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Effects of Parental Care and Body Size on the Reproductive Success of the Paradise Fish *Macropodus opercularis* (L.) in a Small Area

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Wen-Bin Huang and Chih-Chieh Chang (2011) Effects of parental care and body size on the reproductive success of the paradise fish *Macropodus opercularis* (L.) in a small area. *Zoological Studies* **50**(4): 401-408. The paradise fish *Macropodus opercularis* (L.) is a species famous for its predominant behaviors of male parental care, and in this territorial species, a larger male seems to have an advantage in terms of reproductive success. This study was conducted to examine the effect of parental care on the reproductive success of the paradise fish using different parental-care treatments of male-only care, female-only care, bi-parental care, and no-parental care. Hatching success of the paradise fish significantly differed among the 4 parental-care treatments. Hatching rates (HRs) averaged 94% for the male-only and bi-parental care treatments, 82% for female-only care, and 62% for no-parental care. There was no significant difference in HRs between male-only and bi-parental care, suggesting that when the male is present, the female's contribution toward hatching success is insignificant. Also, there were significant positive correlations of egg numbers (ENs) and HRs to standard lengths (SLs) of parental males (SL_{male}). Their regression relationships were: $\ln(EN) = 5.336 \times 10^{-2} SL_{male} + 4.207$ ($r^2 = 0.461$) and $HR = 2.511\ SL_{male} - 53.806$ ($r^2 = 0.661$). These results suggest that reproductive success of the paradise fish primarily depends on male parental care. Based on the HRs, male fish were divided into 2 distinctive size groups: a small- (43.7-45.7 mm) and a large-size group (49.2-62.1 mm) with mean HRs of 48.6% and 90.0%, respectively. Accordingly, a minimum body length of about 49 mm was the size for the male to attain the plateau of a maximum HR. http://zoolstud.sinica.edu.tw/Journals/50.4/401.pdf

Key words: Hatching rate, Egg number, Size effect, Breeding success, Labyrinth fish.

The paradise fish *Macropodus opercularis* (Linnaeus) is a popular aquarium fish. This is due to its vivid red and blue colors and also its interesting reproductive behaviors of bubble-nest construction and parental care. It belongs to the family Osphronemidae in the suborder Anabantoidei (Britz 2001). The paradise fish is native to Taiwan (Tsai 1992, Young 1995), is also widely distributed in China (Yangtze River basin to Hainan I.) and northern Vietnam in East Asia, and has been introduced all over the tropical and subtropical world (Freyhof and Herder 2002). It has a labyrinthiform organ, formed by the expansion of the 1st epibranchial that allows it to breathe in submarginal or even anoxic water (Lagler et al. 1977, Nelson 2006). The paradise fish sexually matures at approximately 6 mo, breeds well in aquaria, and reaches a maximum size of approximately 80-100 mm in standard length (SL; Forselius 1957, Hall 1968). The number of eggs produced per female spawning sequence was reported to vary from a few hundred (Tsai 1992, Huang and Cheng 2006) to nearly a thousand (Hall 1966). Its reproductive season in the wild in Taiwan is from Mar. to Oct. with a peak from May to July (Chen and Fang 1999). Indigenous to Taiwan,

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the fish was widely distributed in lowland areas, in rice paddy fields and lakes, prior to the mid-1970s, but has become rare in the last few decades due to the construction of farmland irrigation canals and ditches and the heavy use of pesticides and insecticides by agriculture (Tzeng 1990, Young 1995, Chen and Fang 1999, Shao and Chen 2003).

Parental care promotes the survival and well-being of the next generation at a cost to the current generation's resources (Gross 2005). In addressing parental care, Gross proposed 2 major questions: "who cares?" and "how much?" In fish species in which males are bigger than females, males are often the predominant providers of parental care (Downhower et al 1983, Pyron 1996). The paradise fish is slightly sexually dimorphic; males are brighter in color and usually larger than females. It was categorized as being of the male-care type with occasional help from the female (Richter 1988, Cole et al. 1999). Female paradise fish often show behaviors of retrieving eggs and bubble-blowing with non-aggressive males (Hall 1968). However, there is no quantitative information on the level of parental care contributed by each sex of the paradise fish during the reproductive cycle.

The body size of a male is one of the important characteristics which a female uses to evaluate a mate's genetic quality and resource-holding potential (Zahavi 1975, Andersson 1994). For example, females of the banggai cardinal fish *Pterapogon kauderni* Koumans adjust their offspring weight according to the size of the male mouthbrooding partner (Kolm 2001). The body size of the male Siamese fighting fish *Betta splendens* Regan may affect reproductive success by influencing the quality of his parental care provided to the brood (Jaroensutasinee and Jaroensutasinee 2001b). Therefore, the size and number of eggs produced by the female may be male size-dependent in fish species with male parental care. In addition, fecundity is usually female size-dependent within a species (Bone and Moore 2008). It was found that the larger that both the male and female are, the more eggs the female produces of the blue gourami *Trichogaster trichopterus* (Pallas) (Degani 1989) and Siamese fighting fish (Clotfelter et al. 2006).

Numerous studies were conducted on the paradise fish and related species in the past. A short summary of those studies was made by Huang and Cheng (2006). In the middle of the last century, studies were mainly concerned with behavior, distribution, endocrinology, osteology, phylogeny, and physical and physiological factors affecting spawning at the family level of anabantoid species, including *M. opercularis*. Later studies focused on behaviors associated with predator avoidance (Csanyi 1985 1986, Gerlai 1993, Miklosi et al. 1997, Miklosi and Csanyi 1999), intraspecific aggression (Kassel and Devis 1975, Francis 1983, Miklosi et al. 1997), environmental toxicants (Yin and Zhang 1986, Zhang and Yin 1986), and the effects of prostaglandin-$F_{2\alpha}$ on reproductive behaviors (Villars et al. 1985, Villars and Burdick 1986). Recently, effects of temperature and floating materials in the non-reproductive season on breeding by the paradise fish were described (Haung and Cheng 2006). Despite much study of the paradise fish, little is known about the effects of parental care and body sizes on its reproductive success.

Gaining a better understanding the effects on reproduction of parental care and the body size of each sex would be beneficial to the knowledge of aquatic enthusiasts and to qualifying brood stocks for conservation and commercial purposes. Therefore, the objectives of this study were to: (1) enumerate the levels of contribution to parental care by the parental male and female of the paradise fish on reproductive success, including bubble nest size, number of eggs produced, and the hatching rate (HR), and (2) test the hypothesis that the larger the body size of the male or female is, the higher the reproductive success will be.

MATERIALS AND METHODS

Experimental fish and conditions

Adult males and females (*n* = 70 each) about 1 yr old of the paradise fish were obtained from the Aquatic Breeding Institute of the Hualien County government in eastern Taiwan in July 2007. After being brought to the laboratory, male and female fish were held separately in two 550-L circular tanks, each 110 cm in diameter and 58.5 cm in height, and equipped with a simple biofilter. They were acclimated for 2 wk at room temperature of 27.2-29.4°C and a daily photoperiod of 12 h of light and 12 h of dark. Water in the tanks was replaced with clean water once a week. The fish were fed frozen bloodworms at about 5% of their weight twice a day at 08:00 and 18:00.

Two experiments, one on parental care and the other on parental body sizes, were conducted to assess the effects of those aspects on the reproductive success of the paradise fish in this study. In both the parental-care and body-size experiments, none of the paradise fish was used more than once. Because the fish alter their appearance after anesthetization (Kodric-Brown 1989), they were not anesthetized to measure the SL. A piece of standard green Styrofoam (3 × 6 cm) was put into each experimental tank as floating material to substitute for natural floating plants, as suggested by Haung and Cheng (2006), to facilitate successful bubble-nest building and reproduction of the paradise fish. During both experimental periods, fish were kept in the same experimental tanks (25 × 20 × 15 cm), containing 5 L of water at a water depth of 10 cm, under the same conditions at which they were acclimatized. Water temperatures ranged 26.9-27.4 and 26.9-28.2°C during the parental-care and body-size experiments, respectively.

Parental-care experiment

Four parental-care treatments were designed: bi-parental care, male-only care, female-only care, and no-parental care. Three replicates were used for each of the 4 parental-care treatments. In order to reduce the interference from body-size effects in this experiment, 12 mature males and 12 mature females of approximately the same size for each sex were selected from the acclimation containers. From these 24 fish, a male and a female were randomly selected as a pair, their SLs were measured, and they were then placed in one of 12 experimental tanks. There was 1 replicate tank for each of the bi-parental care and male-only care treatments in which the female failed to spawn, even though her mate had built a bubble nest in the tank. These were excluded from the data analysis. After spawning, the male and/or female fish were carefully removed from each of the experimental tanks according to their assigned parental-care treatment. After that, the parental-care experiment commenced and lasted until all eggs had hatched.

Each tank was checked twice a day at 08:00 and 18:00 before feeding. Images of the bubble nests with eggs were taken with a digital camera using a standard scale. The size of the bubble nest was measured by calculating its area in the digital image using the software of Motic Images 2000. The egg number (EN) was counted from the image; eggs were solid and light yellow. Hatchlings (newly hatched larvae) were counted and removed from the tanks with a dropper. The HR, calculated as the total number of hatchlings divided by the EN × 100 (%), was used as one of the reproductive variables to assess the effects of parental care on reproductive success.

Body-size experiment

Eighteen males and 18 females of various sizes were selected from the acclimation tanks. From these 36 fish, a male and a female were randomly paired, their SLs were measured, and then they were placed in one of 18 experimental tanks. Both the male and female were kept in the tank until the end of the experiment, when all eggs had hatched. Of the 18 pairs, females in 5 tanks failed to spawn, even though their mates had built bubble nests in the tanks. They were excluded from the data collection and analysis. Means ± standard deviations (SDs) and ranges of paradise fish SLs were 53.7 ± 6.1 and 43.7-62.1 mm for the 13 males and 52.2 ± 2.5 and 48.5-55.6 mm for the 13 females. The HR, bubble-nest size, and EN were used as reproductive variables to assess the effect of parental body size on reproductive success. Data collection in this experiment, such as bubble-nest size, EN, and HR, was the same as in the parental-care experiment.

Data analysis

The non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) by ranks was employed to determine the significance level of differences in HRs at the end of the experiment, as well as in the male and female SLs, bubble-nest sizes, and ENs at the beginning, among the 4 parental-care treatments due to the small sample sizes in the parental-care experiment (Kruskal and Wallis 1952). Dunn's test was used as a post hoc procedure to test differences among the means of the 4 treatments (Dunn 1964). For the body-size experiment, Pearson product-moment correlation coefficients were used to assess relationships between parental body sizes and reproductive variables. A multivariate regression model with a stepwise procedure was applied to analyze the relationship of both parental body sizes, including their interactions, to each of the reproductive variables (Johnson 1998). The model was $Y = Z + SL_{male} + SL_{female} + SL_{male} \times SL_{female} + \varepsilon$, where Y is a reproductive variable, including the HR, EN, and bubble-nest size, Z is a covariate variable, including the EN and bubble-nest size if it was significantly correlated to Y, SL is the standard length, and ε is the error component in the model. The bubble-nest size and EN were logarithmically transformed for normalization in the analyses in both experiments. The software used for the statistical analyses was SPSS (Chicago, IL, USA) for Windows vers. 12.0, and all statistical tests were considered significant at $p \leq 0.05$.

RESULTS

Effects of parental care

HRs significantly differed among the 4 parental-care treatments ($p = 0.046$, $H = 8.018$, $n = 10$) (Fig. 1). The order of mean HRs (± S.D.) was 94.30% (± 2.12%), 94.05% (± 1.63%), 83.23% (± 3.26%), and 61.95% (± 1.45%) for the male-only, bi-parental, female-only, and no-parental care treatments, respectively. Two homogenous groups of HRs were derived from the 4 treatments according to Dunn's post hoc test (Fig. 1). HRs in the male-only and bi-parental care treatments were significantly higher than that in the no-parental care treatment, and insignificantly differed from that in the female-only treatment. Compared to the no-parental care treatment with the lowest HR, an increase of the HR in the female-only care treatment was about 2/3 those of the male-only and bi-parental care treatments. At the beginning of this experiment, SLs of males and females, bubble-nest sizes, and ENs used in this experiment insignificantly differed among the 4 parental-care treatments ($p > 0.05$, Table 1).

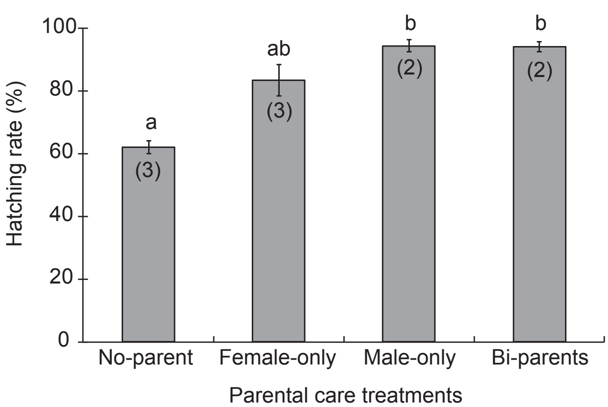

Fig. 1. Mean hatching rates of 4 parental-care treatments of no-parent, female-only, male-only, and bi-parents by the paradise fish. The hatching rate was calculated as the total number of hatchlings divided by the number of eggs times 100 (%). Numbers in parentheses are the sample sizes (number of fish pairs). The length of the perpendicular line indicates the standard deviation. Values with the same letter insignificantly differ according to multiple-range tests by the least significant difference method (α = 0.05).

Effects of body size

Both ENs and HRs were significantly positively correlated to SLs of parental males (EN: $r = 0.679$, $p = 0.011$; HR: $r = 0.813$, $p = 0.001$), but not to those of females ($p > 0.05$) (Table 2). Bubble-nest sizes were not significantly correlated to SLs of either sex, ENs, or HRs. There was a significant correlation between ENs and HRs ($r = 0.702$, $p = 0.007$) (Table 2).

After the stepwise procedures, only the variable of the parental male SL was selected for the final regression models of EN ($r^2 = 0.461$, $F_{1,11} = 9.41$, $p = 0.11$) and HR ($r = 0.661$, $F_{1,11} = 21.44$, $p < 0.001$), while no dependent variable was selected for bubble-nest size ($p > 0.05$). None of the covariates was selected for the final model, even though the EN was significantly correlated to the HR (Table 2). These 2 final models were $\ln(EN) = 5.336 \times 10^{-2}\ SL_{male} + 4.207$ (Fig. 2A) and $HR = 2.511\ SL_{male} - 53.806$

Table 1. Means ± standard deviations of male and female standard lengths (SLs), bubble nest sizes, and egg numbers of the paradise fish and *H* and *p* values of the Kruskal-Wallis test among the 4 parental care treatments at the commencement of the experiment (*n* is the sample size)

Variable	Parental care treatment				*H*	*p*
	No-parent ($n = 3$)	Female-only ($n = 3$)	Male-only ($n = 2$)	Bi-parental ($n = 2$)		
Male SL (mm)	59.2 ± 1.0	59.4 ± 1.4	58.2 ± 0.6	57.6 ± 0.6	5.000	0.172
Female SL (mm)	53.0 ± 1.1	53.8 ± 0.9	53.1 ± 1.1	54.3 ± 0.8	2.218	0.528
Bubble nest size (cm^2)	38.7 ± 13.8	33.1 ± 16.7	26.2 ± 4.0	35.3 ± 15.4	1.309	0.727
Egg number	1376 ± 297	1172 ± 400	1319 ± 473	1030 ± 534	1.073	0.784

(Fig. 3A), where ln(EN) is the log-transformed egg number and HR is the hatching rate. For female SLs, no relationship to EN was apparent (Fig. 2B), and no trend or grouping was found relative to the HR (Fig. 3B). However, for male SLs, 2 different groups of HRs with a break at 46-49 mm were apparent in the scatterplot of SLs of parental males (Fig. 3A). HRs in the tanks of males with SLs of 43.7-45.7 mm were low at 45%-52%, while HRs in tanks of males with SLs of 49.2-62.1 mm were high at 81%-94%. The latter mean (90.0%) was nearly 2-fold higher than the former (48.6%).

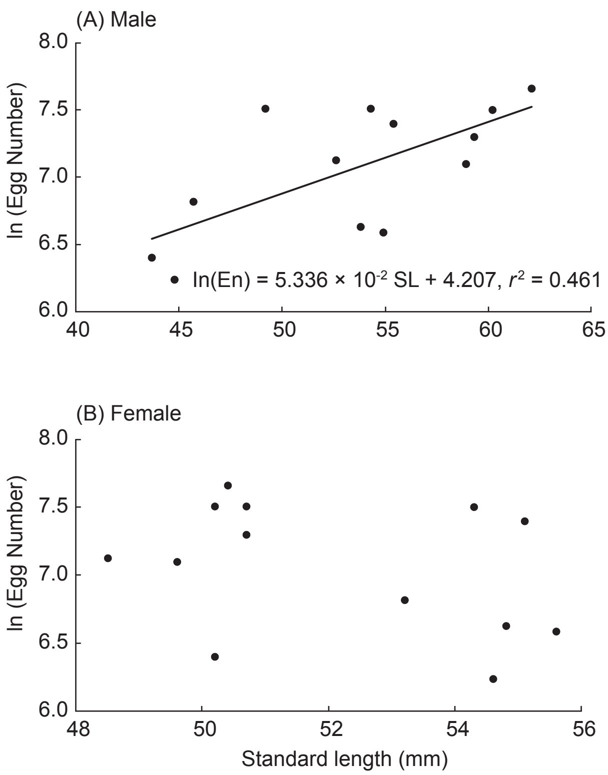

Fig. 2. Relationships between the logarithmic egg number (EN) and standard length (SL) for the male (A) and female (B) paradise fish (n = 13). The regression is shown as a solid line.

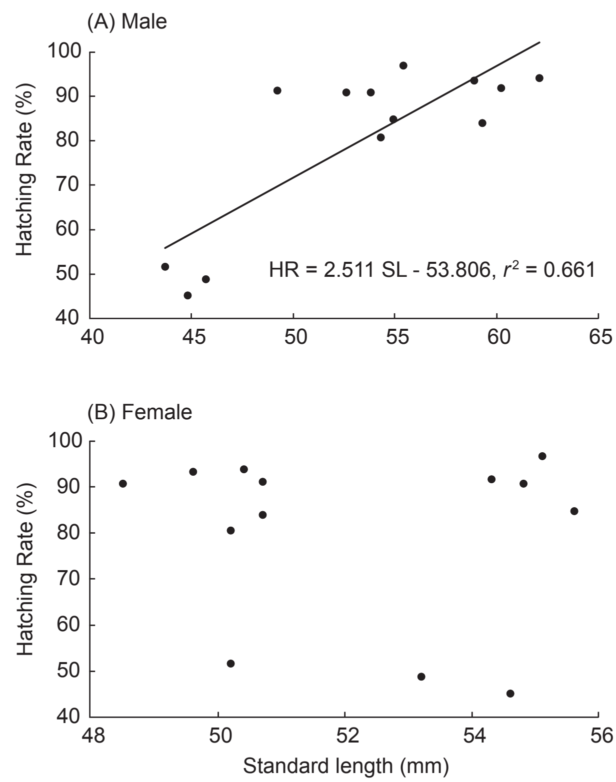

Fig. 3. Scatterplots of hatching rates against standard lengths of male (A) and female (B) parents for the paradise fish (dotted horizontal lines, average hatching rates of the small and large size groups) (n = 13). The regression is shown as a solid line.

Table 2. Pearson correlation coefficient matrix among standard lengths (SLs) of males and females, bubble nest sizes, egg numbers, and hatching rates of the paradise fish (p values in parentheses, n = 13)

	Female SL	Bubble nest size	Egg number	Hatching rate (%)
Male SL (mm)	- 0.078	0.267	0.679*	0.813**
	(0.801)	(0.378)	(0.011)	(0.001)
Female SL (mm)	-	- 0.229	- 0.357	- 0.115
		(0.452)	(0.231)	(0.708)
			0.521	0.339
Bubble nest size (cm^2)		-	(0.068)	(0.177)
Egg number			-	0.702**
				(0.007)

*$p \le 0.05$; **$p \le 0.01$.

DISCUSSION

Effects of parental care

While parental-care behavior maximizes selfish genetic interests of the parent, it is not an all-or-none behavior; rather, it is dynamically adjusted to individual circumstances (Gross 2005). Parental-care behavior is energetically costly (Wootton 1990). The amount of care a parental male provides should depend on the amount of care provided by his mate and vice versa in bi-parental species (Balshine et al. 2002). Cases of parental care behavior dynamically invested in reproduction were found in the convict cichlid *Cichlasoma nigrofasciatum* (Günther) (Galvani and Coleman 1998), guppy *Poecilia reticulata* Peters, and three-spined stickleback *Gasterosteus aculeatus* Linnaeus (Amundsen 2003). In our study, the mean HR of the paradise fish in the male-only care treatment was high (94.30%) and insignificantly differed from that (94.05%) in the bi-parental care treatment (Fig. 1). These results indicate that if the male paradise fish is on nest-care duty, the marginal benefit of a female's input may be insignificant to hatching success. In the female-only care treatment, the mean HR (83.23%) of the paradise fish was higher than that (61.95%) of the no-parental care treatment (Fig. 1). The difference between these HRs indicates that if the male is absent, the female paradise fish can take over the duty of parental care to maintain basic benefits from her energetic investment in this reproduction behavior, even though her efficiency was lower than the male's. It may even be said that a female paradise fish is not static in her parental investment, but instead incorporates the status of her mate.

In general, parental care by males may be favored in fish because external fertilization reduces the chance that a male is providing parental care to offspring it has not fathered (Blumer 1979) or because a male can defend a spawning site that is in short supply (Baylis 1981). Gross (2005) also indicated that the predominance of male parental care in fishes evolved not because the male sex obtains greater benefits from care-giving, but because males pay a lower future cost for the same benefits. Assistant breeding behaviors of retrieving eggs and bubble-blowing by female paradise fish, documented by Hall (1968), were not observed in tanks of the bi-parental treatment, but were seen in tanks of the female-only treatment in our study. Breeding in a small restricted aquarium, in which the male paradise fish showed predominant aggressive behavior, could be a significant reason why the females did not show the assistant breeding behaviors with males in our tanks of the bi-parental treatment. In short, in a small area, parental care from the male is sufficient to attain high reproductive success for the paradise fish.

Effects of parental body size

The EN is an important index of reproductive success in fish. In species of anabantoids, it is known that the larger the parents, the more eggs they produce in the blue gourami (Degani 1989) and Siamese fighting fish (Clotfelter et al. 2006). However, in our study, ENs of paradise fish were only found to be positively and linearly correlated to male body lengths (Table 2, Fig. 2). It is reasonable that a bigger male has an advantage in handling more eggs, possibly because of a greater fanning ability for efficient oxygenation, faster embryo development, and better offspring protection. Fecundity is usually female size-dependent within a species (Bone and Moore 2008). Adult females of the paradise fish can spawn several times in a reproductive season (Tsai 1992). With batch spawning, the EN of the paradise fish was found to be size-independent of the female in our study (Table 2, Fig. 2A), although it is still unclear whether or not the total spawned eggs or fecundity in a reproductive season are size-independent for the female paradise fish. However, due to successful male parental care, a female can focus her time and energy on egg production and spawning, rather than caring for the eggs. By spawning many times, she may spread the costs and risks of reproduction among many partners, large and small. She may further ensure reproductive success by laying more eggs for larger males who provide a higher HR.

The HR of eggs is another important index of reproductive success in fish. While it is known that the larger the males and females, the more fry they produce in the blue gourami (Degani 1989), it is unclear whether the greater number of fry is due to larger HRs or larger ENs, or both. In our study, HRs of the paradise fish were significantly positively correlated to male parental lengths, even though the linear fit was not so good (Table 2, Fig. 3A). However, it is evident that larger individuals of the male paradise fish, the main egg carer, had higher HRs. In addition, we found that a male size of about 49 mm was the minimum size to attain maximum breeding success (Fig. 3A). For the wild Siamese fighting fish, males likely achieve higher reproductive success by being heavier and bigger, and 3 reasons were suggested by Jaroensutasinee and Jaroensutasinee (2001a): (1) to prevent egg cannibalism by their mates, (2) to take care of a large number of eggs, and (3) to be successful in male contests and protect their offspring from predators.

In conclusion, parental-care behavior of the paradise fish had a positive effect on hatching success with the highest HRs among male-only and bi-parental care. The effect of female care on hatching success was low, at about 2/3 that of the male, if the female was single, and was apparently ineffective if the male was on duty. Also, the reproductive success (ENs and HRs) was positively correlated to male length. A body length of > 49 mm was the size at which males attained the plateau of the maximum HR. However, it should be noted that although our findings in the parental-care experiment were statistically significant using a non-parametric method due to the small sample sizes, these findings should be conservatively applied, especially the non-significant results. In addition, there is little information about failed spawning in our study, even though mating success rates, at 83.3% (10/12) and 72.2% (13/18), were high in our 2 experiments. Failed spawning behavior in restricted waters of a small area should be investigated in future research.

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