The relationship between nest aggregation and reproductive behavior in the blenniid fish *Meiacanthus kamoharai*

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Abstract   The effects of nest aggregation on reproductive behaviors were studied in the blenniid fish *Meiacanthus kamoharai*. Behavioral observations were undertaken at two artificially established nest sites with different nest densities (aggregated vs. dispersed), in Tachibanaura Bay, Kochi Prefecture, Japan, in 2018. Significant differences of courtship and intrasexual aggressive behaviors were found between fish in the aggregated and dispersed nest sites. A temporary flux in the operational sex ratio (OSR) at the two experimental sites may account for these differences. Accordingly, such variation in the reproductive behaviors of females and nesting males may reflect a plastic behavioral response to variability in nest distribution.

Introduction

Generally, males compete for females and perform a courtship display for mating due to strong sexual selection (Hoelzer 1990). Conversely, in some species, the direction of sexual selection is reversed from conventional sex roles (Gwynne 1991; Vincent et al. 1992; Kvarnemo and Ahnesjö 1996). The sex roles typically are significantly affected by the operational sex ratio (OSR, the ratio of sexually active males to fertilizable females) (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). Availability for reproductive resource (e.g. spawning substrate) can affect the

OSR (Ahnesjö et al. 2001). If the OSR skews toward females, female–female competition and female courtship will be predominant (Emlen and Oring 1977; Saraiva et al. 2011).

Nest sites are an essential reproductive resource for substrate-brooding fishes (Mück et al. 2013). Furthermore, nest distribution can influence mating systems and reproductive behaviors in a variety of fishes (Reichard et al. 2008; Saraiva et al. 2009, 2011; Ota et al. 2012; Mück et al. 2013). For example, the peacock blenny *Salaria pavo* and the two-spotted goby *Gobiusculus flavescens* has been confirmed that a few large males monopolize multiple nests where available nests are aggregated (Saraiva et al. 2009; Mück et al. 2013). Thus, the OSR will become female-biased leading to sex-role reversal, with female active courting and competing for mating (Borg et al. 2002; Saraiva et al. 2009). Additionally, monopolization of multiple nests in aggregated nest sites by large males was also confirmed in a population of *S. pavo* in Ria Formosa (southern Portugal) where scarcity of adequate nesting substrate (Almada et al. 1994, 1995; Oliveira 1999). Saraiva et al. (2011) compared two populations of *S. pavo* that differed in the degree of nest aggregation and discovered variation in mating systems between them. However, only few field experiments have been conducted to study intra-populational differences in nest aggregation in fishes (Borg et al. 2002).

Meiacanthus kamoharai Tomiyama, 1956 is a blenniid fish inhabiting rocky shores and coral reefs in southern Japan (Yoshino 1984). This species deposits eggs on both natural and artificial substrates, the latter including empty cans, bottles and pipes. The adhesive demersal eggs typically measure 1.25–1.32 mm in long diameter and 0.65–0.80 mm in short diameter (Ohshiro et al. 1976). The species is sexually dimorphic, with more-elongated caudal-fin rays and longer pelvic-fin rays in males compared to females. The spawning season of this species is from June to September. The mating system is suggestive of male-territory-visiting polygamy (Kuwamura 1997), with males assuming exclusive parental care of eggs in the nest (Oyama et al. 2020). Oyama et al. (2020) found that courtship displays were performed by both sexes when females visited or passed a nest occupied by a male; females basically formed a small school consist of 2–5 individuals when visiting male's nests for breeding. Courtship displays by single females towards several males were frequent in June and July, while males clearly exhibited courtship displays in August, which indicates that the courtship roles of individuals could change seasonally during the spawning season (Oyama et al. 2020).

In Tachibanaura Bay, Kochi Prefecture, there were several natural aggregated and dispersed nest sites that were consisted of empty bottles, and nesting males of *M. kamoharai* were observed

in both sites during the spawning season. In particular, multiple nesting males congregated in natural aggregated nest sites. Thus, the specific objective of this study was to examine whether differences in nest aggregation affecting reproductive behaviors of *M. kamoharai*. Here, we conducted field observations at two experimental sites in Tachibanaura Bay, which are characterized by a different density of artificial nest aggregations. Male and female courtship display, intra-sexual aggressive behavior and the numbers of eggs in each nest were compared between two artificially established sites: an aggregated nest site and a dispersed nest site (Fig. 1). It is predicted that the aggregated site will be occupied by multiple males, and the group of gravid females will more frequency visit this site for the purpose of spawning. Our results show, males in the aggregated site did not monopolize multiple nests. Therefore, the temporal OSR will become equal or male-biased with concomitantly more frequent male courtship and male–male aggression behaviors in the aggregated site. Conversely, the temporal OSR will become female-biased with concomitantly increased female courtship and female–female aggression behaviors in the dispersed site.

Material and methods

Observation sites

Field observations of *M. kamoharai* were conducted using SCUBA at the two artificial nest sites in Tachibanaura fishing port (32 ° 49 ' 29 '' N, 132 ° 39 ' 20 '' E), on May 1 and from June 17 to July 2 in 2018. The experimental sites were set at depths of 1–5 m; the substrate consisted of various sized rocks, in addition to sandy gravel, mud and coral (Oyama et al. 2020).

Experimental set-up

To clarify whether differences in nest aggregation affect the frequency of reproductive behavior, artificial nests made of polyvinyl chloride tubes (3 cm inside diameter, length 30 cm) were set on the bottom at an angle of ~90° on May 1, 2018. The inner surface of each nest was covered with a plastic film, the subsequent removal of which enabled the observation of eggs. To create the aggregated site (AS) of nests, ten pipes were set in two lines within an area of 0.5×2 m (nest density, 10 pipes/m^2) (Fig. 1). To create the dispersed site (DS) of nests, five pipes were set in a single line in an area measuring 5×20 m (nest density, 0.05 pipes/m^2) (Fig. 1). The observation sites were set ca. 20 m apart and we did not observe other nests of males *M.*

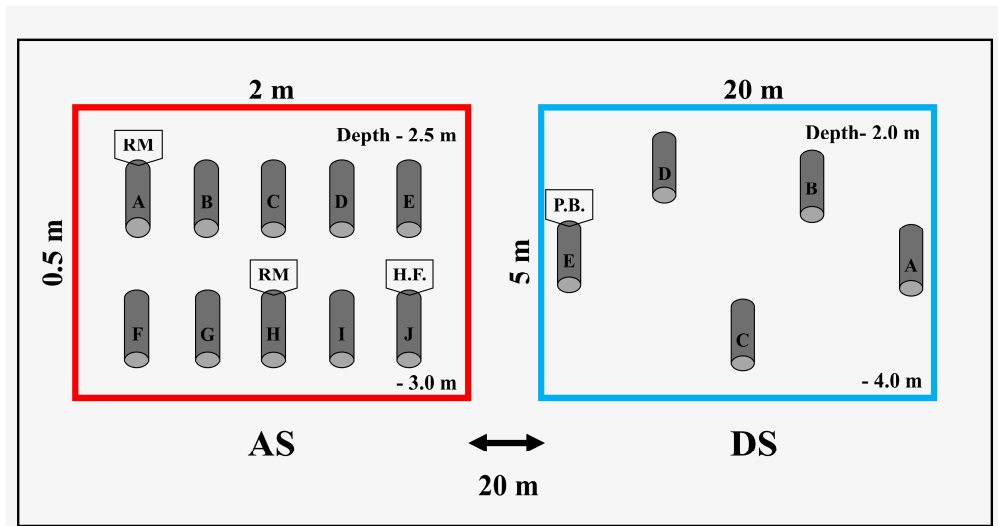*kamoharai* between or near the two artificially established sites.

Figure 1. Top view of the artificial set-up at two observation sites, AS and DS for nesting *Meiacanthus kamoharai* in Tachibanaura fishing port in 2018. Cylinder represent polyvinyl chloride tubes (3 cm inside diameter, length 30 cm) at each site. AS, aggregated site; DS, dispersed site; RM, replaced by newcomer male; H.F., occupied by *Hapalochlaena fasciata*; P.B., occupied by *Petroscirtes bleviceps*.

Behavioral observations

In *M. kamoharai*, the breeding time during the day was reported between 8:00 and 15:00 at the study location (Oyama et al. 2020). Similarly, our observations were conducted for a total of 210 minutes per day (8:00–15:00) between June 19 and July 2, and fish behavior was recorded through visual observations and with an underwater camera (Olympus TG-5). At the AS, five of the ten pipes were simultaneously observed for 30 min, and the other five pipes for the next 30 min. At the DS, each of the five pipes were observed for 30 min. To compare reproductive behavior of *M. kamoharai* between the two-type artificial nest sites, the number of nests occupied by males, courtship displays of both sexes, and instances of male and female intrasexual aggression were recorded. Females showed courtship behavior by approaching a nesting male and displaying her rounded belly at the nest entrance. In male courtship behavior, a male would approach several females near the nest and return to the nest after a signal jump (Abel 1961) or show lateral displays with repeated expanding and folding of all his fins. We defined the count unit of courtship behavior as from the start of the display by male or female to the moment of being received or rejected by the opposite sex (Oyama et al. 2020). Intrasexual aggression of *M.*

kamoharai includes lateral displays and biting each other (Oyama et al. 2020). We determined the count unit of intrasexual aggression as from the start of the display by male or female to the moment of being settled. Adult fish were sexed according to the length of the elongated caudal-fin rays and pelvic-fin rays and abdominal swelling in females (Oyama et al. 2020). Nesting males were individually identified by their characteristic head color pattern.

Clutch size and male receptivity for a female

To compare nesting male reproductive success and the number of males that are ready to mate with females of *M. kamoharai* between two experimental sites, the plastic film in each artificial nest was carefully removed and the clutch was photographed every day, between June 20 and July 2. The film was reattached to the nest immediately after taking a photograph and males went on caring for eggs. Egg numbers were determined from the photographs using the feature of Analyze Particles in Image J software (https://imagej.nih.gov/ij/). Egg developmental stages were determined according to the progress of embryo development, such as the degree of eye development (Karino and Arai 2006). Individuals of male *M. kamoharai* are capable of simultaneously caring for multiple batches of eggs in an approximately similar stage of development, and hatching occurs 10–13 days after spawning (Oyama et al. 2020). Nesting males do not accept additional batches from females when they tend eggs from older batches (e.g. 6 days old, or just before hatching), even if there is available space within their nests to receive further eggs from gravid females. Therefore, a male caring for batches (0–5 days old) with additional space in its nest and having additional batches on the following observation day is here defined as a 'receptive male'. The number of receptive males for spawning on an observation day was divided by the area of each observation site to compare quantitively between two experiment sites. NDRM (the number of daily receptive males = the number of receptive males/one day/m^2) was calculated to compare between the two experimental sites.
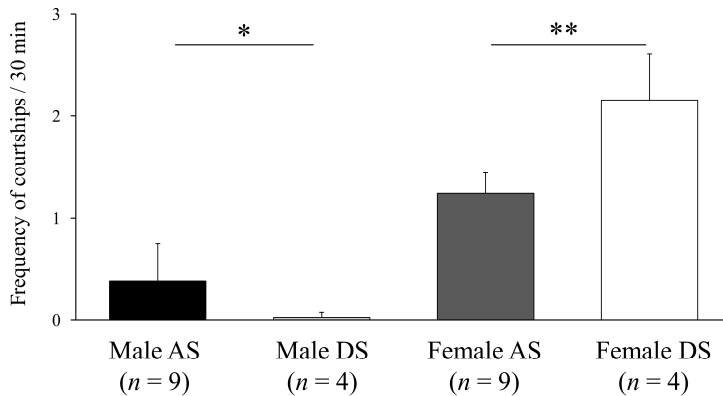
Statistical analyses

Values are presented as mean $\pm$ standard deviation. Male of *M. kamoharai* nest occupation rate between AS and DS was examined using Fisher's exact probability test. The frequency of behaviors per 30 min (courtship displays and intrasexual aggressions in both sexes) between experimental sites were compared by Mann-Whitney *U* test. We further used Mann-Whitney *U* test to analyze differences in the number of eggs in nests and NDRMs between AS and DS. All

tests were two-tailed and the significance level was set at 0.05. All statistical analyses were performed using of R version 3.6.1 (R Core Team).

Results

Behavioral observations

During the observation period from June 19 to July 2, males of *M. kamoharai* occupied nine of the ten available AS pipes (AS-A, B, C, D, E, F, G, H, I); however, two nesting males were replaced by two other males as of June 25 (AS-A, H) (Fig.1). The eggs of the former males did not remain in the inner surface area of the pipes AS-A and AS-H when nesting males exchanged. At four of the available five DS pipes, the same males occupied the nests for the entire period (DS-A, B, C, D) (Fig. 1). All nesting males occupied one pipe and there was no male who occupied two or more pipes at both sites. However, nesting males could care for multiple batches simultaneously in one nest (Oyama et al. 2020). Even though *M. kamoharai* was predominantly occupying the artificial pipes at both sites for reproduction, one pipe in AS was monopolized by a blue-lined octopus, *Hapalochlaena fasciata*, (AS-J, Fig. 1) and one pipe in DS was occupied

Figure 2. The frequency of *Meiacanthus kamoharai* male and female courtship displays, per 30-minute observation period, for fish at nests in an artificially aggregated site (AS) and a dispersed sites (DS), in Tachibanaura Bay, Kochi Prefecture, Japan, from June 19 to July 2, 2018. * $P < 0.05$, ** $P < 0.01$, Mann-Whitney U test. Values are presented as the mean + 1 standard deviation of mean. Male, male courtship behavior; Female, female courtship behavior.
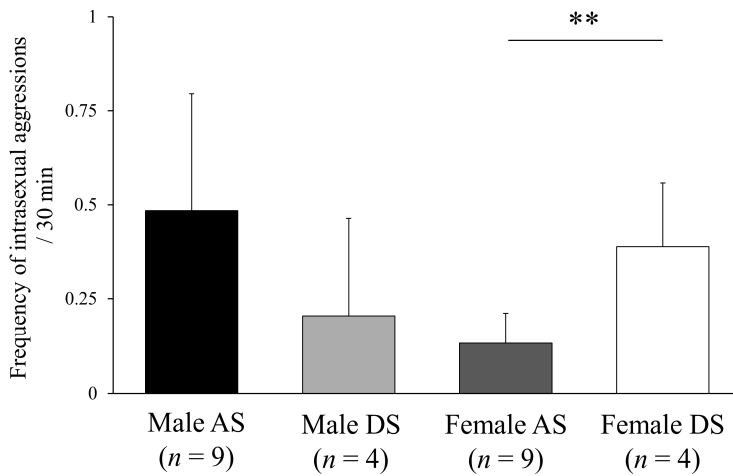
Figure 3. The frequency of *Meiacanthus kamoharai* intrasexual aggressions, per 30-minute observation period, for fish at nests in an artificially aggregated site (AS) and a dispersed sites (DS), in Tachibanaura Bay, Kochi Prefecture, Japan, from June 19 to July 2, 2018. ** $P < 0.01$, Mann-Whitney U test. Values are presented as the mean + 1 standard deviation of mean. Male, male–male aggressive behavior; Female, female–female aggressive behavior.

by combtooth blenny, *Petroscirtes bleviceps*, (DS-E, Fig. 1). Thus, the occupancy rate was always 100% in both experimental sites throughout the entire observation period comprising three different species including a cephalopod. There was no significant difference in male *M. kamoharai* occupation rate of nests between AS and DS (AS, 9/10; DS, 4/5; Fisher's exact probability test, $P > 0.05$).

Female courtship displays were less frequent around the AS nests as compared with the DS nests (AS: mean $\pm$ SD = 1.24 $\pm$ 0.20, range = 0.91–1.45, $n = 9$; DS: 2.16 $\pm$ 0.45, 1.82–2.82, $n = 4$; Mann-Whitney U test, $U = 0.0$, $P < 0.01$) (Fig. 2). In contrast, male courtship displays were more frequent at the AS than at the DS nests (AS: 0.38 $\pm$ 0.36, 0.00–0.64, $n = 9$; DS: 0.02 $\pm$ 0.05, 0.00–0.09, $n = 4$; Mann-Whitney U test, $U = 32.5$, $P < 0.05$) (Fig. 2). Overall, females were more engaged in courtship behavior compared to males (female: 1.52 $\pm$ 0.52, 0.91–2.82, $n = 13$; male: 0.27 $\pm$ 0.34, 0.00–1.18, $n = 13$; Mann-Whitney U test, $U = 91.0$, $P < 0.005$) (Fig. 2).

Female–female aggression was less frequent at the AS than at the DS (AS: 0.13 $\pm$ 0.08, 0.00–0.27, $n = 9$; DS: 0.39 $\pm$ 0.17, 0.27–0.64, $n = 4$; Mann-Whitney U test, $U = 1.0$, $P < 0.01$) (Fig. 3). Nevertheless, the frequency of male–male aggression did not differ significantly between the observation sites (AS: 0.48 $\pm$ 0.31, 0.00–1.00, $n = 9$; DS: 0.20 $\pm$ 0.26, 0.00–0.55, $n = 4$; Mann-Whitney U test, $U = 28.0$, $P > 0.05$) (Fig. 3).

Clutch sizes and numbers of receptive males

The number of eggs did not significantly differ between the two artificial sites (AS: mean $\pm$ SD = 11,827 $\pm$ 905 eggs, range = 10,508–12,854, $n = 7$; DS: 10,409 $\pm$ 1,853, 8,434–12,858, $n = 4$; Mann-Whitney U test, $U = 8.0$, $P > 0.05$). NDRM was significantly lager at the AS site (2.08 $\pm$ 0.86 males, 1.00–4.00, $n = 13$) than at the DS site (0.01 $\pm$ 0.01 males, 0.00–0.03, $n = 13$; Mann-Whitney U test, $U = 0.0$, $P < 0.001$).

Discussion

The effects of nest aggregation on mating system and reproductive behavior have been reported for *S. pavo*, *G. flavescens*, the cichlid *Telamatochromis vittatus*, and European bitterling *Rhodeus amarus* (e.g. Reichard et al. 2008; Saraiva et al. 2009, 2011; Ota et al. 2012; Mück et al. 2013). In the present study, it was found that there was a significant difference in the frequency of several reproductive behaviors of *M. kamoharai* between the AS and the DS nests. These variations in the reproductive behavior of females and nesting males might reflect a plastic behavioral response to variability in nest distribution.

In general, as the resource required to attract mates become aggregately distributed in the natural environment, the potential of resource monopolization for obtaining additional mates increases (Emlen and Oring 1977). Saraiva et al. (2009) reported that nest site monopolization by few dominant males of *S. pavo* occurred in nest aggregated site. However, in this study, male of *M. kamoharai* always utilized only one pipe as a nest in both AS and DS, and nest site monopolization (i.e. occupation of > one pipe) was not observed.

Meiacanthus kamoharai was the main occupant of artificial pipes. However, two other species successfully occupied and defended their pipes for shelter/reproduction. This shows that nesting sites are a limiting resource and that there is competition for access.

Female courtship behavior and female–female aggressive behavior were more frequent around the DS site than the AS site (Fig. 2, 3). The field observations were conducted during the female courtship season (June and July), when females actively court nesting males for mating opportunities (Oyama et al. 2020) (Fig. 2). These results indicate that competition among females in *M. kamoharai* for mating partner was enhancing in the DS nests. In some blenniid species, females show a tendency to decrease mate selectivity when their mating opportunities are limited (Oliveira et al. 1999; Neat et al. 2002). In the common goby *Pomatoschistus microps*, a scarcity

of suitable nesting structures increases simultaneous female visits and female courtships (Borg et al. 2002). In contrast, Saraiva et al. (2009, 2011) reported the frequency of female courtship behavior was higher in the aggregated nest than dispersed nest site population in the blenniid fish *S. pavo*. It is considered that the two ecological features in *M. kamoharai* are the cause of the difference between this study and previous study in *S. pavo*. In *M. kamoharai*, monopolization of multiple nests by dominant males did not occur on aggregated site, and females often approach the nests of males by the school consist of two to five individuals (Oyama et al. 2020). Thus, when a female school visits nests where males guarded, the operational sex ratio (OSR) may temporarily become male-biased at AS nests and female-biased at DS nests. Therefore, competition among females may be higher around DS nests than at AS nests and female courtship behavior and intra-sexual aggressive should be comparatively more prevalent at DS nests.

The frequency of male courtship displays was higher at the AS than at the DS nests (Fig. 2). This result suggests that male courtship behavior is affected by the OSR at both types of site. Although competition among male for breeding was more probable at the AS than at the DS nests, the frequency of intra-sexual aggressive behavior did not significantly differ between the two observation sites. It was reported that the male of the mottled blenny *Istiblennius enosimae* showed aggressive behavior against potential egg predators who entered an area of about 50 cm around the nest hole (Murase and Sunobe 2009). The territorial area where males take aggressive behavior might have been narrower than the distance between neighboring nests at the AS. Thus, the frequency of male–male aggressive interaction did not significantly differ between the two observation sites.

The numbers of eggs in the nests guarded by males did not significantly differ between the two observation sites. Therefore, variations in nest distribution likely have little effect on the reproductive success of nesting males or mate preference by females to nesting males, in the season studied. In contrast, the number of daily receptive males will be more abundant at the AS. This result suggests that there is a possibility that temporal OSR become male-biased at AS.

In conclusion, this study experimentally examined the effect of nest density on male and female reproductive behaviors in the blenniid fish *M. kamoharai*. The results show several differences in some behavioral parameters between sites with artificial aggregated nests and dispersed nests. A temporarily flux in the OSR at the two observation sites may account for these results. Therefore, we concluded that males and females of *M. kamoharai* can plastically adjust their reproductive behavior according to nest density.

Acknowledgements

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