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Behaviour

ALGAL-DIET ENHANCES SEXUAL ORNAMENT, GROWTH AND
REPRODUCTION IN THE GUPPY

by

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Short title: KARINO & HAIJIMA: EFFECT OF ALGAE-INTAKE ON
GUPPIES

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Summary

Carotenoids often influence male coloration, and the carotenoid-based coloration is used for female mate choice in some animals. The positive influence of carotenoids on a variety of physiological systems in animals is also known. Because animals have to obtain carotenoids from their food, the conspicuousness of carotenoid-based coloration may indicate the male's foraging ability for carotenoid-rich food. It is possible, therefore, that females can obtain benefits of the high-foraging ability and, thus, health or vigor of their offspring through their mate preference for the male coloration. In order to test this prediction, we examined the influence of algae-intake (carotenoid-resource in nature) on guppies. By algae-supplement, not only male orange coloration but also growth of both sexes and female reproduction were enhanced compared with fish in control groups, although they got the same amount of energy from their food. However, carotenoid-supplementation did not show the positive influence on growth and reproduction, though male orange coloration became conspicuous. It is possible to explain the difference of the influence on growth and reproduction between algae- and carotenoid-supplement groups in two ways. First, some kinds of carotenoids within algae other than carotenoids used for carotenoid-supplementation play an important role for enhancement of growth and reproduction. Second, other substances (e.g., non-digestible carbohydrates) within algae influenced growth and reproduction through the positive effect on digestive system. Since algae-intake enhanced not only male orange coloration but also growth and reproduction, female guppies may obtain benefits through mate preference for males having brighter coloration and, hence, greater foraging ability for algae.

Introduction

Carotenoid-based coloration is known as one of major sexual signals in animals (Andersson, 1994; Olson & Owens, 1998). Since animals can not synthesize carotenoids de novo, they must obtain carotenoids from food such as plants and algae (Mayne, 1996; Olson & Owens, 1998). Therefore, carotenoid-based coloration is thought as an honest signal of foraging ability and may signal the superiority of the individual. In fact, individuals having most conspicuous carotenoid-based coloration are chosen by the opposite sex as the best mate in a variety of birds and fishes (Hill, 1991; Johnson et al., 1993; Bakker & Mundwiler, 1994; Wedekind et al., 1998; Craig & Foote, 2001). Moreover, conspicuousness of carotenoid-based coloration often influences on male-male competition for accessing mates or other resources (Evans & Norris, 1996; Pryke et al., 2002). In addition, because parasites affect host health as well as carotenoid-based coloration, brightness of carotenoid-based coloration also indicates the ability of parasite resistance of the individuals (Milinski & Bakker, 1990; Lozano, 1994; Skarstein & Folstad, 1996; McGraw & Hill, 2000).

On the other hand, carotenoids also have several biological functions in animals and the amount of dietary-carotenoids influences on a variety of physiological processes such as nervous, immune, digestive, endocrine and reproductive systems (Krinsky et al., 1989; Olson, 1993; Olson & Owens, 1998; Blount et al., 2002). For example, carotenoids play an important role in free-radical absorption and, thus, may function in cancer prevention (Burton & Ingold, 1984; Bendich, 1993; Mayne, 1996). Therefore, animals necessarily acquire carotenoids and the greater amount of carotenoid-intake may favor their

physiological systems.

This suggests that carotenoid-based coloration reflects the males' foraging ability for carotenoid-rich foods and carotenoid-intake contributes not only to male sexual attractiveness but also to health and vigor in both sexes. If foraging ability could be inherited by the offspring from their fathers, females can obtain indirect benefits by choosing their mates on the basis of coloration through both sexual attractiveness of their sons and vigor or health of their offspring. However, no integrated information is available about the influence of the diet containing carotenoids both on male sexual ornaments and on other fitness components such as growth and reproduction. In order to test this prediction, therefore, we examined the influence of algal-intake on carotenoid-based coloration, growth and reproduction in the guppy (Poecilia reticulata).

Guppies are live-bearing fish native to streams and rivers of South America (Houde, 1997). Guppies show remarkable sexual dichromatism: males have conspicuous orange, black and iridescent color spots on their bodies and caudal fins. It is well known that female guppies choose their mates by conspicuousness or size of orange spots of males (Houde, 1987; Kodric-Brown, 1989; Houde & Endler, 1990). Kodric-Brown (1989) revealed that conspicuousness of orange coloration of male guppies depended on the amount of dietary-carotenoids. Guppies in the natural habitat obtain carotenoids mainly through benthic algae, and algae are known as a scarce resource in the natural environment in which guppies live (Endler, 1980; Dussault & Kramer, 1981; Grether et al., 1999). Moreover, Houde & Torio (1992) documented the effect of parasite infection on orange coloration of male guppies: in

parasitized males conspicuousness of the orange coloration decreased. Therefore, orange coloration in male guppies may indicate both foraging ability and resistance to parasites.

In the present study, we examined the influence of the intake of algae and carotenoids on growth and reproduction, and on male orange coloration. We conducted three treatments of split-brood experiments to examine (1) the effect of algae-intake, (2) the effect of carotenoid-supplementation, and (3) the effect of the amount of food intake without algae- and carotenoid-supplements. If algae or carotenoid intake enhances not only male orange coloration but also growth or reproduction, it suggests that females obtain indirect benefits from mate preference on the basis of orange coloration.

Methods

Study animals

We used guppies of a feral population in the Hiji River (26°43'N, 128°11'E) in Okinawa Island, southern Japan. It is known that guppies introduced into new habitats flexibly change the conspicuousness of male traits, female preferences and life history traits (Endler, 1980; Reznick *et al.*, 1990). This occurs within several years according to the new environment (Endler, 1980; Houde, 1997). Guppies were introduced into Okinawa in 1970s (Kouchi, 1997), and thus might have been subjected to natural and sexual selection for a sufficient long period to adapt to these new habitats. For instance, size of male orange spots is highly variable among individuals within the

population (Karino & Haijima. 2001), and degrees of spot size and individual variation of spot size are similar to those of native populations (cf. Houde & Endler, 1990; Winemiller et al., 1990; Houde, 1992). In addition, females of this population also show mate preference for orange coloration (Karino & Shinjo, unpubl. data).

We collected guppies in the Hiji River in March 2000 and March 2001. Collected specimens and their descendants were reared in 40-60 l aquariums with circulating water at 26-28°C with a 12:12 h light:dark regime in the laboratory. We used the second and third generation descendants of wild-caught individuals for experiments.

Experimental design

Body size and orange spot size are highly heritable traits in this guppy population (Karino & Haijima, 2001). In order to minimize the genetic effect, we divided full siblings from a single brood into two groups for each experiment. Guppies fertilize internally. To obtain full siblings, we had to control fertilization and kept virgin females separately from males from 4-5 weeks after birth. Four months after birth, a virgin female and a given male were transferred to an aquarium until the female became pregnant. The fry of a single brood was reared 5 weeks in an aquarium and was fed twice daily with newly hatched brine shrimp nauplii and commercial food. Five weeks after birth, the young fish expressed secondary sexual traits such as male gonopodium (the anal fin modified as a sperm-transfer organ), and the sex of each individual could be determined using these traits. The fish were anesthetized in 2-phenoxyetanol solution and marked individually with

subcutaneous injections of fluorescent elastomer (Northwest Marine Technology).

As an experimental apparatus, we used an aquarium (30 x 16 x 14 cm) for each brood. The aquarium was separated into two compartments by an opaque plastic board in the middle. The board had a window (10 x 7.5 cm) and the window was sealed by fine mesh. This window allowed water circulation between the two compartments to minimize environmental effects on the fish by the treatment (e.g., oxygen supply by algae) but prevented movements of fish as well as food between the compartments. The full siblings from a single brood were divided into two groups; each group was composed of 3 males and 2 females. One group of fish was placed into one compartment of the aquarium, and the other group into another compartment of the same aquarium. They were reared in this aquarium for 3 months. During this experimental period, two groups from a single brood were fed a different quality or a different quantity of food. We washed the inside of the aquarium and exchanged water once per 4-7 days, and added algal-growth suppresser (S-3; Nisso) into the water to prevent algal growth within the aquarium.

At the start and the end of the experiment, we measured body size (standard length; SL) of each individual using vernier caliper to the nearest 0.1 mm. The fish was anesthetized in 2-phenoxyetanol solution when measured. Since females prefer males with larger apparent size in this guppy population (Karino & Matsunaga, 2002), we also measured total length (TL) of males.

We also measured orange spot patterns of males at the end of the experiments, because male guppies fully express their secondary sexual traits about 4 months from birth. We recorded both the right and left sides of the

anesthetized male by means of a digital camera (Nikon Coolpix 950). The distance between the lens of the camera and the fish was fixed at 10.0 cm, and distance and angle of the light (Tokin 23 Watt) to the fish were also fixed. In order to calibrate, the scale and color plates were displayed on the background of the fish. We anesthetized the fish using 2-phenoxyethanol solution as lightly as possible and recorded them as soon as they stopped moving, because black spots increase in size and may obscure parts of the orange spots in heavily anesthetized fish (Houde, 1992). Some males jumped out from the aquariums and died between body size measurement and digital camera recording; in such cases we excluded the data from the analysis of orange spot patterns. The recorded images were put into a Macintosh computer and analyzed by Photoshop 6.0 (Adobe). We counted the number of orange spots both on body and tail (caudal fin) of the right and left sides of the male. We also measured area of all orange spots on body and tail as well as total body and tail area. To quantify coloration of orange spots, we measure hue ($^{\circ}$), saturation (%) and brightness (%) of six points of each spot, and calculated the average of the six points as the score of each spot. We conducted this color-quantification for all orange spots on body and tail of both right and left sides of the males (see Karino & Haijima, 2001 for a detailed method).

For statistical analysis, the total area of orange spots on body and tail were divided by the total area of body and tail to obtain the relative orange spot area. The number and relative area of orange spots were averaged between the right and left sides of the fish. Hue, saturation and brightness of orange spots were also averaged for all spots of the fish.

In addition, in order to quantify the degree of

orange spot expression of males in the earlier stage, we compared the digital camera image of the male at one month from the start of the experiment with that at the end of the experiment. Then, we scored orange spot expression of the male at one month as follows: 2, all spots were expressed; 1, some spots were expressed but others were still not expressed; 0, no spot was expressed.

We observed the fish in the aquarium everyday and recorded the date when the female delivered the first offspring. When the female delivered the next brood during the experimental period, we also recorded the date and calculated inter-delivery period between the first and second deliveries.

We conducted the following 3 experiments to examine the effect of diet on growth of males and females, male orange spot patterns and female reproduction.

Experiment 1

In order to examine the effect of algal-diet, one group from a single brood was fed food containing algae (algae-supplement) and another group was fed food without algae (control), but the fish of both groups obtained the same amount of energy from those foods. To obtain algae of similar quality, we put a fine plastic mesh sheet (8 x 8 cm) into a container with dechlorinated water as well as water from the original river about 4 weeks. The mesh sheet was equally covered by a species of unicellular green algae (S. Mayama and A. Kuriyama, pers. comm.) after 4 weeks. These algae were known as an important carotenoid resource in guppies (Dussault & Kramer, 1981; Grether et al., 1999).

In the preliminary experiment, we brushed off all algae from the mesh sheet and measured wet and dry weights of algae (mean \pm SE; 557.4 \pm 40.3 mg and 69.5 \pm 5.0 mg, respectively, N = 35) on a single mesh sheet. Then, we gave another mesh sheet covered by algae together with a given amount of commercial fish food (Tetramin; crude protein = 46 %, crude fat = 8 %, crude fiber = 2 %, crude ash = 11 %; TetraWerke) to 5 individual guppies for 2 days. We measured wet weights of the mesh sheet with algae before and after the presentation to guppies. We, then, calculated the decrement of algal wet weight by guppy foraging during the 2 days (85.8 \pm 2.2 mg, N = 35) and estimated dry weight (10.7 \pm 0.3 mg) using above-mentioned data. The energy of the algae and the commercial food were measured (17600 J/g and 20160 J/g, respectively; dry weight; mean values from 10 samples) by Tokyo Metropolitan Industrial Technology Research Institute.

From these results of the preliminary experiment, we adjusted the amount of the commercial food to the total amount of energy intake of an individual guppy (241.9 J/day) for both algae-supplement (algae on a mesh sheet and commercial food) and control (commercial food only) groups. During the experiment, we exchanged the mesh sheet with algae to a new one every second day for the algae-supplement group, and we also exchanged the mesh sheet without algae in a similar way for the control group.

We replicated this experiment for 20 broods.

Experiment 2

To examine the influence of carotenoid intake on guppies, one group from a single brood was fed commercial food with

carotenoids (carotenoid-supplement) and another group was fed only commercial food (control) during experimental period. In this experiment, we used another commercial food (CE-2; crude protein = 25 %, crude fat = 5 %, crude fiber = 4 %, crude ash = 7 %; Clea Japan) than in experiment 1, because this food contained no carotenoids. Therefore, we expected that the effect of experimental carotenoid supplementation would be clearly observed.

In the preliminary experiment, we tried several kinds of carotenoids and combinations among them. Then, we chose the combination of 100 ppm of β -carotene with 50 ppm of lycopene for carotenoid-supplement to 5 individuals of guppies, since this combination influenced most clearly male orange coloration. The energy of the commercial food with carotenoid-supplement and that without carotenoid-supplement was 19010 J/g and 19030 J/g, respectively (mean values from 10 samples; Tokyo Metropolitan Industrial Technology Research Institute).

We adjusted the amount of food to the total energy-intake of fish both in carotenoid-supplement and in control groups (228.4 J/day for an individual). We replicated this experiment for 21 broods.

Experiment 3

Finally, we examined the influence of the quantity of the same quality food without algae or carotenoid-supplement on guppies. One group (full-fed group) was fed the commercial food (CE-2) for 228.4 J/day per individual, similar to the control group of experiment 2. Another group (half-fed group) got only 50 % of this amount of the commercial food (114.2 J/day/individual) of the full-fed group. We

predicted that the quantity of food would possibly affect growth and/or reproduction but not male orange coloration. If the result of this experiment would confirm this prediction, only foraging ability for carotenoid-rich foods will be expressed by male orange coloration. We conducted 14 replications for this experiment.

Statistical analysis

For each sex, all data were averaged within each group. Before analysis, percent data such as relative orange spot area, saturation and brightness of male orange coloration were arcsine transformed (Zar, 1999). We adopted parametric tests because all of the averaged data showed a normal distribution (Kolmogorov-Smirnov one-sample test, $p > 0.05$). To compare the two groups in each experiment, we conducted paired t-tests.

When both body size and male coloration or female reproduction were significantly different between the two groups, the difference of male coloration or female reproduction could possibly not directly be caused by diet-treatment but indirectly generated through the difference of body sizes. To confirm this possibility, we conducted ANCOVA for body size (SL) as a covariate when both body size and male coloration or female reproduction were different between the two groups. When ANCOVA was conducted, we did not use the averaged data for each group but used data of each individual, because the relationship between body size and degree of male coloration or female reproduction was individually different even within a group.

Results

Experiment 1

At the start of the experiment, body sizes of males were not different both in SL (algae-supplement group, mean \pm SE = 11.4 ± 0.1 mm, control groups, 11.5 ± 0.1 mm; paired t-test, $t_{19} = -1.58$, $p = 0.13$) and TL (algae-supplement group, 15.9 ± 0.2 mm, control groups, 16.1 ± 0.2 mm; $t_{19} = -1.82$, $p = 0.09$) between the two groups. However, males of algae-supplement groups grew larger than males of control groups at the end of the experiment (SL, $t_{19} = 2.52$, $p = 0.02$, Fig. 1a; TL, $t_{19} = 2.43$, $p = 0.03$, Fig. 2a). Therefore, algae-supplementation might account for the growth differences of about 11 % (0.4 mm) in SL and 10 % (0.6 mm) in TL on the average. A similar tendency was shown for females: SL of females did not differ between algae-supplement and control groups at the start of the experiment (algae-supplement group, 11.7 ± 0.1 mm, control groups, 11.9 ± 0.1 ; $t_{19} = -1.90$, $p = 0.07$). However, females of algae-supplement groups grew larger than females of control groups at the end of the experiment ($t_{19} = 7.54$, $p < 0.001$; Fig. 3a) and growth of females of algae-supplement groups was about 17 % higher (1.9 mm) than females of control groups.

In male orange spot patterns, the degree of spot appearance at one month was significantly different between the two groups (Table 1). Males of algae-supplement groups expressed their orange spots faster than males of control groups. However, the number and relative area of orange spots were not different between the two groups (Table 1). Hue and saturation of orange spot coloration significantly differed between the two groups (hue, paired t-test, $t_{18} = 3.89$, $p = 0.001$; saturation, $t_{18} = 4.02$, $p < 0.001$; Fig. 4).

That is, males of algae-supplement groups had spot coloration closer to orange (i.e., about 40° in hue) and more conspicuous (a greater value in saturation). Brightness of orange spots was not significantly different between the two groups ($t_{18} = -1.79$, $p = 0.09$; Fig. 4). Since body sizes of males were different between the two groups (Fig. 1a), we conducted ANCOVA with body size as a covariate for hue and saturation of orange spots. In hue, the effect of both diet and body size were not significant (diet, $F_{1,81} = 3.42$, $p = 0.07$; body size, $F_{1,81} = 1.59$, $p = 0.21$). On the other hand, the difference of diet significantly influenced saturation of male orange spots ($F_{1,81} = 5.23$, $p = 0.02$), whereas body size did not affect saturation ($F_{1,81} = 0.68$, $p = 0.41$). Therefore, the conspicuousness of male orange spot coloration, at least for saturation, was directly enhanced by algae-intake. Although orange spot appearance scores at one month were also significantly different between the two groups, ANCOVA could not be adopted because individual data of the spot appearance score were not normally distributed (Kolmogorov-Smirnov one-sample test, $p < 0.001$).

Females of algae-supplement groups delivered the first offspring earlier than females of control groups (paired t-test, $t_{19} = -4.19$, $p < 0.001$; Fig. 5). Some females delivered the second broods during the experiment and the inter-delivery period of females of algae-supplement groups (mean \pm SE; 28.8 ± 0.5 days) was shorter than those of control groups (36.3 ± 2.6 days, $N = 7$ broods; paired t-test, $t_6 = -2.92$, $p = 0.03$). When ANCOVA was conducted with body size as a covariate, the date of the first delivery was significantly influenced by body size ($F_{1,70} = 6.62$, $p = 0.01$), whereas the effect of diet was not significant ($F_{1,70} = 0.14$, $p = 0.71$). The effect of body

size was also significant for the inter-delivery period ($F_{1,23} = 14.58$, $p < 0.001$) in contrast to that of diet ($F_{1,23} = 1.00$, $p = 0.33$). The enhancement of female reproduction in algae-supplement groups, thus, was achieved through larger body size and was not a direct effect of algae-intake.

Experiment 2

SL of males did not significantly differ between carotenoid-supplement groups (mean \pm SE = 10.9 ± 0.2 mm) and control groups (10.8 ± 0.1 mm; paired t-test, $t_{20} = 0.87$, $p = 0.39$) at the start of the experiment. Similarly, TL of males of carotenoid-supplement groups (15.5 ± 0.3 mm) also did not differ from those of control groups (15.4 ± 0.2 mm; $t_{20} = 0.47$, $p = 0.64$). At the end of the experiment, their body sizes also showed no significant difference between the two groups in both SL ($t_{20} = 0.53$, $p = 0.60$; Fig. 1b) and TL ($t_{20} = -0.99$, $p = 0.33$; Fig. 2b). This situation was the same in females; SL of females did not differ between the two groups at both the start (carotenoid-supplement groups, 11.4 ± 0.2 mm; control groups, 11.3 ± 0.2 mm; $t_{20} = 1.52$, $p = 0.14$) and the end ($t_{20} = 0.23$, $p = 0.82$; Fig. 3b) of the experiment. Therefore, carotenoid-supplement did not influence on the growth of guppies.

The score of male orange spot appearance at one month did not differ between the two groups (Table 1). Also, the number and relative area of orange spots were not different between males of the two groups (Table 1). In contrast, male spot coloration of carotenoid-supplement groups showed greater values in both hue and saturation than those of control groups (hue, paired t-test, $t_{19} = 3.33$, $p = 0.004$; saturation, $t_{19} = 4.59$, $p < 0.001$; Fig. 4).

Brightness of male orange spots did not differ between the two groups ($t_{19} = -1.29$, $p = 0.21$; Fig. 4).

In 9 broods, females delivered offspring. The date of the first delivery was not different between females of carotenoid-supplement and those of control groups (paired t-test, $t_8 = -0.80$, $p = 0.45$; Fig. 5). Females delivered the second broods during the experiment in only two cases of carotenoid-supplement groups, but no female delivered the second offspring in control groups.

Experiment 3

Body sizes of males did not differ between the two groups at the start of the experiment (SL, full-fed groups, mean \pm SE = 11.2 ± 0.2 mm, half-fed groups, 11.2 ± 0.2 mm; paired t-test, $t_{13} = -0.14$, $p = 0.90$; TL, full-fed groups, 15.7 ± 0.3 mm, half-fed groups, 15.8 ± 0.3 mm; $t_{13} = -0.35$, $p = 0.73$). However, males of full-fed groups grew larger than males of half-fed groups at the end of the experiment (SL, $t_{13} = 3.00$, $p = 0.01$, Fig. 1c; TL, $t_{13} = 5.88$, $p < 0.001$, Fig. 2c). Thus, male growth in SL and TL was about 18 % (0.5 mm) and 24 % (1.4 mm) higher, respectively, on full-fed groups than half-fed groups on the average. Females of full-fed groups also grew larger than those of half-fed groups at the end of the experiment ($t_{13} = 9.86$, $p < 0.001$; Fig. 3c), although body sizes of females were not different between the two groups at the start of the experiment (full-fed groups, 16.1 ± 0.4 mm, half-fed groups, 16.3 ± 0.4 mm; $t_{13} = -0.75$, $p = 0.47$). Therefore, the treatment of diet-quantity accounted for the difference of about 45 % (3.0 mm) of female growth.

The score of spot appearance, number and relative

area of orange spots did not differ between males of the two groups (Table 1). In orange spot coloration, hue, saturation and brightness were also not different between males of full-fed and half-fed groups (hue, paired t-test, $t_{13} = -1.21$, $p = 0.25$; saturation, $t_{13} = 0.64$, $p = 0.54$; brightness, $t_{13} = -0.33$, $p = 0.75$; Fig. 4). Therefore, the amount of food-intake without algae or carotenoid-supplement did not influence male orange spot patterns.

In 8 cases out of 14 broods, females delivered offspring. Females of full-fed groups delivered the first offspring earlier than females of half-fed groups (paired t-tests, $t_7 = -4.24$, $p = 0.003$; Fig. 5). When ANCOVA was conducted with body size as a covariate, the amount of food did not influence the first delivery date of females ($F_{1,25} = 0.60$, $p = 0.44$) but the effect of body size was significant ($F_{1,25} = 19.99$, $p < 0.001$). This indicates that larger females began reproduction earlier. In only 3 cases in full-fed groups, females delivered second broods, and no female got a second delivery in half-fed groups.

Discussion

The present study demonstrates that algae-intake increased the conspicuousness of orange spot coloration of male guppies. This positive effect on male orange spot coloration was similar to carotenoid-supplementation in this study as well as in previous studies (Kodric-Brown, 1989; Grether, 2000). In addition, larger body size did not contribute to orange spot coloration of males in algae-supplement groups, i.e., the conspicuousness of their orange spots was not a by-product of their faster growth. Therefore, carotenoids within algae may primarily function

for the increment of conspicuousness of male orange spot coloration. These results consist with our prediction that conspicuousness of orange spot coloration is an indication for foraging ability for algae or other high quality foods containing carotenoids in male guppies. In contrast, both algae- and carotenoid-supplements did not influence on the number and size of male orange spots. It is well known that orange spot number and size of male guppies are highly heritable traits in native populations (Houde, 1992) as well as in this study population (Karino & Haijima, 2001). The number and size of orange spots, thus, are not reliable indicators of the male's condition such as quality and quantity of food the male took in.

Algae-intake also enhanced growth of both male and female guppies. In addition, reproduction of females was also positively influenced by algae-supplement, although the positive effect was achieved indirectly through growth enhancement by algae-intake. Similarly, fish in full-fed groups in experiment 3 displayed a faster growth in both sexes, and the females reproduced earlier. In contrast with fish in full-fed groups in experiment 3, guppies in algae-supplement groups in experiment 1 got the same amount of gross energy through their food than those in control groups. Therefore, the enhancement of growth as well as reproduction by algae-intake was caused by the effect of some substances within the algae instead of the amount of energy-intake. Alternatively, it is also possible that guppies increased foraging or digestive efficiency by algal-supplement. On the other hand, carotenoid-supplement did not influence growth and reproduction of guppies in experiment 2. This result was inconsistent with our prediction that carotenoid-intake may positively influence fish growth or reproduction because carotenoids play an

important role in physiological systems such as digestive and reproductive systems in animals (Mayne, 1996; Olson & Owens, 1998).

Why did algae-intake enhance growth and reproduction in guppies although carotenoid-supplement in experiment 2 did not show such positive effects? It is possible to explain this in two ways that are not mutually exclusive. First, the positive effect of algae-intake may be caused by other carotenoids within the algae instead of β -carotene and lycopene. Grether et al. (1999) reported that algae in native streams of guppies contained not only β -carotene but also many other carotenoids and at least lutein and zeaxanthin as well as β -carotene were assimilated and usable by fish. Therefore, it is possible that these other carotenoids within algae positively influenced growth of guppies in experiment 1 in this study. Second, besides carotenoids, other substances within algae may influence physiological systems and enhanced growth in guppies. For instance, it has recently been suggested that non-digestible carbohydrates including dietary fibers within algae and plants have an important function in physiological systems, especially in digestive system, of animals. The non-digestible carbohydrates can accelerate the assimilation ability and prevent several diseases through gut fermentation and through their effects on intestinal microflora (Blaut, 2002; Gudiel-Urbano & Goñi, 2002; Puupponen-Pimiä et al., 2002; Rosamond, 2002). It is possible that non-digestible carbohydrates and other substances such as phenolics within algae positively influenced physiological systems in guppies and enhanced their growth.

In either way, algae-intake enhanced not only exaggeration of the male sexual ornament (orange spot

coloration) but also growth of both males and females. In addition, faster growth through the effect of algae-intake resulted in enhancement of female reproduction. The positive effect on growth may also benefit male reproductive success, because females prefer males with larger total length as their mates in this population (Karino & Matsunaga, 2002) as well as in native populations (Reynolds & Gross, 1992; Endler & Houde, 1995). Furthermore, it is possible that algae-intake increases the resistance against parasite infection because carotenoids positively influence the immune system of animals (Krinsky et al., 1989; Bendich, 1993; Olson & Owens, 1998). The results of this study and of previous studies (Endler, 1980; Kodric-Brown, 1989; Grether, 2000) suggest that conspicuousness of orange spot coloration in male guppies is an honest signal of their foraging ability for high quality food such as algae. Therefore, if foraging ability of males would be a heritable trait, female guppies could obtain indirect benefits by choosing males with more conspicuous orange coloration than their companions.

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Figure legends

Fig. 1. Male standard length at the end of the experimental period in experiment 1 (a), experiment 2 (b) and experiment 3 (c). Columns show mean and bars indicate SE. * $P < 0.05$ (paired t-test).

Fig. 2. Male total length at the end of the experimental period in experiment 1 (a), experiment 2 (b) and experiment 3 (c). Columns show mean and bars indicate SE. * $P < 0.05$, *** $P < 0.001$ (paired t-test).

Fig. 3. Female standard length at the end of the experimental period in experiment 1 (a), experiment 2 (b) and experiment 3 (c). Columns show mean and bars indicate SE. *** $P < 0.001$ (paired t-test).

Fig. 4. Coloration of male orange spots in each experiment. Hue (a), saturation (b) and brightness (c) are shown. Solid columns indicate algae- and carotenoid-supplement groups in experiments 1 and 2 or full-fed groups in experiment 3, respectively. Open columns show control groups in experiments 1 and 2 or half-fed groups in experiment 3, respectively. Means are given \pm SE. ** $P < 0.01$, *** $P < 0.001$ (paired t-test).

Fig. 5. Date of the first delivery of females in each group. Solid columns show algae- and carotenoid-supplement groups in experiments 1 and 2 or full-fed groups in experiment 3, respectively. Open columns indicate control groups in experiments 1 and 2 or half-fed groups in experiment 3, respectively. Means are given \pm SE. ** $P < 0.01$, *** $P < 0.001$ (paired t-test).

TABLE 1. Comparison of male orange spots between treatment groups. Mean \pm SE are shown, and relative spot areas were arcsine-transformed. Statistical analysis was conducted by paired t-test

Experiment 1 (N = 19)	Algae-supplement group	Control group	P
Spot appearance score	1.60 \pm 0.11	1.34 \pm 0.11	0.03
Number of spots	2.99 \pm 0.16	2.87 \pm 0.15	0.41
Relative spot area	0.11 \pm 0.01	0.10 \pm 0.01	0.33
Experiment 2 (N = 20)	Carotenoid-supplement group	Control group	p
Spot appearance score	1.29 \pm 0.14	1.10 \pm 0.15	0.32
Number of spots	2.77 \pm 0.16	2.97 \pm 0.20	0.31
Relative spot area	0.11 \pm 0.01	0.10 \pm 0.01	0.64
Experiment 3 (N = 14)	Full-fed group	Half-fed group	p
Spot appearance score	1.21 \pm 0.16	0.98 \pm 0.19	0.14
Number of spots	2.99 \pm 0.25	2.96 \pm 0.22	0.79
Relative spot area	0.12 \pm 0.01	0.11 \pm 0.01	0.38

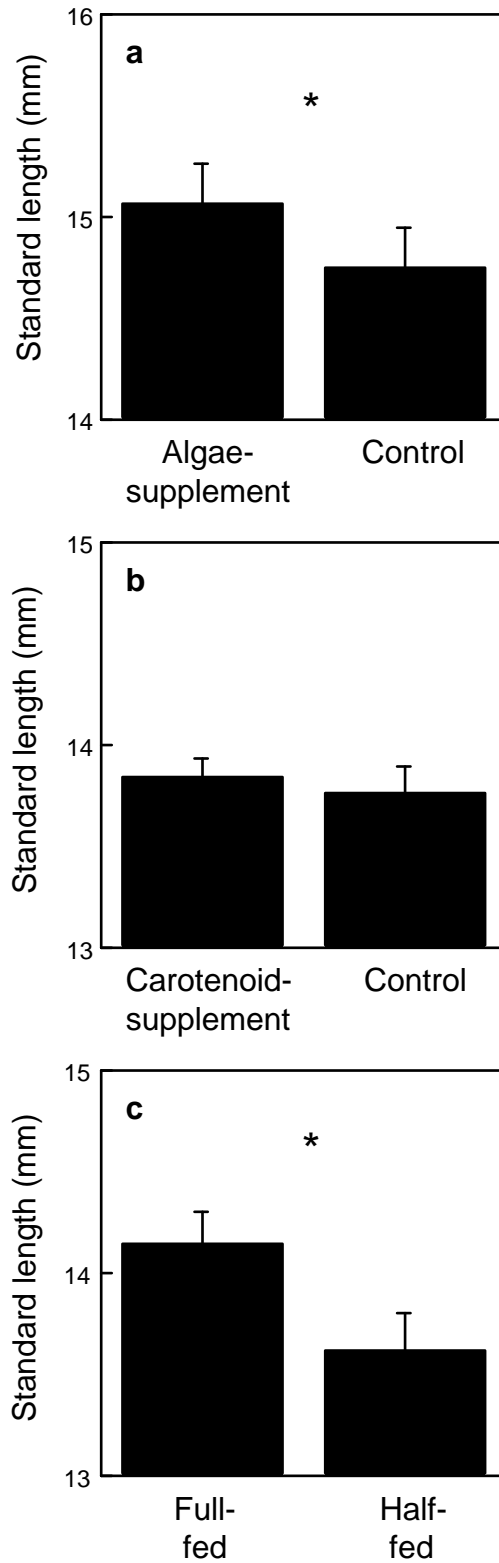


Fig. 1. (Karino & Haijima)

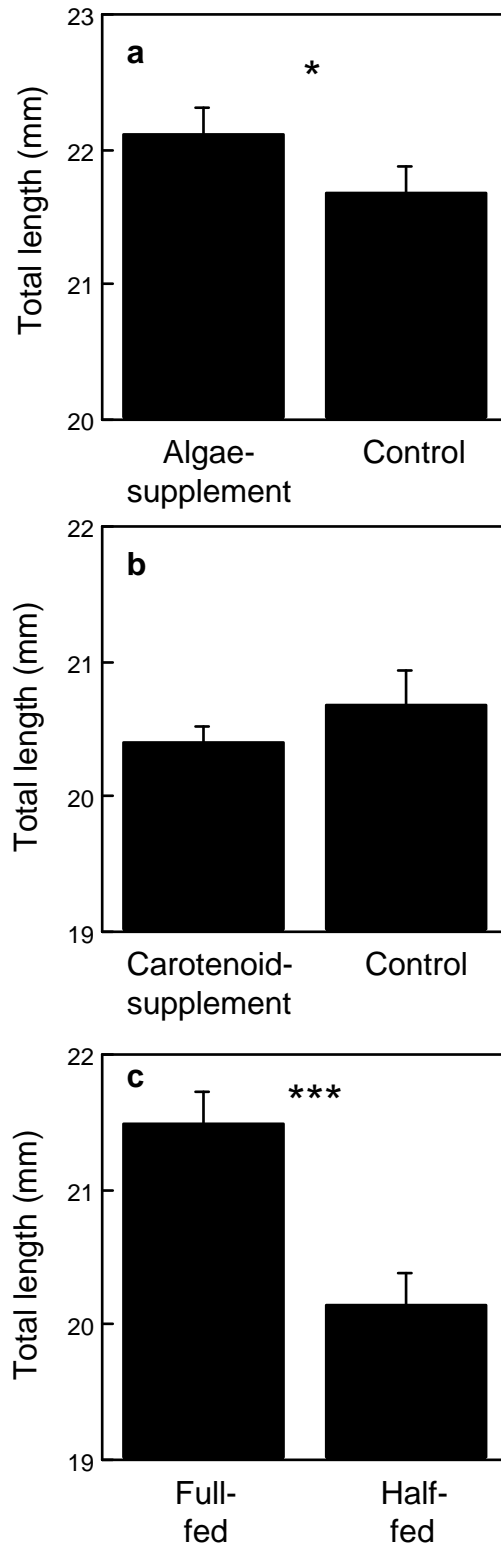


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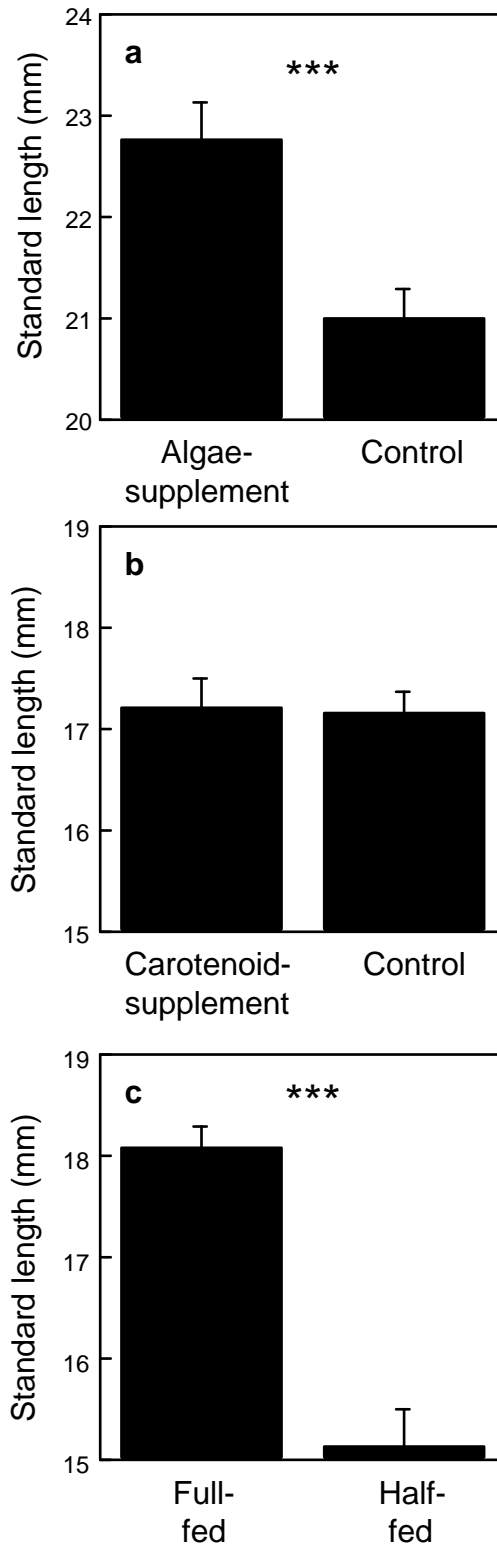


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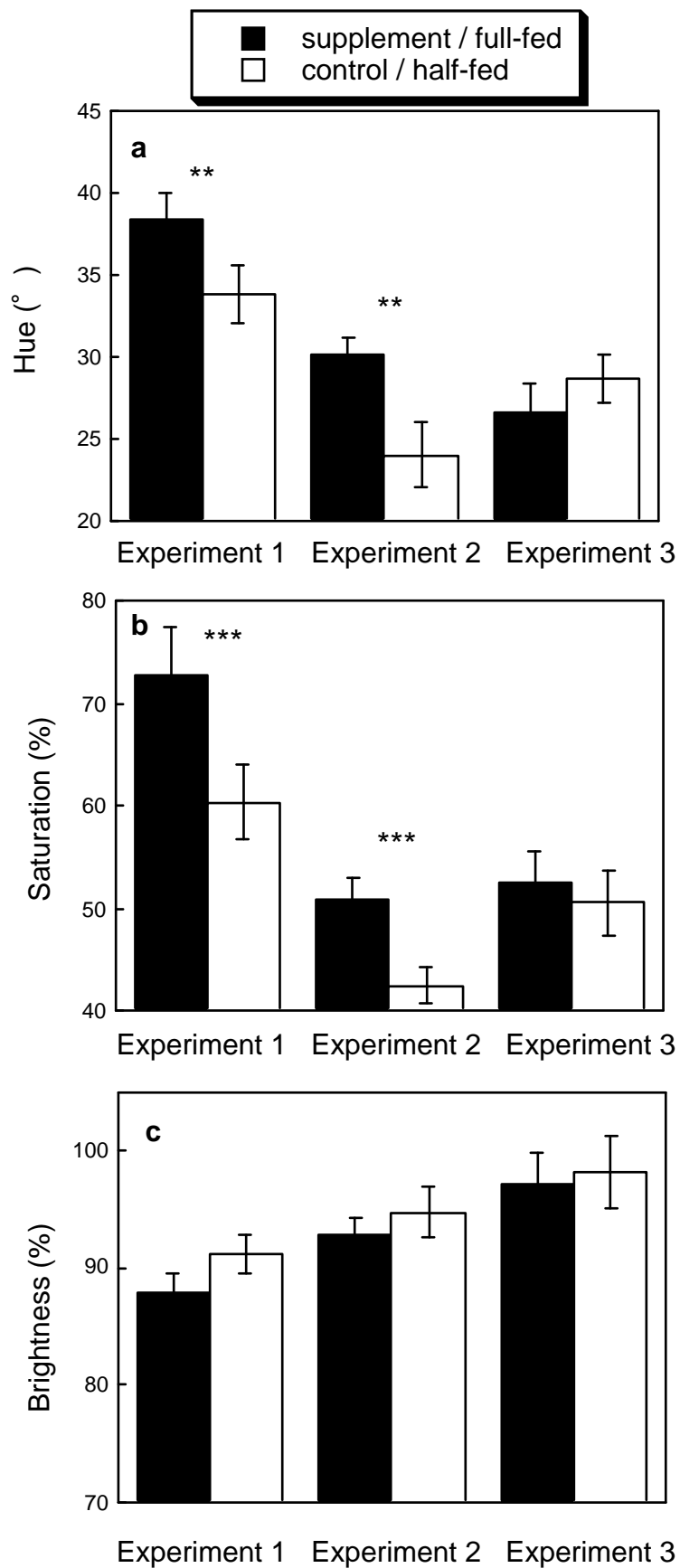


Fig. 4. (Karino & Haijima)

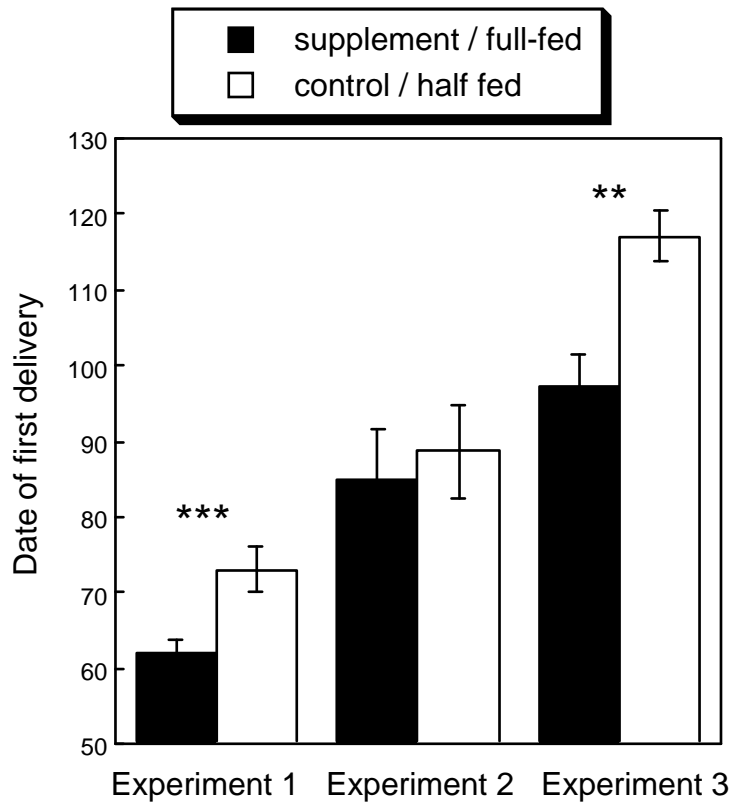


Fig. 5. (Karino & Hajjima)