INTRODUCTION

Mature males of the medaka Oryzias latipes, egg-laying killifish which inhabit freshwater in Japan, Korea and China, have a much larger anal fin than females.1–3 This sexual dimorphism implies that the male fin has a mating-related function. The male anal fin is not a copulatory organ, but it appears to be important in stimulating the female into oviposition, because during spawning the male envelops the female with the anal and dorsal fins.4–6 Beyond this, however, the function of the anal fin in males during spawning is unknown. In the present study, we experimentally manipulated the size of the anal fin in males to examine the fin's role in the fertilization of spawned eggs. Our hypothesis is that the anal fin in male medaka is important for efficient fertilization, suggesting selection for a larger anal fin.

MATERIALS AND METHODS

The mating behavior of medaka is described below. The male follows the female and initiates the first phase of courtship, known as the ‘quick circle’.7 If the female is receptive, the male envelops her with his dorsal and anal fins, the bodies of the two fish being parallel. Both then quiver, as the eggs and sperm are released. If the female is unreceptive to the male, she usually performs ‘head-up’ movements, in which the forepart of the body is raised.8 Females usually lay 20 to 40 eggs which adhere to her ventral surface until attached to aquatic vegetation.1 In the laboratory, reproductive activity can be induced by manipulating the photoperiod, and spawning induced by prior isolation followed by pairing the two sexes.1,6 Under appropriate conditions of temperature and diet, medaka mate daily, or healthy females produce a new clutch every day for 3 or 4 months.1,9

Experimental fish were collected from an inlet of Suwa Lake, Nagano Prefecture, Japan (36°01'N, 138°06'E), in May 1995 and held in stock aquaria (about 10 males and 10 females in each). The stock aquaria were maintained at 28°C on a 14 h light:10 h dark photoperiod (‘lights-on’ at 6:00), and the fish were fed twice a day with brine shrimp (Artemia sp.) and Tetramin flake food (Tetra, Germany). The experiment was conducted between 10 October and 21 December 1995 in aquaria (30×17×22 cm) with the same temperature, photoperiod and dietary conditions as the stock aquaria. The experimental aquaria were divided into two similarly sized compartments with a removable clear Plexiglas partition, which separated a male and female until the start of the mating trial.

SUMMARY: The anal fin of the medaka Oryzias latipes is sexually dimorphic, with mature males developing a larger anal fin than females. To determine the functions of the male anal fin during mating, an anal fin-removal experiment was conducted. Males were mated with females in successive treatments in which different portions of the anal fin were removed. Male medaka with half of their anal fin removed fertilized a significantly lower proportion of eggs than did males with a normal anal fin. The results show that the anal fin in male medaka is important for efficient fertilization, suggesting selection for a larger anal fin.

KEY WORDS: fertilization success, function, Oryzias latipes, sexual dimorphism, size manipulation.
We conducted three successive anal fin treatments on each male: no-removal, edge-removal and half-removal (Fig. 1). We did not include a ‘whole-removal’ treatment in the present experiment, because some males fail to mate when the entire fin is removed (Koseki Y & Takata K, unpubl. data), probably due to abnormal mating behavior of males and/or rejection by females. We used six males and six females. Anal fin size correlates positively with body size (Koseki Y & Takata K, unpubl. data) and fecundity may increase with body size for females. To minimize possible effects of body size on the experiment, fish of similar standard length (SL) were selected (range of SL was 29.3–31.0 mm for males and 28.5–31.7 mm for females).

In the ‘no-removal’ treatment, where the anal fin was not manipulated (control), each female was placed in an aquarium compartment and each male was introduced into a separate compartment. The six pairs were allowed to mate by removing the Plexiglas partition, followed by a 1-day period of acclimation. One day was considered sufficient for females to produce a new clutch and for males to replenish sperm, based on the fact that fertilization rates of relatively large males (>27.5 mm SL) do not decline for up to six matings in 1 day.1,10 The mating trials lasted until spawning and the required time from introduction to mating was determined. Immediately after spawning, all released eggs which adhered to a female’s ventral surface were collected, and the numbers of released and fertilized eggs (see Iwamatsu11 for distinction between fertilized and non-fertilized eggs) were counted after 1 or 2 hours using a binocular microscope, for determination of the fertilization rate (FR: the number of fertilized eggs divided by the number of spawned eggs). Following the first mating trials, males were replaced with each other and each new pair was acclimatized for 1 day with the Plexiglas partition. The pairs were allowed to mate the next day. In this manner, each male was mated with the six females.

In the ‘edge-removal’ treatment, the same protocol as described above for the no-removal treatment was followed, except for the anal fin manipulation described below. The distal edges (~1 mm) of the anal fin of the six males used in the no-removal treatment were removed to examine the effect of fin removal itself. The anal fin was removed with a blade while the fish was anesthetized with 0.1% 2-phenoxyethanol. Each male was then placed in an isolation aquarium containing ~0.0003% methyleneblue for a 2-day recovery period before the following mating trials. During these 2 days, no apparent regeneration of the fin occurred.

In the ‘half-removal’ treatment, the anal fins of males were made to approximate the size of the anal fin of females or slightly smaller, by removing the distal half (Koseki Y & Takata K, unpubl. data). We allowed males to recover from the fin manipulation as in the edge-removal treatment, and then followed the same protocol for mating trials as in the no- and edge-removal treatments.

All trials in the three treatments were conducted between 6:00 and 12:00. FR data were arcsine-transformed for Pearson’s correlation coefficient and two-way ANOVA.12 For each male, Friedman’s test was applied to compare the required time for spawning among treatments because of non-normality of the data. The significance levels of Friedman’s tests were adjusted by the sequential Bonferroni method.13

**RESULTS AND DISCUSSION**

All observed pairs (four trials were missed) in the three treatments spawned within 1 hour of the start of trials (range, 1–60 min; mean, 3.38 min). No difference was observed in the required time among treatments for all males (Table 1, Friedman’s tests, \( n = 4–6 \), \( \chi^2 = 0.15–6.71 \), all \( P \) values were non-significant at 5% level after the sequential Bonferroni method. In part, this result suggests that the mating behavior and activity of females and males were not affected by the extent of fin removal, although no quantitative behavioral data were measured in the present study.14 Since males whose anal fin was completely removed often fail to mate, Egami and Nambu4 and Uematsu5 suggested that the male anal fin is important for stimulating oviposition by females. Our result implies that a half-size anal fin (or similar size to female) is sufficient to initiate spawning or mating itself. Therefore, the function of the male anal fin as a stimulatory organ alone may not adequately explain the sexual dimorphism in anal fin size.

There was a significant difference in FR among treatments (Table 1, two-way ANOVA, \( F_{2,90} = 7.43, P < 0.01 \)). No significant correlation between FR and the number of eggs released was found (\( n = 108, r = 0.09, P > 0.05 \)). Multiple comparisons revealed that FR in the half-removal treatment was significantly lower than that in the no- and edge-removal treatments (Tukey’s HSD methods, \( P \)-values <0.05), while there was no significant difference between the no- and edge-removal treatments.
Role of the anal fin in male medaka

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Table 1  Required times for spawning (mode, range), numbers of eggs released (mean±SD) and fertilization rates (FR: the number of fertilized eggs divided by the number of spawned eggs; mean±SD) for the six males (A–F) in the three anal fin-removal treatments

<table>
<thead>
<tr>
<th>Treatment variable</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>No removal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required time (min)</td>
<td>2.5, 1–4 (6)</td>
<td>1, 1–3 (6)</td>
<td>1, 1–5 (5)</td>
<td>2, 1–3 (6)</td>
<td>1.5, 1–36 (6)</td>
<td>1, 1–5 (6)</td>
</tr>
<tr>
<td>No. eggs</td>
<td>22.0±6.8 (6)</td>
<td>25.7±4.1 (6)</td>
<td>22.5±6.1 (6)</td>
<td>24.7±5.5 (6)</td>
<td>23.3±6.6 (6)</td>
<td>23.0±2.5 (6)</td>
</tr>
<tr>
<td>FR (%)</td>
<td>92.5±9.7 (6)</td>
<td>98.2±2.6 (6)</td>
<td>66.7±25.5 (6)</td>
<td>93.4±6.5 (6)</td>
<td>94.4±6.3 (6)</td>
<td>97.9±4.7 (6)</td>
</tr>
<tr>
<td>Edge removal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Required time (min)</td>
<td>2, 1–2 (5)</td>
<td>1, 1–2 (6)</td>
<td>1, 1–2 (6)</td>
<td>1, 1–7 (5)</td>
<td>1.5, 1–4 (6)</td>
<td>1, 1–2 (6)</td>
</tr>
<tr>
<td>No. eggs</td>
<td>24.5±7.3 (6)</td>
<td>30.7±6.3 (6)</td>
<td>24.3±5.7 (6)</td>
<td>26.0±4.9 (6)</td>
<td>30.2±5.1 (6)</td>
<td>28.5±4.6 (6)</td>
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<tr>
<td>FR (%)</td>
<td>95.3±7.5 (6)</td>
<td>96.0±5.9 (6)</td>
<td>72.9±14.8 (6)</td>
<td>95.6±5.7 (6)</td>
<td>93.0±8.5 (6)</td>
<td>97.0±3.7 (6)</td>
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<tr>
<td>Half removal</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Required time (min)</td>
<td>2.5, 1–7 (6)</td>
<td>1, 1–2 (6)</td>
<td>1, 1–2 (5)</td>
<td>1.5, 1–60 (6)</td>
<td>5.5, 1–21 (6)</td>
<td>2.5, 1–13 (6)</td>
</tr>
<tr>
<td>No. eggs</td>
<td>22.5±3.3 (6)</td>
<td>24.5±7.0 (6)</td>
<td>23.3±4.1 (6)</td>
<td>24.2±5.1 (6)</td>
<td>18.0±5.2 (6)</td>
<td>22.7±8.4 (6)</td>
</tr>
<tr>
<td>FR (%)</td>
<td>86.4±19.1 (6)</td>
<td>86.9±13.3 (6)</td>
<td>68.7±20.1 (6)</td>
<td>80.6±21.1 (6)</td>
<td>75.2±25.2 (6)</td>
<td>86.6±13.0 (6)</td>
</tr>
</tbody>
</table>

Numbers of trials are in parentheses.

(Tukey’s HSD method, P > 0.05). That is, males with a half-sized anal fin had a lower FR relative to males with a normal anal fin, and this was not caused by the handling effects of removal. Therefore, the results suggest that one of the functions of the anal fin in male medaka is to ensure fertilization of released eggs. The male medaka envelops the female with its anal and dorsal fins.1 This behavior may allow a male to position its gonopore closer to the female gonopore and prevent the sperm from scattering. Therefore, it is possible that selection favors a larger anal fin in males. Details such as the correspondence between variation in the anal fin size observed in nature and the FR will require further study.

ACKNOWLEDGMENTS

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