform any parental care for the eggs (e.g.,

fanning and mouth-cleaning). Fertilized eggs (3.9 ± 0.02 mm egg diameter, range = 3.6–4.09, $n = 27$) were milky white and had a smooth-faced spherical shape.

Sneaking behavior by an NT-male (sneaker) was observed once (Fig. 5c; Online Resource 4). The sneaker was wandering in the tank and when the sneaker approached the T-male's territory, the sneaker did not perform body wiggling, like females, even though the T-male was exhibiting it toward him. As a result, the sneaker was able to stay in the T-male's territory without being attacked. Eventually, a female visited and spawned an egg mass after the T-male's courtship. As soon as the T-male started looking for the egg mass, the sneaker also began looking and released sperm toward the egg mass when he located it. The T-male then began to attack the sneaker, and the sneaker immediately left the territory.

Male–male competition

Nine competitive interactions between T-males and NT-males were observed. Aggression by T-males toward NT-males and vice versa was observed. When the T-male suffered a defeat, he was robbed of his territory by the NT-male. These competitive interactions involved a T-male that was smaller than the NT-male ($n = 2$; T-male won in both cases), an NT-male that was smaller than the T-male ($n = 3$; T-male won in one and lost two cases), and a T-male and an NT-male that were the same size ($n = 4$; T-male won two and lost two). The outcome of these competitions (win or lose) did not depend on SL ($\chi^2 = 1.8$, $P = 0.18$, $\phi = 0.26$, $n = 5$) or whether the male was territorial or non-territorial ($\chi^2 = 1.11$, $P = 0.74$, $\phi = 0.03$, $n = 9$). However, there was a significant difference in the frequency of body wiggling between the winner and the loser ($W = 80$, $P < 0.01$, $r = 0.93$, $n = 18$) (Fig. 7). This result indicates that the body wiggling of males is also an aggressive display in this species.

Skew of reproductive success among males

When two males established adjacent territories and spawned on the same day, the number of spawnings by each male was seemingly skewed (Table 1). On five of the six observation days, one male did not, or could not, spawn at all.

Table 1 Number of spawning in two males during each observation day

Date	Number of spawnings	
	Male A	Male B
14 Oct.	2	0
21 Oct.	6	0
25 Oct.	6	1
18 Nov.	2	0
25 Nov.	1	0
29 Nov.	3	0

Male A and *Male B* indicate males which spawned more and less frequently, respectively

Discussion

In this study, we provide a detailed description of the reproductive behavior of a deep-sea snailfish. To the best of our knowledge, this is the first report to observe the reproductive behavior of deep-sea fish under free-living conditions. As *in situ* behavioral observations of deep-sea fish are difficult, our results provide valuable insights into the reproductive biology of deep-sea fishes. Our results show that *C. pellucidus* males establish territories around spawning substrate and perform body wiggling as a courtship behavior. A similar courtship behavior has been observed in not only Liparidae species, *Liparis atlanticus*, which reportedly reproduces in shallow areas (Detwyler 1963) but also Cottidae species, such as *Hemilepidotus gilberti* (Hayakawa and Munehara 1996). It suggests that this may be a conservative behavioral component of courtship in Cottoidei species. By contrast, the courtship display of *L. atlanticus* and *H. gilberti* males which involves expansion of all fins (Detwyler 1963) was not observed in *C. pellucidus*. In addition, the sexual dimorphism seen in *L. atlanticus*—only the males' anterior five or six dorsal rays are elongated (Detwyler 1963)—did not observed or was not apparent in *C. pellucidus*. Such visual information would be of little use in the dark deep-sea compared with brighter shallow areas. The absence of apparent sexual dimorphisms in *C. pellucidus* suggests that their courtship display may be independent of visual signals result from adaptations to the deep-sea environment and optimizing reproductive investment.

Because visual information is largely unavailable in the deep-sea, except for bioluminescent species, some deep-sea fish use other sensory cues to seek potential mates. In deep-sea anglerfish, the well-developed eyes and olfactory structures of free-living males suggest that they rely on olfactory and/or bioluminescent cues to locate conspecific females (Pietsch 2005, 2009). In *Cyclothone microdon*, the olfactory organ and other parts of the olfactory system (e.g., olfactory rosette, olfactory bulb, telencephalon) are much more developed in males than in females, suggesting that these structures are used to detect pheromones from females (Marshall 1967). In *C. pellucidus*, the body-wiggling display (i.e., hydrodynamic signals received by the mechanosensory lateral line) may play various roles in communication among individuals in reproductive and competitive contexts. Some studies have shown, or suggested, that lateral line signals are used for communication in fish reproduction (Satou et al. 1994a, b; Marchesan et al. 2000; Medina et al. 2013) and competition (Enquist et al. 1990; Butler and Maruska 2015, 2016). Our results suggest that T-males of *C. pellucidus* wiggle their body to court females. Because the hydrodynamic signal propagates in multiple directions over long distances, body wiggling may be a way for T-males to make their presence known to females under light-limited conditions. This behavior was also used in the context of male–male competition. The strength and/or frequency of hydrodynamic signals generated by body wiggling and fin flapping could represent physical traits such as muscle mass and energy reserves. Because the individuals that exhibited more frequent body wiggling tended to win male–male competitive interactions, there is a possibility that *C. pellucidus* males assess the strength of opponents based on these signals. Butler and Maruska (2015) suggested that an African cichlid fish, *Astatotilapia burtoni*, uses similar mechanosensory lateral line cues to assess opponents during male–male competition to reduce dangerous physical damage. In the relatively noiseless and dark deep-sea environment, hydrodynamic signals generated by body wiggling of *C. pellucidus* may be the

most effective way to communicate, leading to the expansion of this communication to multiple contexts in this species. Future behavioral studies of deep-sea fish may reveal other sensory modalities unique to the deep-sea environment for intra- and interspecific communication.

One of the most notable findings of this study is the observation of sneaking behavior in a deep-sea fish. Sneaking is a reproductive strategy (or tactic) in which males (usually subordinate) fertilize eggs by dashing into the spawning event between a female and another male (usually dominant), to circumvent direct competition with dominant males. Although sneaking behavior has been found in many fishes (Taborsky 1994, 2008), this is the first report from a deep-sea fish and demonstrates the existence of alternative reproductive strategies (or tactics). Generally, sneaking is the best alternative reproductive behavior for subordinate males of a species in which a few dominant or territorial males monopolize most reproductive opportunities (e.g., Taborsky 1994; Taru et al. 2002; Ota and Kohda 2006; Stoltz and Neff 2006). The *C. pellucidus* males studied also established territories, and females visited and spawned within these areas. The sneaking strategy (or tactic) allows males who cannot (or do not) establish a territory to obtain reproductive success, which evolve and sustains this strategy in *C. pellucidus* populations.

There appear to be three deep-sea specific factors that facilitate the evolution of sneaking in *C. pellucidus*. First, the body-wiggling courtship of T-males provides a situation that favors the sneakers. The T-males of *C. pellucidus* frequently exhibited body-wiggling behavior within their territory throughout our observation periods. This hydrodynamic signal may attract females but would also enable sneaker males to locate the T-male's territory and simultaneously assess the behavior and some physical traits of the T-male. This situation is advantageous for sneakers competing for fertilization opportunities with T-males. A similar situation, in which indiscriminate signals are received by an unintended receiver and cause suffering to the exhibiter, has been observed in fireflies that exhibit luminescent courtship signals in the dark (Lloyd 1965; 1975). Indiscriminate sending of information may facilitate the evolution of different strategies to exploit it. Second, it may be relatively easy for sneakers to deceive T-males based on their behavior. T-males usually exhibited body wiggling toward approaching individuals regardless of their sex. If the approaching individual responded with body wiggling (i.e., the individual was male), the T-male began to physically attack him. In contrast, if the approaching individual did not exhibit any behavioral response (i.e., the individual was female), the T-male continued courtship. These results indicate two significant findings: (1) there is a high possibility that T-males determine the sex of the approaching individual based on their behavioral response and using the mechanosensory lateral line system instead of visual cues; (2) T-males will not physically attack an approaching individual if they cannot identify that individual as a male. The sneaker mimics the female's behavior (i.e., no response to the T-male's body wiggling), which likely enables him to stay in the T-male's territory until spawning occurs. Female mimicry is one strategy (or tactics) of sneaker males among fishes (e.g., Warner and Robertson 1978; Brantley and Bass 1994; Gonçalves et al. 1996). If the above two hypotheses are correct, employing female mimicry would facilitate sneaking in *C. pellucidus* because it is difficult for T-males to identify the sex of sneakers in a way that does not depend on the sneakers' behavior. Such T-male's ambiguous behavioral response to approaching individuals has not been reported in not only Liparidae

but also Cottidae species inhabiting in the shallow area: T-male usually showed different behavior depending on the sex of the approaching individual from the first encounter. For example, T-males perform agonistic behavior toward approaching males while they perform courtship behavior toward females (e.g., Kierl and Johnston 2010). Therefore, this ambiguous decision-making of T-male should be one of the specific adaptation to the dark deep-sea. Third, the fertilization procedure of *C. pellucidus* is seemingly vulnerable to sneaking. After the female spawns an egg mass, T-males seek the mass using the filamentous rays of the lower pectoral-fin lobe. Two snailfishes, *Liparis inquilinus* (Able and Musick 1976) and *Careproctus rastrinus* (Sakurai and Kido 1992), have taste buds in the filamentous rays in the lower pectoral-fin lobe and use these to seek food. *Careproctus pellucidus*, which is related to these species, may have the same chemosensory organs in those rays. Our observations suggest that T-males recognize eggs based on contact with the chemoreceptors. This egg-seeking process must be responsible for the time interval (average 10.9 s, range = 0–167 s in the present study) between spawning by females and sperm release by T-males, and it seemingly allows sneakers to intercept fertilization without much difficulty. As visual cues are limited in the deep-sea environment, we consider it highly plausible that the sneaking strategy (or tactic) may be employed by other species whose behavior has not yet been observed.

We found a skew in reproductive success between two males that established adjacent territories. In many animals, the theory of sexual selection, including intra- and intersexual selection, has successfully explained the skew of reproductive success among intraspecific same-sex individuals (e.g., Andersson 1994; Davies et al. 2012). In many fishes, females choose males based on some indicator of increased reproductive success (e.g., body size, morphology, courtship activity, and quality of territory), a process known as mate choice (Warner 1988; Côte and Hunte 1989; Basolo 1990; Milinski and Bakker 1990; Andersson 1994; Davies et al. 2012; Matsumoto et al. 2012). Although it is only preliminary data, our results indicate the possibility of female mate choice in this species. As mentioned above, T-males of *C. pellucidus* appeared to perform body wiggling to court females. This behavior may provide females with information about the quality of males (e.g., some of the physical traits, muscle mass, and energy reserves), just as Sargent et al. (1998) and Passos et al. (2016) reported for other fishes. This hypothesis needs to be tested by comparative studies of male behavioral and morphological traits in situations where reproductive success varies among males. Even so, the present results provide clues to the existence of mate choice in deep-sea fishes.

The presence (or absence) of parental care appears to vary depending on the species. Detwilyer (1963) observed that male *L. atlanticus* fanned eggs with their fins and guarded eggs. DeMartini (1978) also reported that *Liparis fucensis* males handled eggs—males gathered up to several clutches attached to algae and stones into a single mass—but it was unclear whether that could be classified as parental care. Able and Musick (1976) reported that *L. inquilinus* did not perform any parental care. The present study showed that *C. pellucidus* did not perform any behaviors that are generally considered parental care (e.g., fanning, nipping, skimming, cleaning, and guarding). There is insufficient information on post-spawning behavior in snailfishes to explain the relationship between mode of parental care and habitat depth in snailfishes. Notably, our observations showed that males of *C. pellucidus* guard their territory against

conspecific males. Further study is needed to examine whether the observed behavior is parental care or territorial defense.

Several previous studies have investigated the reproductive behavior of deep-sea dwelling fishes using *ex situ* observation (e.g., Chernova 1987; Ferry-Graham et al. 2007; Tsuruwaka and Shimada 2011). Needless to say, *in situ* behavioral observations are the best way to understand the reproductive biology of species and these studies have advanced our understanding of the reproductive biology of deep-sea fish. The present study used *ex situ* behavioral observation and the results indicate that the unique reproductive biology of *C. pellucidus* does not depend on visual information and uses other sensory modalities. Improvements in fish breeding and management technology should make it possible to elucidate the ecology of fishes that live in extreme environments.

Declarations

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Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable institutional guidelines for sampling, care and experimental use of animals were followed.

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Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author, TM, on reasonable request.

Code availability

Not applicable

Authors' contributions

Toshiaki Mori: Conceptualization, Methodology, Validation, Investigation, Writing – Original Draft, Visualization. **Kazuya Fukuda:** Conceptualization, Methodology, Formal analysis, Writing – Original Draft, Writing – Review and Editing, Visualization. **Syouko Ohtsuka:** Investigation. **Shinya Yamauchi:** Conceptualization, Methodology, Resources. **Tatsuki Yoshinaga:** Conceptualization, Supervision.

Consent to participate

Not applicable

Consent for publication

Not applicable

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Figures

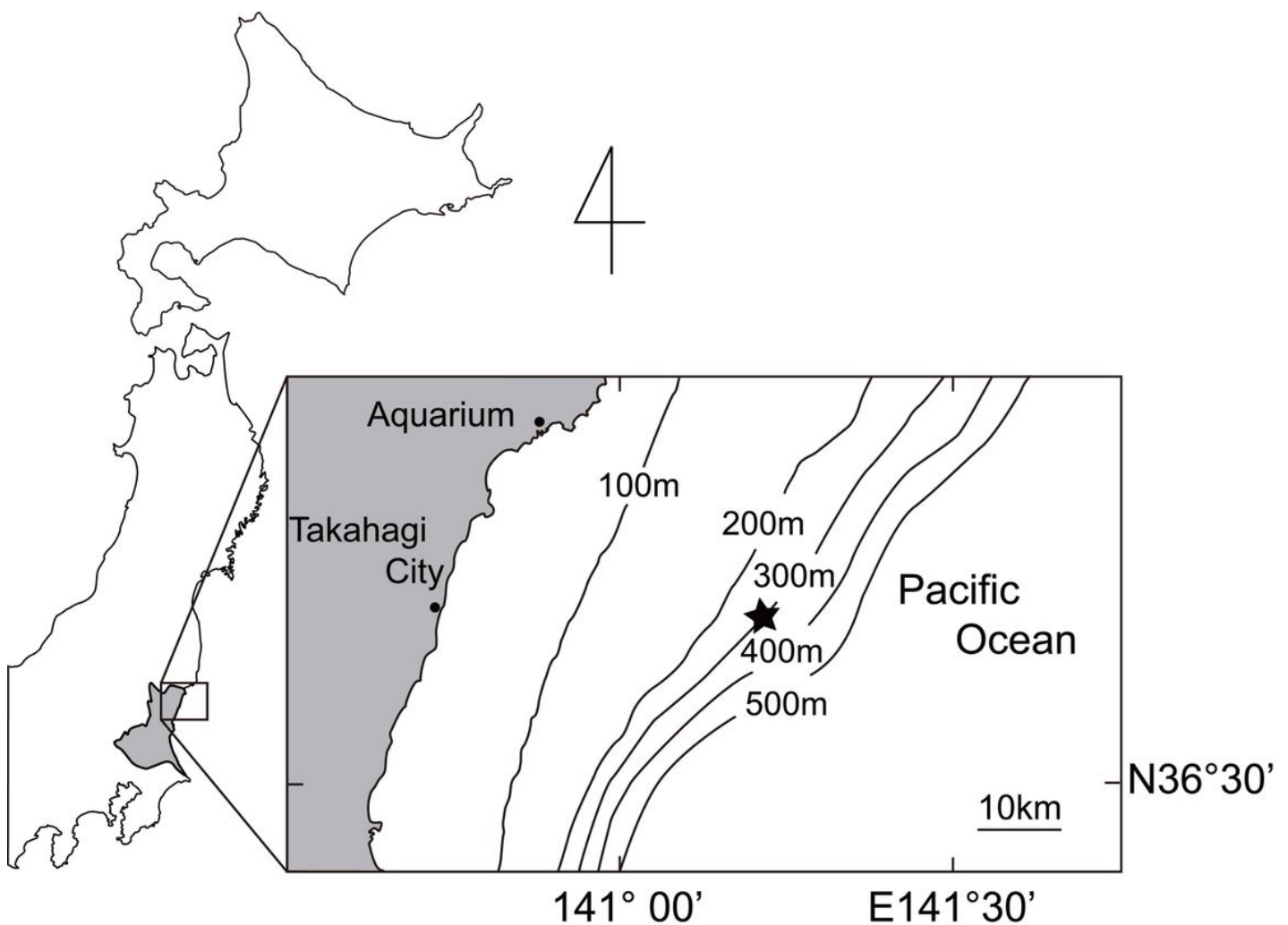


Figure 1

Collected location at offshore of Ibaraki Prefecture, Japan. Solid line with a numeric number water depth, star collected point, circle aquarium location where the present study conducted

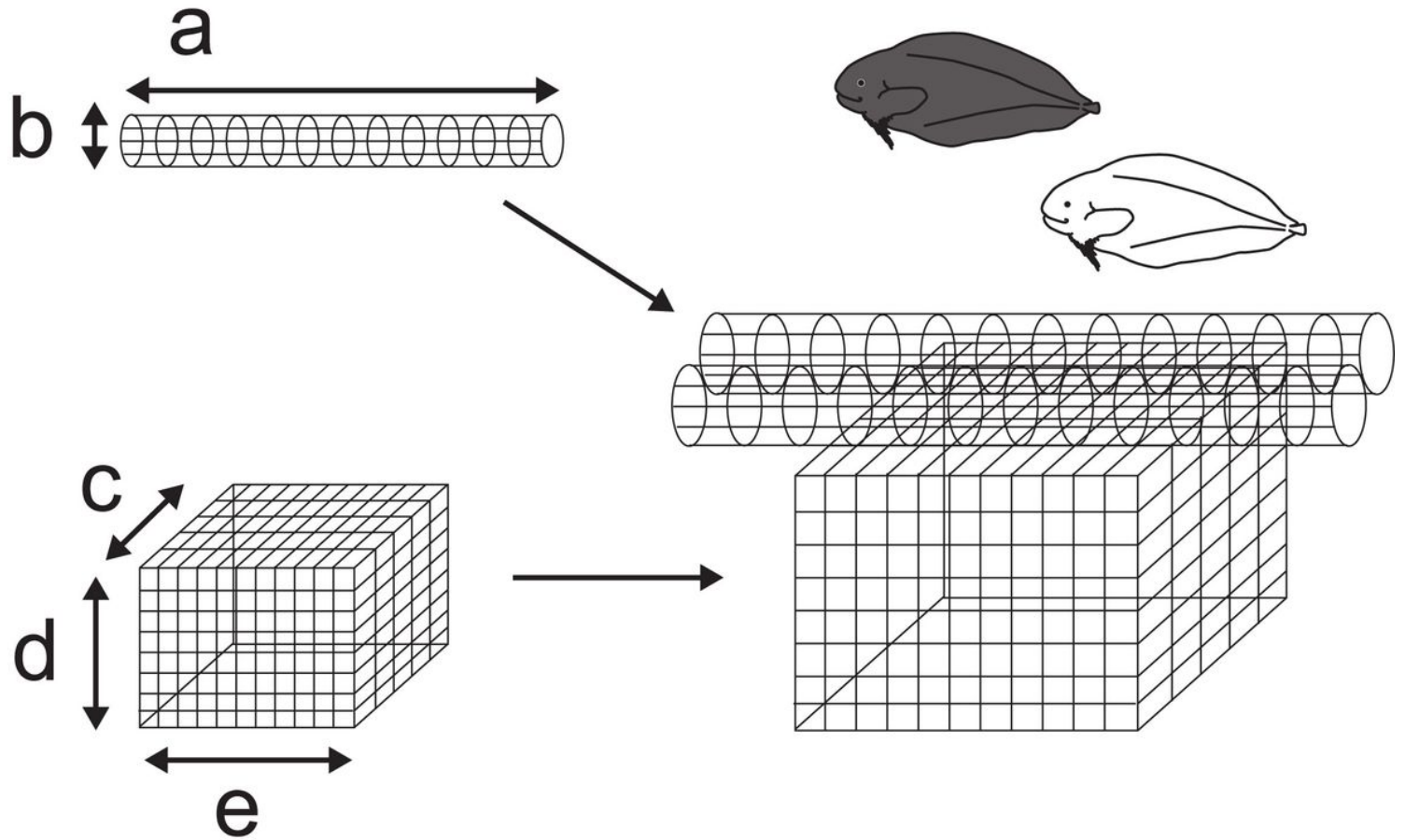


Figure 2

Schematic diagram of the artificial spawning substrate. It consisted of combining a cube and two columns made of polyethylene net (10 × 10 mm mesh size). a 280 mm length, b 40 mm inside diameter, c 220 mm depth, d 160 mm height, e 220 mm width

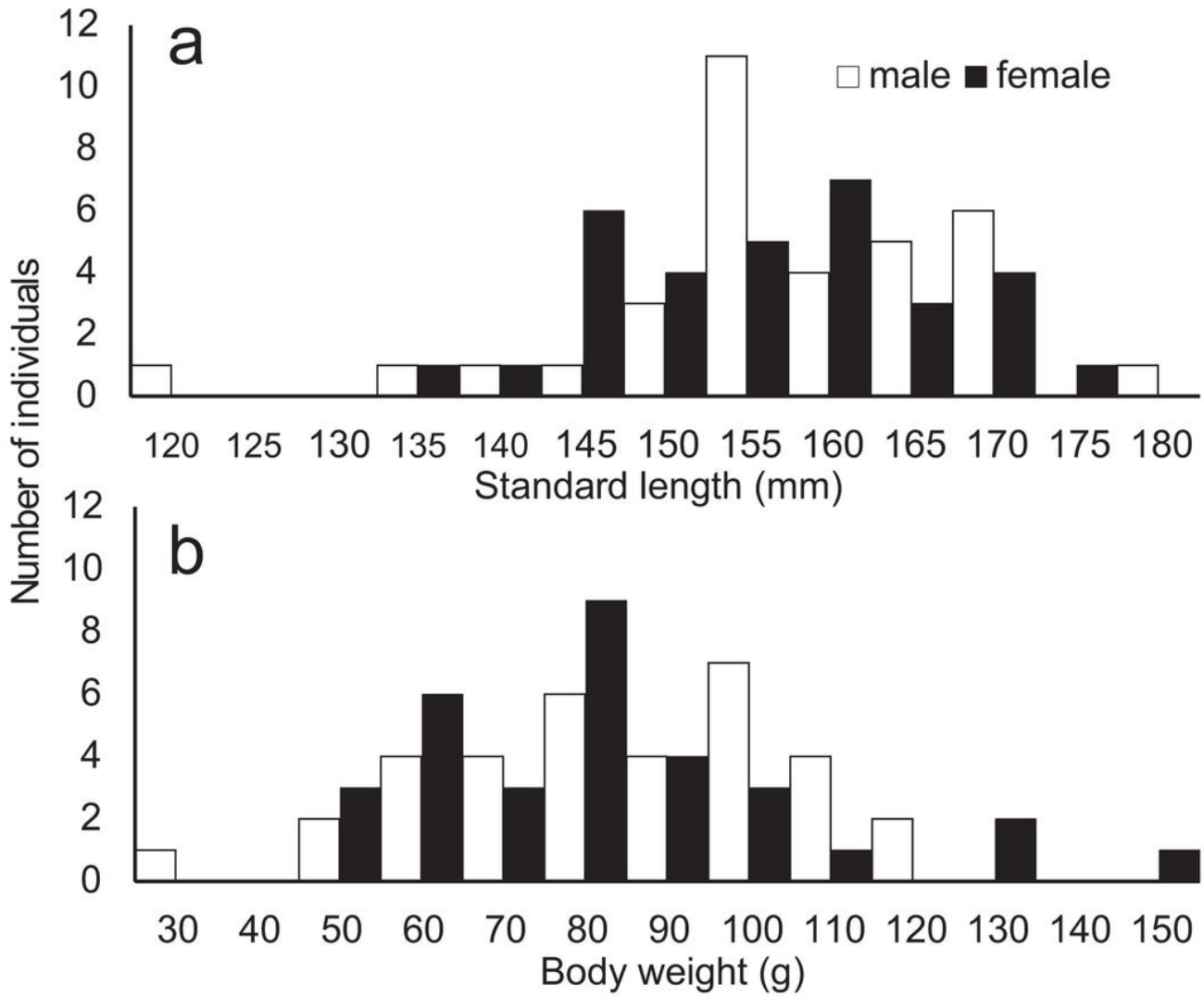


Figure 3

Standard length (a) and body weight (b) distributions of male and female *Careproctus pellucidus* (n = 66). White bar male, black bar female

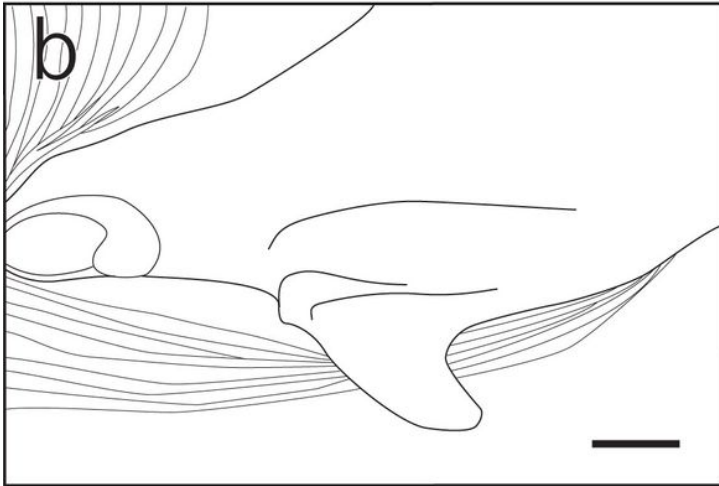


Figure 4

Shape of the urogenital papilla. a, living *Careproctus pellucidus* in the aquarium. Black solid square indicates the rough position of a urogenital papilla. b, shape of the male urogenital papilla. c, shape of the female urogenital papilla. Bar = 1.0 mm.

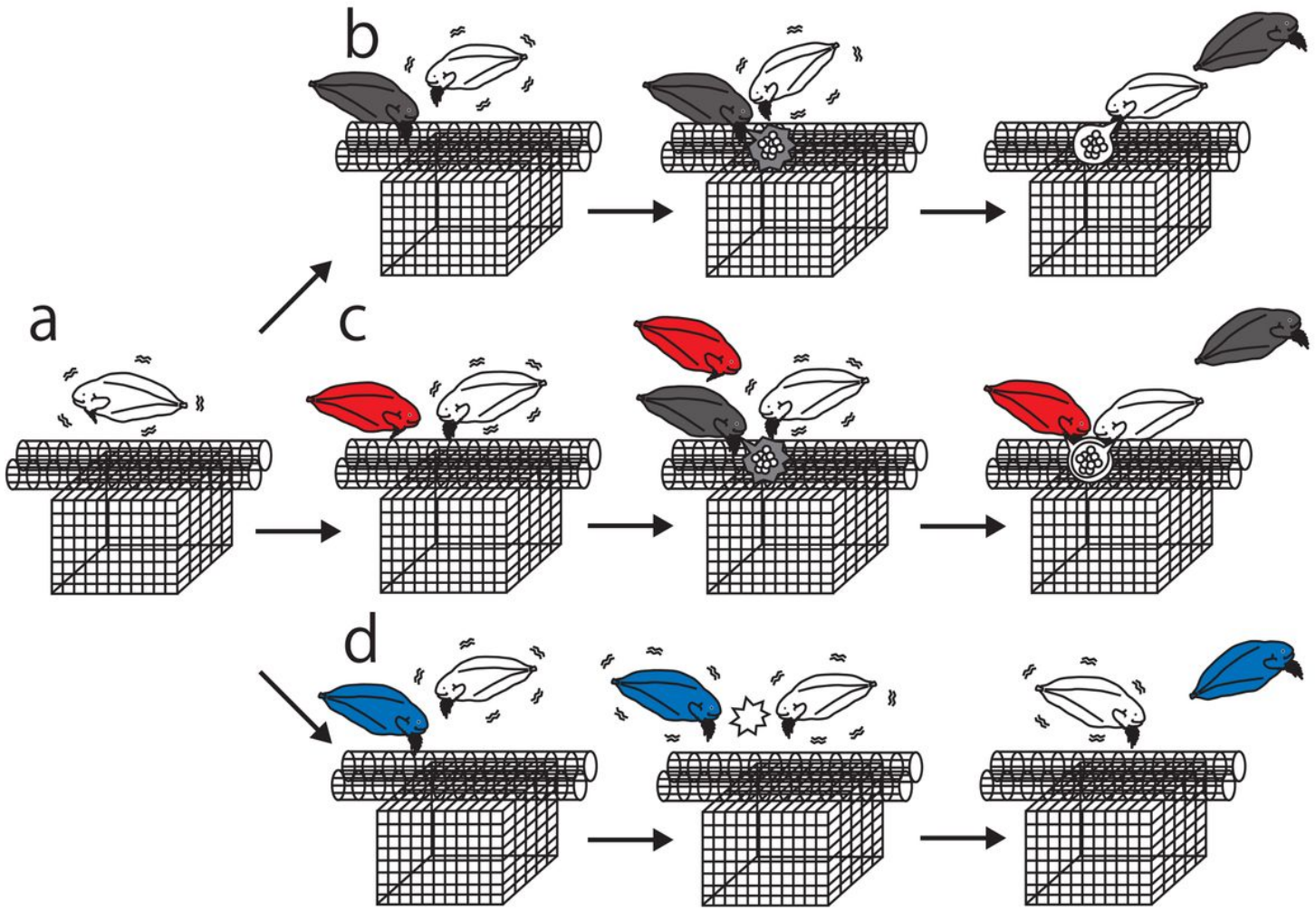


Figure 5

Behavioral sequence of pair spawning (b), sneaking (c), and male–male competition (d) beginning with territorial behavior of male (a). See text for a detailed explanation. white individual territorial male, dark gray individual female, blue individual non-territorial male, red individual sneaker, gray balloon mark spawning with sticky ovarian cavity fluid, white balloon mark sperm releasing

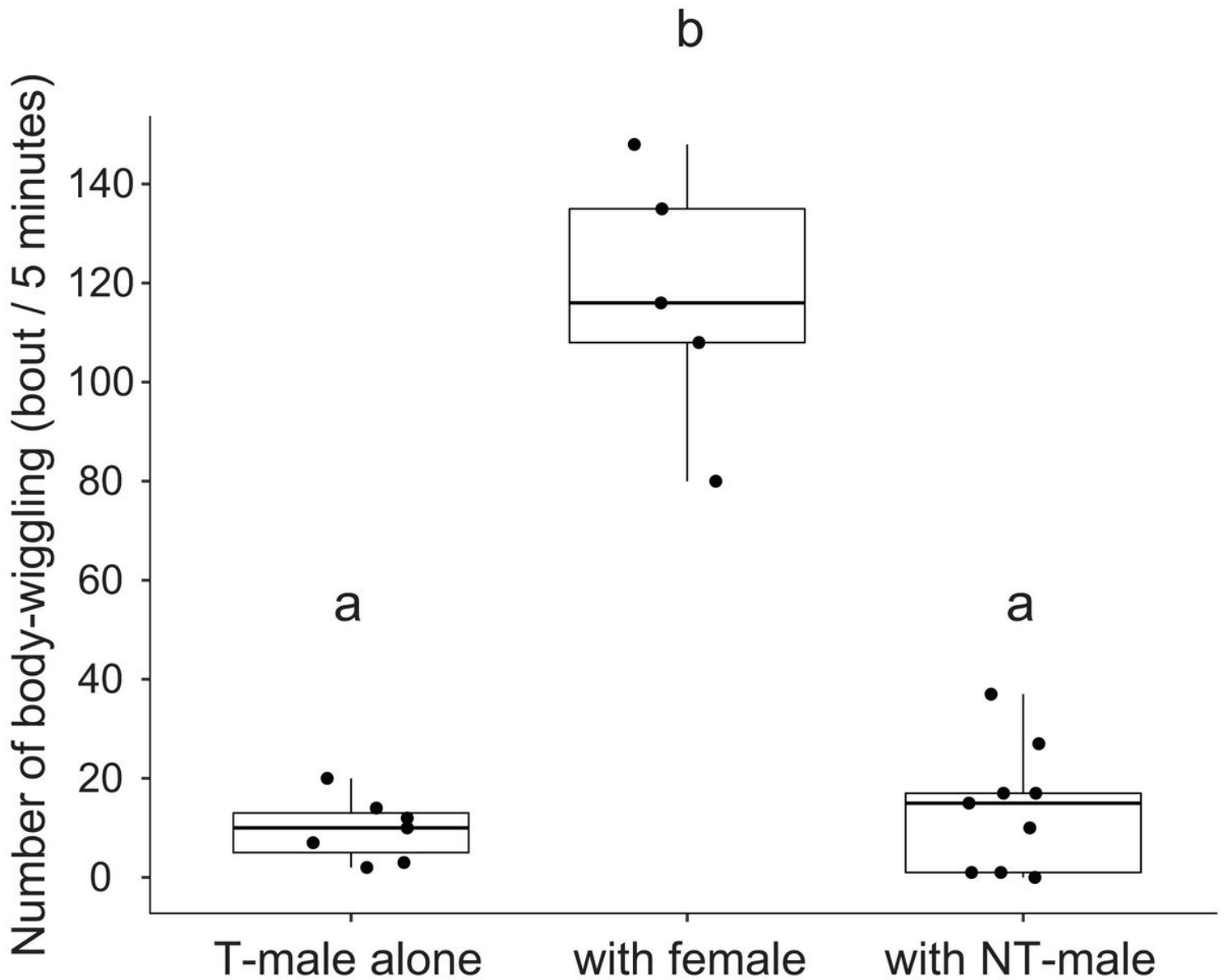


Figure 6

Frequency of body wiggling behavior of territorial male (T-males) in three situations: T-male alone (n = 7), T-male with female (n = 5), and T-male with non-territorial male (NT-male) (n = 9). Data not sharing the same letter are significantly different ($P < 0.05$) by Tukey-Kramer test. All data are shown in plots. Box plots indicate first and third quartiles, a horizontal line in the box indicates the median, and the whiskers indicate high and low values.

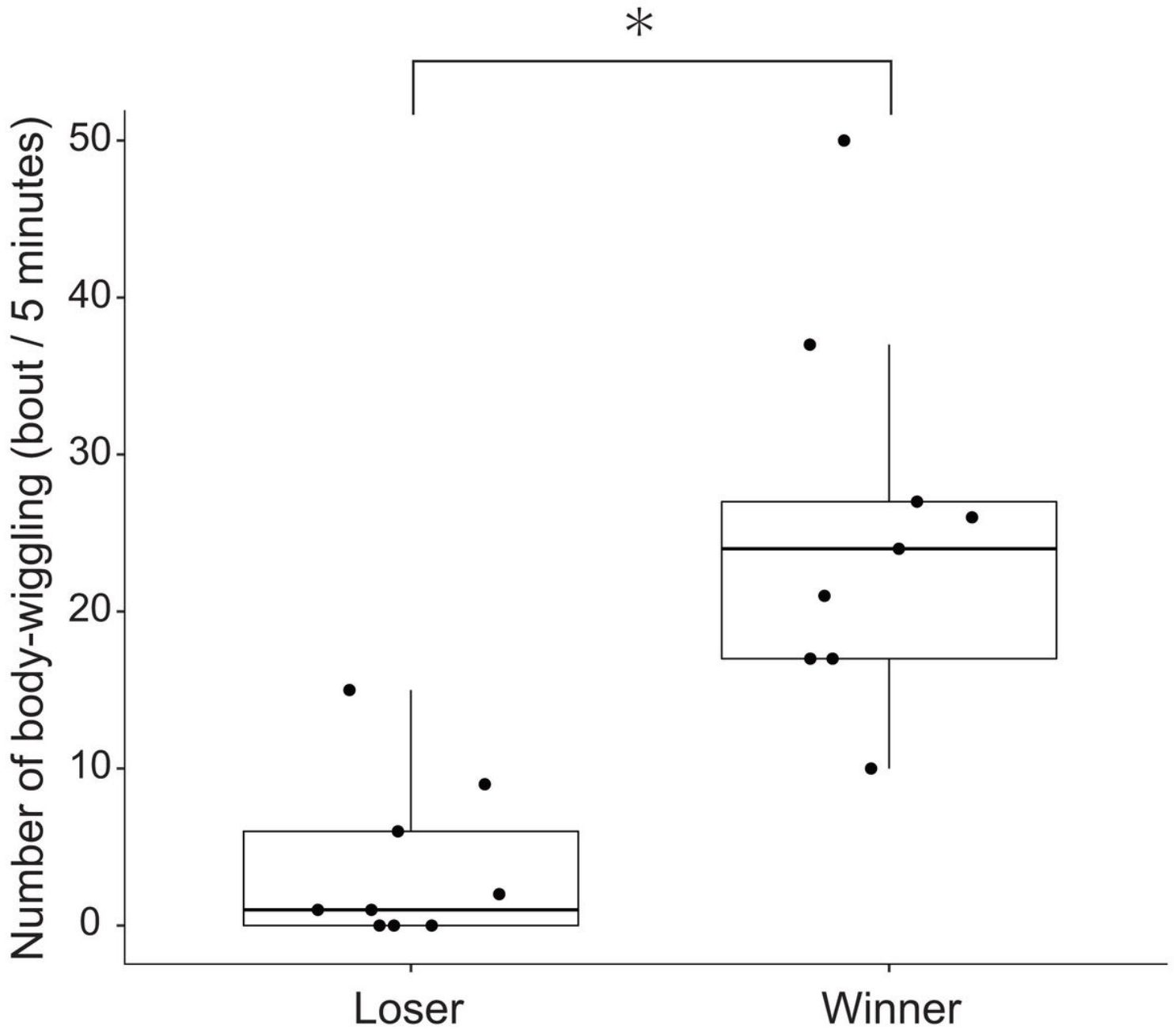


Figure 7

Frequency of body wiggling behavior of winners and losers in male–male competition (n = 18). There is a significant difference (* $P < 0.05$) based on the Wilcoxon Rank Sum test. All data are shown in plots. Box plots indicate first and third quartiles, horizontal line in the box indicates the median, whiskers indicate high and low values, and plot on the outside of the whiskers indicate an outlier.

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