



# Seasonal change in male reproductive investment of a fish

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**Abstract** Many animals are sexually dimorphic, whereby males may display brighter body coloration and more distinctive ornamentation than females. Fishes in temperate regions markedly change their energy allocation toward reproduction in response to the seasonal environment. Seasonal change in reproductive investment affects the expression of sexually dimorphic traits in males through gonadal weight change. Here, we report seasonal changes in body size, testis weight and sexual dimorphism of the fins (anal fin length and dorsal fin length) in the northern medaka *Oryzias sakaizumii*. Fish were collected periodically from a wild population (Aomori). Gonad weight increased from May to July in both males and females, corresponding to the reproductive season. Moreover, during this period, the degree of sexual dimorphism in fin length increased. To investi-

gate the relationship between testis weight and individual differences in male fin length, we analyzed relationships among morphological traits using structural equation modeling. In the reproductive season, increased testis weight was associated with longer fin length in males, but the relationship disappeared after the reproductive season. These observations suggest that the sexually dimorphic fin in this fish is a mating signal. Results from a mating experiment also support this view. Males with larger size and/or longer fin attracted more mates than those with smaller fins, suggesting that sexual selection operates through females choosing to mate with males having longer fins.

**Keywords** Life-history adaptation · Seasonality · *Oryzias latipes* species complex · Sexual dimorphism · Indicator signal

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## Introduction

Fishes in temperate regions generally reproduce only for specific, limited periods of the year in response to the physical environmental changes, particularly temperature and photoperiod ('reproductive seasonality'; Winemiller and Rose 1992; Vila-Gispert et al. 2002). In species exhibiting reproductive seasonality, energy allocation to reproduction differs between reproductive and non-reproductive seasons. In fishes, gonadal weight as a percentage of body weight, that is, the gonad soma index (GSI), increases during the reproductive season (e.g., Wootton 1998). The seasonal change in gonad

weight is most remarkable in females but, in many species, it is also observed in males (e.g., Awaji and Hanyu 1987). Moreover, males often express sexual characters that act as mating signals only in the reproductive season; for example, nuptial coloration (Kodric-Brown 1998), muscle mass for vocalization (Nguyen et al. 2008), elongation of fins (Torricelli et al. 2000), and breeding tubercles of Cyprinidae (Dugas et al. 2016).

Seasonal change in gonad development is considered to physiologically control the expression of the sexual characters. Androgens that are produced in the testis (e.g., Williams and Carroll 2009) mediate the expression of sexually dimorphic traits in various fish species, for example, mating territoriality in the tilapia (Oliveira and Almada 1999), red-throat coloration in three-spined sticklebacks (Kurtz et al. 2007), and anal and dorsal fin elongation in medaka (Kawajiri et al. 2015). In general, testis size positively correlates with body weight. Individual variation in nutrient condition would be expected to cause a positive association between relative testis weight (standardized by size) and sexual characters (Simmons et al. 2017). However, the opposite association is often observed in some taxa (e.g., Oliveira and Almada 1999; Fernandes et al. 2010; Simmons et al. 2017). Simmons et al. (2017) point out that resource-allocation trade-off might occur if both testis and sexual characters are energetically costly. When the allocation of nutrients is the predominant mechanism determining the association, a negative association between these factors would be expected. Periodic environmental changes in temperature are likely to constrain gonadal development and/or nutrient condition. Investigation of the association between testis and sexual characters in temperate fish should provide insight into the determination of individual variation of sexual characters in the wild.

We investigate seasonal changes in the association between testis weight and the sexually dimorphic fin length in the northern medaka *Oryzias sakaizumii*. This species shows sexual dimorphism in the anal and dorsal fins, with males having longer fins than females (Kawajiri et al. 2014; Fig. 1). Although both females and males exhibit increased GSI in the reproductive season (Awaji and Hanyu 1987), the annual change in the sexual characters in the wild population has not been previously investigated. We examine the hypothesis that the sexually dimorphic fins are condition-dependent mating signals reflecting the male's investment in the

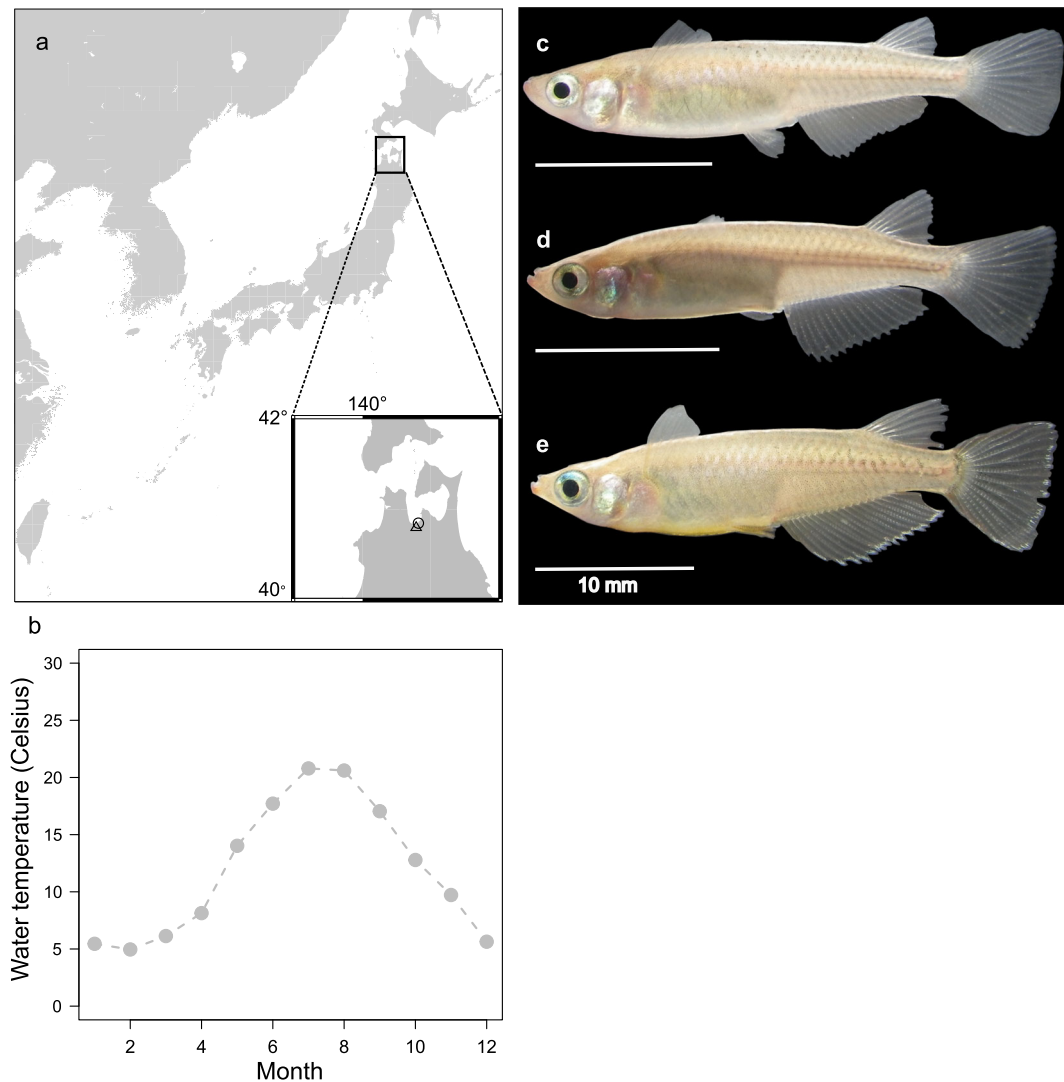
testis. First, we consider whether the increase in size of male sexual characters is greater during the reproductive season than during the non-reproductive season. Second, we determine whether male fin length is positively associated with relative testis weight in the reproductive season. Finally, we assess whether males with longer fins are preferred by females for mating. To clarify the seasonal change in the sexually dimorphic fins, we repeatedly collected fish from the same population before, during and after the reproductive season. Seasonal changes in morphological traits were measured, including body length, body weight, gonad weight, and lengths of the sexually dimorphic anal and dorsal fins.

We also examined the mate preference for the male fin length in a mating experiment. The male dimorphic fins are used to enclose the female during spawning (Egami and Nambu 1961; Koseki et al. 2000); males with longer fins appear more acceptable to females in mating experiments (Egami and Nambu 1961; Fujimoto et al. 2014; Katsumura et al. 2014). However, previous studies on female mate preference have employed experimental fin shortening (Egami and Nambu 1961) or have used F<sub>2</sub> progeny males from an intercross between populations having different average fin lengths (Fujimoto et al. 2014). In principle, sexual selection by mate choice operates on individual variation within a population. However, to date the importance of intra-population variation in fin length has not been examined in wild populations. This is an important gap in the knowledge required to determine whether sexual selection by mate choice is the evolutionary cause of the longer fins in medaka. If dimorphic fins reflect male reproductive investment, males with longer fins should tend to mate with more females. Therefore, we examined the relationship between the male fin length and the number of females mated. Our results are discussed in terms of the mechanism of sexual selection acting on fin length in this fish.

## Materials and methods

### Fish and collection site

Medaka, or *Oryzias latipes* species complex, are small freshwater fish distributed throughout Japan, Korea, and China (Iwamatsu 2006). Mitochondrial DNA studies have revealed that Japanese populations of the *O. latipes* complex consist of two genetically distinct



**Fig. 1** Geographic location of collection sites. The circle and triangle on the map indicate collection sites in 2004 and 2013, respectively (a). The annual change of water temperature at the collection site, 2004 (b). The photographs of fish show a female

collected in April, 2013 (c), a male collected before the reproductive season in April, 2013 (d), and a male collected during the reproductive season in May, 2013 (e)

groups. A ‘northern Japan group’ is distributed along the Sea of Japan coast of eastern Japan and a ‘southern Japan group’ is distributed along the Pacific coast of eastern Japan and over western Japan. The ‘northern Japan group’ has recently been described as northern medaka *O. sakaizumii* (Asai et al. 2011).

Medaka show clear reproductive seasonality in the wild. The length of the reproductive season is mainly determined by physiological mechanisms. The most suitable water temperature for growth is considered to be approximately 26–28 °C (Yamahira et al. 2007). Ovarian maturation is initiated by warmer temperatures

and longer photoperiod (Koger et al. 1999; Shimmura et al. 2017). Hence in the wild, the reproductive season is limited to the warm season, and GSI increases from April to August (Awaji and Hanyu 1987). Because the collection site in this study was located near to the limit of the northern distribution of this fish in Japan (Fig. 1), the reproductive season was expected to be shorter than in other areas in mainland Japan.

The life span of medaka in the wild is considered to be less than 2 years, based on seasonal change in their size distribution (Awaji and Hanyu 1987; Egami et al. 1988), and their median life span in the aquarium

(Gopalakrishnan et al. 2013). This was the case in the Aomori population studied here, based on the seasonal change in the size distribution (Yamahira et al. unpublished data). Newly recruited fish can be roughly distinguished by their size (standard length < 20 mm or body weight < 0.16 g). Adult 1-year-old fish reproduce in summer and almost all die before the following reproductive season (Edeline et al. 2016). Seasonal reduction in GSI late in summer might determine both juvenile recruitment and the change in condition of 1-year-old adults, for example, resorption of the gonads or energy depletion, or both.

Mating usually occurs between one male and one female. They align side-by-side, and the male wraps the female's body with his dorsal and anal fins, placing the vents of the two individuals in close proximity. The pair then quiver for < 1 min while the female externally releases eggs, which are then fertilized by the male. Females sometimes abandon egg release by disengaging themselves from the male (Fujimoto et al. 2014). In the reproductive season, mature females spawn each day in the morning. The female bears fertilized eggs on the genital pore for several hours afterward (Iwamatsu 2006). Males are able to fertilize multiple females daily (Howard et al. 1998; Weir and Grant 2010). Sperm depletion has rarely been observed, except for the small males (Howard et al. 1998).

#### *Measurement of fish morphology*

From March to November 2004, samples were collected once or twice a month from the habitat in Aomori (Mayajiri, Aomori city: 40° 50' N, 140° 49' E) (Fig. 1a). The fish were collected using landing nets (approximately 770 cm<sup>2</sup>, 2-mm mesh) and/or four-armed scoop nets (60 × 60 × 40 cm or 90 × 90 × 55 cm, 0.5-mm mesh). In each month, 15–20 males and females were collected, that is, a total of 150 males and 165 females. Collected fish were individually placed into a small acrylic container and photographed from the side using a digital camera (Coolpix 4500, Nikon, Tokyo, Japan). The distance from the container and camera was fixed. Digital pictures were later downloaded to a personal computer, and standard length (SL) and anal fin length (AFL) of each fish was measured to < 0.01 mm (Adobe, Illustrator 10.0.3 with Baby Universe, BPT-Pro2 or Inkscape ver. 0.92). After photographing the fish, they were anesthetized with MS-222 (aminobenzene methanesulfonate, FUJIFILM Wako Pure Chemical

Corporation, Osaka, Japan) and then fixed in 5% neutralized formalin. Their weights were measured with an electronic balance (GR-202, A & D Weighing, Thebarton, Australia) with an accuracy of 0.0001 g. The wet body weight (BW) of each fish was measured after wiping the surface of the body with a paper towel. After the BW measurement, the abdomen was opened, the organs, including the gonads, were removed, and the gonad weight was measured. The gonad somatic index (GSI) was calculated by the following formula.

$$GSI = \frac{\text{Ovary or Testis weight}}{\text{Body weight}} * 100$$

#### *Seasonal change in anal and dorsal fin length*

To assess the temporal stability of the initial pattern in fin length, we collected additional individuals during April to July 2013 and observed seasonal change in fin length. We also measured dorsal fin length in the dataset, because we were unable to measure dorsal fin length in most of the individuals photographed in the 2004 collection. We collected fish approximately every 2 weeks in Kuwabara, Aomori City (40° 49' N, 140° 48' E), using the same procedure as in 2004. We randomly chose 20 adult females and 20 adult males (i.e., > 20 mm SL). Fish were photographed and SL, AFL and DFL were measured following the method described above (Optio × 90, RICOH, Tokyo, Japan). In 2013, we collected and measured totals of 138 males and 127 females. After photographing them, the fish were released at the collection site. Considering the habitat area and the population density, a few percent of individuals could have been re-measured. To assess seasonal change in anal and dorsal fin lengths, we calculated AFL/SL and DFL/SL. Preliminary analysis of fin length showed that allometric scaling between SL and fin length did not change throughout the season (supporting information in Fig. S1). Both AFL/SL and DFL/SL were suitable indices for adjusting for individual size difference. The significance of each variable was tested using the *F*-test in the analysis of variance (ANOVA), which considered sex, collected month and the interaction between sex and month as explanatory variables. The analysis was performed separately for the 2004 and 2013 datasets.

If the male reproductive investment changes seasonally, the correlation between testis weight (TW) and

AFL in males may vary between reproductive and non-reproductive seasons. We examined seasonal change in the correlation between the TW and AFL using structural equation modeling (SEM) to correct for effects of body size. SEM assumes a partial correlation between SL and BW, and partial regressions between SL and AFL, BW and TW, and TW and AFL. Each variable was standardized by Z-transformation to standardize the scale of the variance. Estimation of SEM parameters was conducted using “lavaan” ver. 0.6–5 of the R package (Rosseel 2012). To clarify seasonal change in the relationship between TW and AFL, the dataset was divided and analyzed before and after the reproductive season, based on the seasonal changes in the female GSI (reproductive season: March to July,  $N=61$ ; non-reproductive season: August to November,  $N=50$ ). In this analysis, we excluded fish whose SL, AFL, BW, or TW could not be measured, and also fish in which SL was  $<20$  mm, which may be sexually immature (Iwamatsu 2006).

#### *The relationship between male reproductive success and morphology*

To examine the relationship between male reproductive success and fin length, we conducted a mating experiment. The experimental fish were collected in Kuwabara in June, 2015. After the collection, fish were kept in buckets and transported to the Asamushi Marine Biology Education Center, Tohoku University. On the day of collection, we chose adult individuals based on their standard length (i.e.,  $>20$  mm SL). One male and five females were assigned to each container ( $28 \times 26 \times 24.5$  cm, water volume 10 L,  $26 \pm 1$  °C, and natural day length). The fish were allowed to mate freely until the next morning (ca. 9:00–11:00). We replicated this experiment 14 times using different individuals. We collected the eggs borne by each female and determined the fertilization rate in each clutch. The fertilization rate of each female ( $N=49$ ) was calculated as the number of fertilized eggs divided by the total number of eggs produced. The number of mates for a male was defined as the number of females that bore fertilized eggs ( $N=14$ ). After egg collection, the fish were photographed and SL, anal fin length and dorsal fin length were measured following the method described above (Optio  $\times 90$ , RICOH, Tokyo, Japan).

The male characters that affected male reproductive success were determined using the Wald test in the

generalized linear model (GLM) (Bolker et al. 2009). In the models, SL, AFL (or DFL) and the ratio AFL/SL (or DFL/SL) of each male were treated as explanatory variables. To avoid multicollinearity (Graham 2003), the analysis did not use AFL and DFL at the same time because the lengths of these sexually dimorphic fins were strongly correlated (Table S1; Fujimoto et al. 2014). We treated the number of mates and fertilization rate as the response variables. To estimate the effect of male traits on the number of mates, the presence or absence of eggs in five females was assumed to follow a binomial distribution in the GLMs (Bolker et al. 2009). Similarly, to estimate the effect of male traits on fertilization rate, we performed a generalized linear mixed model (GLMM). In this model, fertilization rate was assumed to follow a binomial distribution, and male individuals were treated as a random effect. The analysis was performed with R ver. 3.6.1 (R Core Team 2019), and the GLMM was performed using the “lme4” package, ver.1.1.21 (Bates et al. 2015).

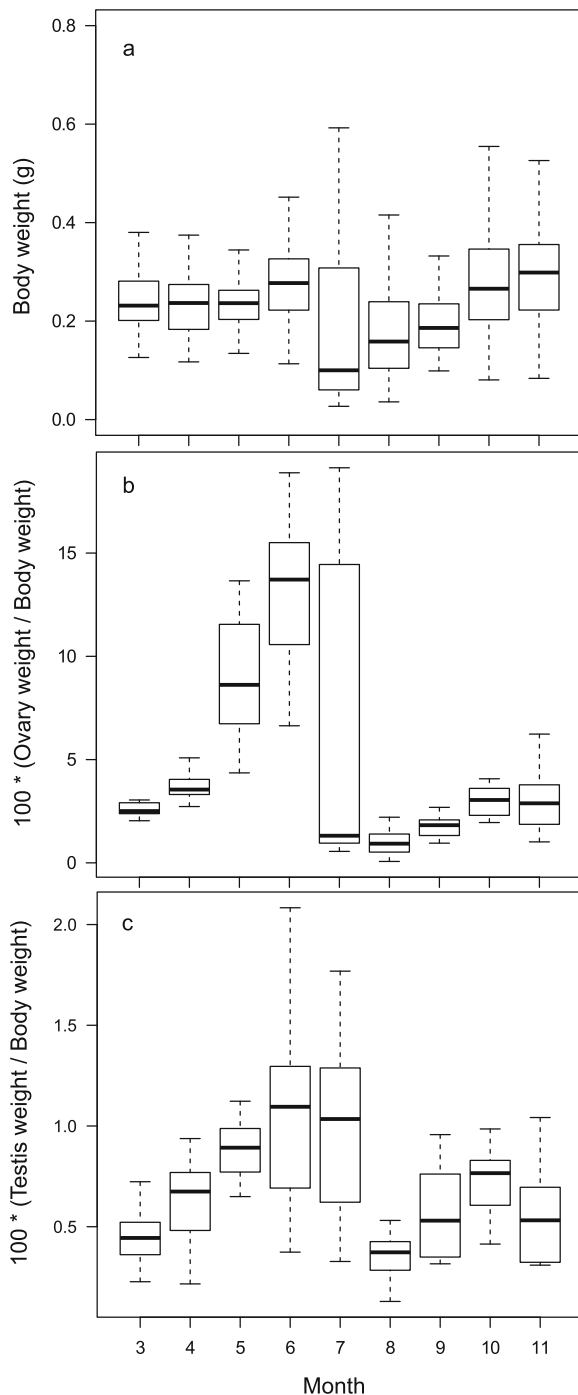
## Results

Seasonal changes in body weight, gonad weight and fin length

Female GSI increased from May to July and peaked between June and July, with a median value of 13.7% at the peak (Fig. 2b). Thereafter, the GSI sharply declined from July to August, to a median value of 0.93% in August. In males, GSI also increased from May to July (Fig. 2c). Seasonal changes in female GSI clearly showed that the reproductive season for this population extended from May to July. The increase in GSI was not influenced by size distribution because the median body weight was relatively constant in this period (0.20–0.24 g; Fig. 2a). The decrease in median GSI between July and August mainly resulted from recruitment of sexually immature juveniles (Body weight  $< 0.16$  g), but adult 1-year-old females (BW  $> 0.16$  g) also decreased their GSI in August.

The fin length in males increased during the reproductive season in both 2004 and 2013 collections (Fig. 3). Male anal fin length and dorsal fin length showed similar seasonal changes; the average fin length of fish collected in July was approximately 1 mm longer than in April in all size ranges (Fig. S1). Because the seasonal change in fin length was





**Fig. 2** Monthly distribution of body weight (a), female GSI (b), and male GSI (c), in 2004. Circles and triangles represent individual females and males, respectively

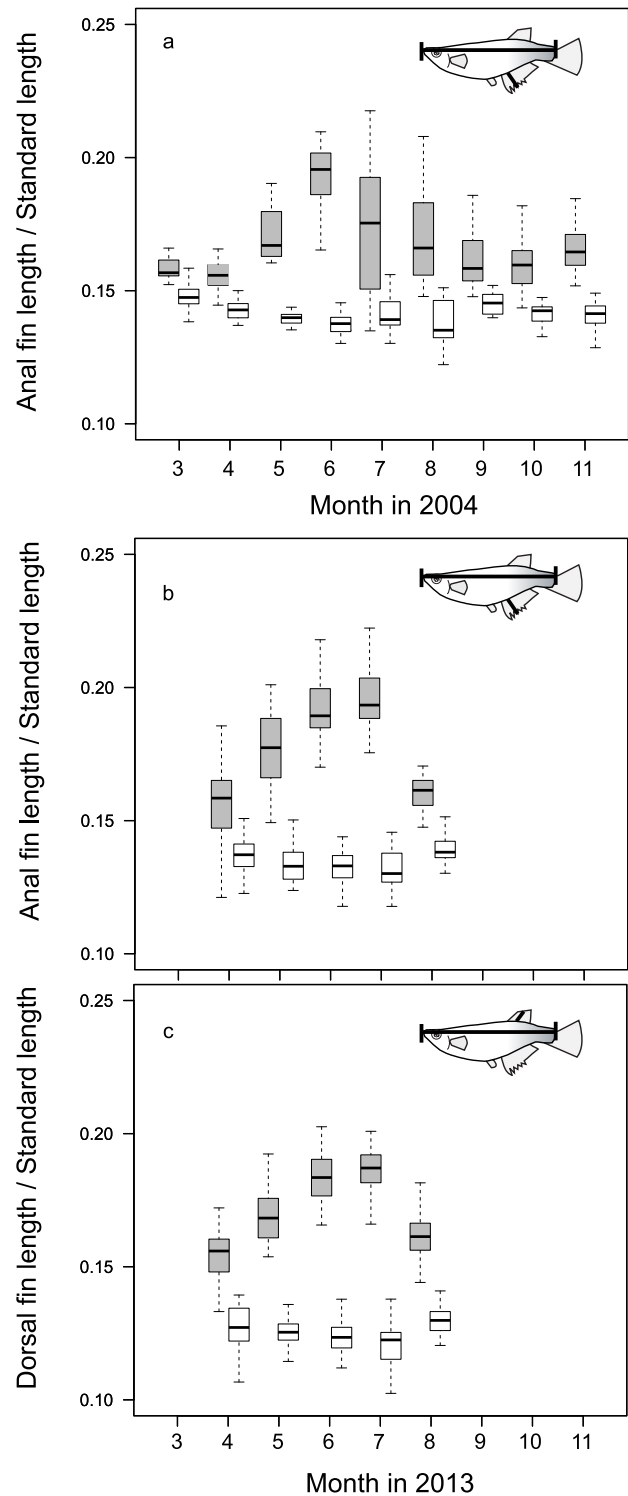
smaller in females than in males, sexual difference in AFL/SL increased as the reproductive season progressed in both the 2004 collection (Sex:  $F_{1, 265} = 388.70$ ,  $P < 0.001$ ; Month:  $F_{8, 265} = 5.457$ ,  $P < 0.001$ ; Sex  $\times$  Month:  $F_{8, 265} = 10.742$ ,  $P < 0.001$ , Fig. 3a) and the 2013 collection (Sex:  $F_{1, 293} = 1115.34$ ,  $P < 0.001$ ; Month:  $F_{4, 293} = 29.74$ ,  $P < 0.001$ ; Sex  $\times$  Month:  $F_{4, 293} = 46.86$ ,  $P < 0.001$ , Fig. 3b). DFL/SL showed the same trend as AFL (Sex:  $F_{1, 292} = 1677.26$ ,  $P < 0.001$ ; Month:  $F_{4, 293} = 25.45$ ,  $P < 0.001$ ; Sex  $\times$  Month:  $F_{4, 293} = 44.88$ ,  $P < 0.001$ , Fig. 3c). The difference in median male fin length observed during the reproductive season did not persist after the reproductive season (Fig. 3) because of the death of adult 1-year-old fish. One adult male with AFL ratio near 0.2 was present in each month from September to November but no such individuals were present in March and April.

Before and during the reproductive season, increased testis weight was associated with longer AFL in males. The partial regression coefficient between the TW and AFL was positive after correcting for body size effects by SEM (slope  $\pm$  SE =  $0.57 \pm 0.09$ ,  $Z = 6.17$ ,  $P < 0.001$ , Fig. 4a, c, Table S2). In SEM, the partial regression coefficient between BW and AFL was also positive, suggesting body size and testis weight affect AFL, independently. In contrast, after the reproductive season, the partial regression coefficient between testis weight and AFL became almost zero, and was not statistically significant (slope  $\pm$  SE =  $0.04 \pm 0.08$ ,  $Z = 0.51$ ,  $P = 0.610$ , Fig. 4b, d, Table. S2).

#### The effect of male morphology on reproductive success

Males with larger size or longer fins significantly acquired more mates (Table 1, Fig. S2). AFL/SL and DFL/SL did not significantly affect the number of mates. We included multiple variables in the GLM to evaluate the relative importance of body size or fin length (SL + AFL or SL + DFL). In these analyses, neither SL + AFL (SL: slope  $\pm$  SE =  $0.23 \pm 0.30$ ,  $Z = 0.75$ ,  $P = 0.45$ , AFL: slope  $\pm$  SE =  $0.49 \pm 0.92$ ,  $Z = 0.54$ ,  $P = 0.59$ ) nor SL + DFL (SL: slope  $\pm$  SE =  $0.30 \pm 0.40$ ,  $Z = 0.74$ ,  $P = 0.46$ , DFL: slope  $\pm$  SE =  $0.29 \pm 1.59$ ,  $Z = 0.18$ ,  $P = 0.85$ ) were significant, indicating a confounding effect between SL and fin length. For all morphological characters, the effects on fertilization rate were not significant (Table. 2, Fig. S3). The overall mean fertilization rate was  $96.2 \pm 8.1\%$  (mean  $\pm$  SD).

**Fig. 3** Seasonal change in the sexually dimorphic fins, anal fin length in 2004 collection (**a**) and 2013 collection (**b**), and dorsal fin length in 2013 collection (**c**). The colors in the gray and white box represented the males and females, respectively



## Discussion

### Seasonal change in gonad weight and fin length

Seasonal changes in the GSI clearly showed that the reproductive season for this population extends from May to July (Fig. 2). The annual changes in the water temperature may explain the reproductive seasonality in this fish (Fig. 1). Medaka become less active at low water temperatures and do not feed below 10 °C. Starvation stress in winter is widely believed to be a cause of reproductive seasonality in temperate fish (e.g., Conover 1992; Sogard 1997). Indeed, the population used in this study lost an average of 8% body weight throughout the winter (Yamahira, unpubl. data). Because the average body weight did not increase during March to May (Fig. 2a), the starvation stress induced by the low temperature seems to continue until May. We infer that low temperatures in spring physiologically restrict the onset of reproduction in this population.

The sexually dimorphic features in males—anal fin length and dorsal fin length—also change seasonally. The degree of sexual dimorphism became more prominent as the reproductive season progressed (Fig. 3). The timing of fin elongation in males roughly coincided with the timing of their increase in GSI (Figs. 2c, 4), indicating that the change in fin lengths predominantly reflected their physiological condition. In medaka, androgens are associated with the expression of sexual dimorphism in the fins (Ogino et al. 2014; Kawajiri et al. 2015). Thus, ontogenetic change in the testosterone/estradiol ratio may be the mechanism of expression of sexual dimorphism in fin length (Kawajiri et al. 2015). The seasonal change in testis weight is considered to affect the expression of sexual dimorphism in AFL and DFL, mediated by sex steroid hormone metabolism. We found that increased testis weight was associated with longer AFL in males in the reproductive season, but the correlation was weak in the non-reproductive season (Fig. 4). These observations suggest that fin length in the reproductive season is a condition-dependent mating signal that reflects the male's reproductive investment.

### Male fin length functions as a mating signal

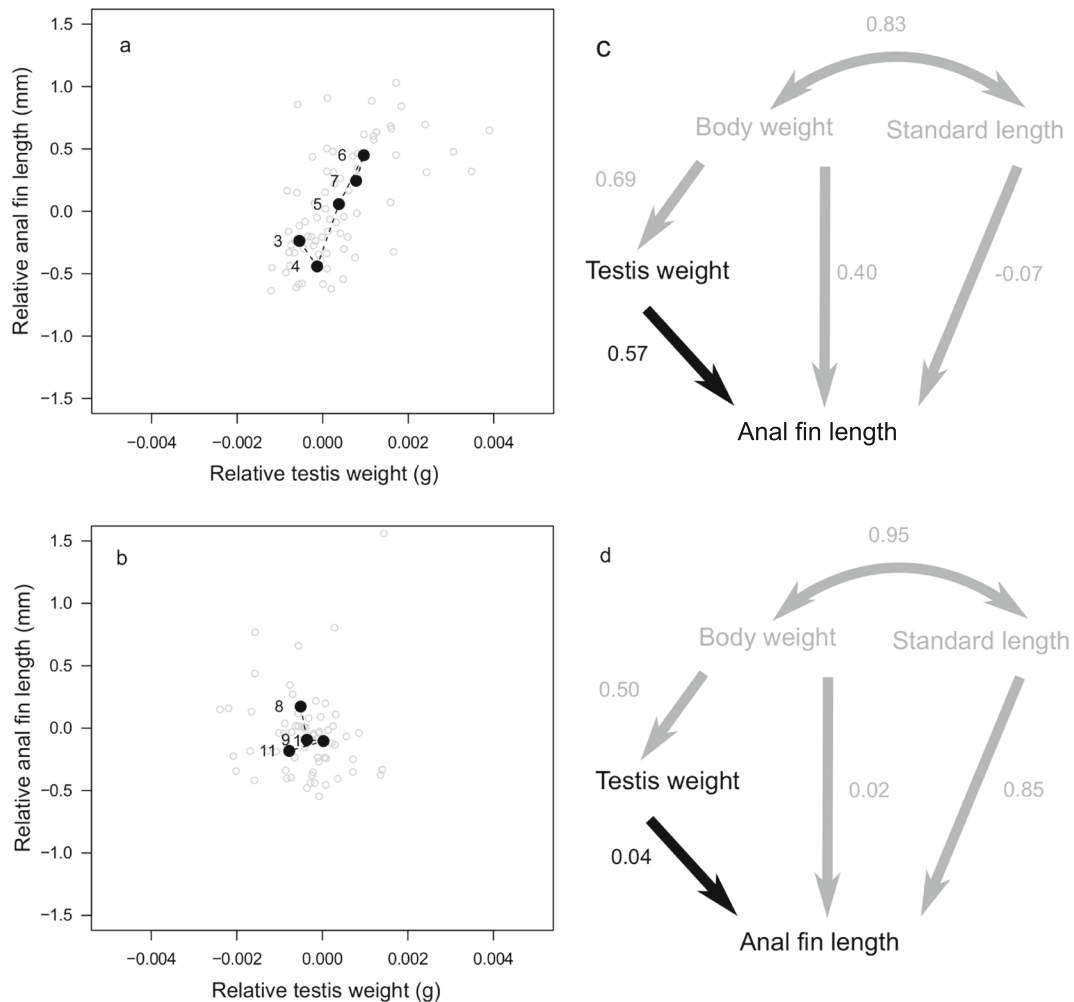
The mating experiment showed that larger size or longer fins in males were associated with higher mate numbers (Table. 1, Fig. S2), implying that fin length functions as

a mating signal. Many empirical studies on fish species with ornamented fins (e.g., swordtails, cichlids, and gobies) demonstrated that females preferred males with this trait (e.g., Basolo 1990; Karino 1997; Suk and Choe 2002). Medaka likewise show female mate preference for larger body size (Howard et al. 1998) and longer fins (Fujimoto et al. 2014; Katsumura et al. 2014); males with longer anal and/or dorsal fins are less likely to be rejected by females (Fujimoto et al. 2014). This observation in the medaka suggests that female mate preference is the mechanism that determines individual differences in mate number. Although our data did not clearly identify the effect of body size and fin length on female mate choice, we demonstrated that variation in male body size and/or fin length did affect female mate choice, even within the phenotypic variation of a wild population.

Why do females prefer larger body size and/or longer fins? Because AFL and DFL were correlated with both body size and relative testis weight in the reproductive season (Table S1, Fig. 4), male fin length may function as an honest signal of the body weight and/or the nutrient condition of males. The studied population is distributed near the northern limit of the species. Because their growth rate sharply declines below 20 °C (Yamahira et al. 2007), individual growth is strongly constrained at low temperatures (Fig. 2). Selection pressure would favor faster growth of the body size in higher latitudes. Previous studies on latitudinal variation in juvenile growth rate, support this view, that is, populations at higher latitudes have higher growth rates (e.g., Yamahira et al. 2007; Yamahira and Takeshi 2008). If the high growth rate or the superior food acquisition ability (or both) are the physiological causes of the larger size and longer fins, females may indirectly assess the genetic quality of males using fin length. In that case, female preference facilitates local adaptation (Servedio and Boughman 2017). However, genetic association between fin length and these physiological characters has not been investigated, and further study is needed.

In the light of the wrapping behavior associated with the male's anal and dorsal fins during the egg spawning, it has been hypothesized that these elongated fins might represent copulatory organs, facilitating successful egg fertilization (Egami and Nambu 1961; Koseki et al. 2000). The direct benefit from a high fertilization rate is another possible mechanism for selection of females showing mate preference for male characters (Andersson 1994; Møller and Jennions 2001; Weir and Grant 2010; Smith et al.





**Fig. 4** Seasonal change in the correlation between relative TW and relative AFL in males (**a, c**: fish collected in March to July, 2004; **b, d**: fish collected in August to November, 2004). The filled circles and numbers in a and b represent the mean values in each month. The values in c, d show the estimated parameters in the

structural equation model. Bidirectional arrows represent partial correlation coefficients between SL and BW, and the unidirectional arrows represents partial regression coefficient between AFL and other factors

2014). This hypothesis is not consistent our result. Differences in AFL among the males did not affect the fertilization rate (Fig. S3, Table. 2), and the average fertilization

**Table 1** Results of Wald test in GLM, dependence of the number of mates on SL, AFL, DFL, AFL/SL and DFL/SL

Character	Slope	SE	Z	P
Standard length (SL)	0.37	0.16	2.31	0.02
Anal fin length (AFL)	1.10	0.49	2.25	0.03
Dorsal fin length (DFL)	1.39	0.63	2.21	0.03
AFL / SL	28.87	18.96	1.52	0.13
DFL / SL	37.50	30.18	1.24	0.21

**Table 2** Results of Wald test in GLMM, dependence of the fertilization rate on SL, AFL, DFL, AFL / SL and DFL / SL. Male individual was treated as a random effect in this analysis, but the variance estimates were omitted

Character	Slope	SE	Z	P
Standard length (SL)	0.21	0.17	1.27	0.20
Anal fin length (AFL)	0.05	0.62	0.08	0.94
Dorsal fin length (DFL)	0.61	0.75	0.82	0.41
AFL / SL	-15.34	22.42	-0.69	0.49
DFL / SL	-0.77	40.81	-0.02	0.99

rate was >90%. However, our experiment has evaluated male fertility only under limited circumstances. Further studies need to evaluate the function in challenging situations for males, such as when sperm depletion is likely to occur in small males (Howard et al. 1998) and/or mate guard in sperm competition, i.e., sneaking by other males (Koya et al. 2013).

### Sexual selection affects male fin length

Mate number largely explained the variance in male reproductive success, that is, total fertilized eggs obtained by a male ( $r=0.81$ ,  $t=4.78$ ,  $df=12$ ,  $p<0.001$ ), and therefore sexual selection favors males having larger size or longer fins (or both). On the basis of the inter-population variation, previous studies argued that sexually dimorphic fins evolved through sexual selection (Fujimoto et al. 2014; Katsumura et al. 2014), and our result supports that scenario. If the intra-population variation in fin length has a genetic basis, average fin length in the population would be expected to increase in response to sexual selection pressure. Indeed, the averages of anal and dorsal fin lengths differ genetically among populations of *O. sakaizumii* and its congeners (Kawajiri et al. 2014; Katsumura et al. 2014). This interspecific variation shows that fin lengths are not phylogenetically constrained, and that evolution can occur by sexual selection. Genes related to sex steroid metabolism, such as estrogen receptor and *CYP11B1*, are reported to be the molecular basis of the inter-population variation (Kawajiri et al. 2014; Kawajiri et al. 2015; Katsumura et al. 2014).

However, in contrast to interspecific variation, the genetic basis of condition-dependent expression in the mating signal remains unclear. Kawajiri et al. (2014) reported some candidate regions for inter-population variation in fin length, which affect the ontogenetic timing of the expression of the secondary sexual characters. Genes that affect the age and size at which secondary sexual character are expressed may have evolved in life-history adaptation to different environmental conditions among populations. Thus, the genetic basis of inter-population variation is a good starting point for exploration of the basis of environmental condition-dependent expression of fin length. Future

research should quantify gene-by-environment interactions using rearing experiments that manipulate factors such as temperature and food resource. Such studies will contribute to the understanding of the genetic basis of condition-dependent mating signals and their evolution.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10641-021-01059-x>.

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**Code availability** R scripts used for statistical analysis are available from the Dryad Digital Repository (<https://doi.org/10.5061/dryad.dv41ns1vj>).

**Authors' contributions** S. F., M. Y. and K. Y. designed the research project; S. F., K. Y. and S. T. collected the fish and performed the experiments; S. F. and M. Y. analyzed the data; and S. F., K. Y. and S. T. wrote the manuscript. All authors discussed results and commented on the manuscript.

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### Declarations

**Ethics approval** Act on welfare and management of animals in Japan does not include any species of fishes. Animal experiment rule in the university of the Ryukyus also does not include fishes. All individuals were anesthetized by lethal concentration of MS-222 (ethyl 3-aminobenzoate methanesulfonate)

**Consent to participate and publish** All authors agree to participate in and publish this manuscript.

**Conflicts of interest/competing interests** The authors declare no competing interests.

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