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**The Relationship between the Algal-foraging Ability and Expression of Sexually
Selected Traits in the Male Guppy**

Kenji Karino* and Shinya Shinjo

*Department of Biology, Tokyo Gakugei University, Nukui-kita 4-1-1, Koganei,
Tokyo 184-8501, Japan*

Short title: Algal-foraging Ability in Male Guppies

*Corresponding author: Kenji Karino, Department of Biology, Tokyo Gakugei
University, Nukui-kita 4-1-1, Koganei, Tokyo 184-8501, Japan

Phone: +81-42-329-7520;

Fax: +81-42-329-7737;

E-mail: kkarino@u-gakugei.ac.jp

Section: Ecology

Abstract

In the guppy *Poecilia reticulata*, males exhibit orange spots on their body and tail, and the orange spot patterns are often criteria of female mate choice. The orange spot coloration of the males is determined by the intake of algae, a natural source of carotenoids. Therefore, it is considered that males exhibiting conspicuous orange coloration possess high algal-foraging ability. In the present study, we examined the influence of the algal-foraging ability with regard to an algal-searching ability and algal-foraging frequency on the expression of the orange spot patterns and on other sexually selected traits in male guppies. The males exhibiting a greater performance in terms of both the algal-searching ability and algal-foraging frequency grew larger. The size of the orange spots of the males also increased with the algal-foraging ability. However, neither the algal-searching ability nor the algal-foraging frequency influenced the coloration of the orange spots of the males. In this experiment, it is possible that the shortage of carotenoids due to limited carotenoid supply prevented the males from completely developing their orange spots to the intrinsic size. The results of this study suggest that in male guppies under a carotenoid-limited situation, the allocation of carotenoids is based toward the enlargement of the size of the orange spots rather than the enhancement of coloration. Since both the body size and orange spot patterns of the males contribute to their sexual attractiveness to females, the high algal-foraging ability may enhance their mating success.

Keywords: carotenoid-based coloration, foraging behavior, growth, *Poecilia reticulata*, sexual selection

INTRODUCTION

In some animals, females choose their mates on the basis of the carotenoid-based coloration of the males (Kodric-Brown, 1989; Milinski and Bakker, 1990; Craig and Foote, 2001; Hill, 2002; Bourne *et al.*, 2003; MacDougall and Montgomerie, 2003). Since animals cannot synthesize carotenoids *de novo*, they have to acquire carotenoids through their foods (Krinsky *et al.*, 1989; Olson and Owens, 1998). Therefore, it is often considered that the conspicuousness of the carotenoid-based coloration of males is an indicator of their ability to forage foods that are sources of carotenoids (Endler, 1980). Hence, the females may mate with males that have a high foraging ability for carotenoid-rich foods via their mate choice based on the carotenoid-based coloration.

It is known that carotenoids play important roles in a variety of physiological processes in animals, such as nervous, immune, and endocrine systems (Krinsky *et al.*, 1989; Olson, 1993; Olson and Owens, 1998). Therefore, individuals with a high foraging ability for foods that are sources of carotenoids can enhance their viability. For example, since carotenoids are recognized as free radical scavengers and stimulants of the immune system (Krinsky *et al.*, 1989; Lozano, 1994; Olson and Owens, 1998; Amar *et al.*, 2004), individuals possessing a higher foraging ability for carotenoid-rich foods may be more capable of maintaining their health and vigor than other individuals.

The guppy *Poecilia reticulata* is a live-bearing poeciliid fish that is native to the streams of Trinidad and adjacent parts of South America (Houde, 1997; Magurran, 2005). Male guppies exhibit bright colored spots, such as orange, black, and iridescent,

on their body and tail (caudal fin). It is well known that female guppies choose their mates on the basis of the orange spot coloration (Kodric-Brown, 1989; Grether, 2000) as well as other male traits, *e.g.*, the size of the orange spots (Houde, 1987) and body size (Reynolds and Gross, 1992). Since dietary carotenoids determine the orange spot coloration of males, the conspicuousness of this coloration may indicate their foraging ability for algae that serve as a natural source of carotenoids (Endler, 1980; Kodric-Brown, 1989; Grether *et al.*, 1999). Algae are known to be scarce in the natural habitats of the guppy (Endler, 1980; Grether *et al.*, 1999). Hence, the females can produce higher-quality offspring possessing a higher algal-foraging ability via their mate choice based on the orange spot coloration of the males (Endler, 1980; Karino *et al.*, 2005, 2007). Moreover, it can be predicted that male offspring that inherit the high algal-foraging ability may enhance their sexual attractiveness to females, such as conspicuous orange spot coloration. However, the direct contribution of the algal-foraging ability to the expression of orange spot patterns and other sexually selected traits in male guppies has not been examined in detail thus far.

In the present study, we examined the relationship between the algal-foraging ability and the expression of orange spot patterns in male guppies during the period of sexual maturation. During the experiment, we restricted the availability of algae to the males. During the temporary supply of algae, we observed two aspects of algal-foraging behavior of the males, *i.e.*, an algal-searching ability and the frequency of algal-foraging behavior. Subsequently, we analyzed the relationship between the scores of the algal-foraging ability of the males and the expression of their sexually selected traits including orange spot patterns at the end of the experiment. We predicted the following results for this experiment. (1) The males exhibiting the high

algal-foraging ability will represent conspicuous coloration of the orange spots. (2) The males that show a higher algal-foraging ability will grow faster because algal diet enhances the growth of the guppy (Karino and Haijima, 2004). (3) Since the size of the orange spots in male guppies is primarily determined by genetic factors (Houde, 1992; Brooks and Endler, 2001), the algal-foraging ability of the males will not influence the size of these spots.

MATERIALS AND METHODS

Subject animals

We used laboratory-reared descendants of wild-caught guppies from the Hiji River (26°43'N, 128°11'E), Okinawa, southern Japan, where guppies were introduced at least 30 years ago. In this feral population, the males exhibited high individual variation in their secondary sexual traits such as the sizes of the orange spots, body and tail (Karino and Haijima, 2001). The degrees of size and individual variation of the orange spots are similar to those observed in some native populations (cf. Houde and Endler, 1990; Winemiller *et al.*, 1990). The females in this population exhibit mate preferences on the basis of the orange spot patterns and total length of the males (Karino and Matsunaga, 2002; Karino and Shinjo, 2004).

To obtain the test males, we paired males and females that were caught from the Hiji River in October 2003. Each pair was placed in an isolation tank. The parental fish were removed from the tank after the birth of their offspring. The offspring were fed newly hatched brine shrimp nauplii once daily. When the sex of each fish could be determined based on the development of the gonopodium in males at

5-6 weeks after birth (Houde, 1997), we chose 34 males from different parental pairs. The males were anesthetized using a 2-phenoxyethanol solution and marked with subcutaneous injections of fluorescent elastomer (Northwest Marine Technology, USA) for individual discrimination. At the beginning of the experiment, the males did not express most of their orange spots (see also Karino and Haijima, 2004).

Experimental procedure

We used 5 aquariums (30.0 × 16.5 × 22.0 cm) for the experiment. In order to prevent any disturbance, we placed opaque boards on 4 sides of the aquariums. A part (16.0 × 9.0 cm) of one of the opaque boards was removable and functioned as a window for observation from the outside of the aquarium during the supply of algae (Fig. 1). The window was closed all times, except during the observation period of the algal-supply trials. We also placed two opaque boards within the aquarium (Fig. 1) in a similar manner to that described by Karino *et al.* (2005). A part of one opaque partition (5.0-cm width) could be removed at the beginning of the algal-supply trials by using a nylon filament. We covered the bottom of the aquarium with a 2-3 cm thick layer of gravel. An 18-watt daylight lamp was placed 20 cm above the aquarium with 12-h light and 12-h dark photoperiods, and the water temperature was maintained at 24±1.5°C. We washed the inside of the aquariums and exchanged water once a week, and added an algal-growth suppressor (S-3, Nisso, Japan) in the water to prevent algal growth within the aquarium.

At the beginning of the experiment, we placed 6-7 test males into each experimental aquarium. We reared the test males in the experimental aquariums for a 90-day experimental period. The males were fed a diet containing almost no

carotenoids (CE-2, Clea Japan, Japan) once daily, except during the algal-supply trials once every 3 to 4 days. Therefore, the test males obtained most of the carotenoids from algae during the algal-supply trials.

The removable part of the opaque partition within the experimental aquarium was generally removed. Therefore, the test males could freely move within the aquarium. At the beginning of the algal-supply trial, we replaced the removable part of the opaque partition, and all test males were placed in one compartment (A in Fig. 1) of the aquarium. A plastic mesh sheet (4.0×4.0 cm) that was covered by a species of unicellular green algae (see Karino and Haijima, 2004 for details) was placed at the bottom of the center of the opposite compartment (B in Fig. 1). Then, we removed the removable part of the opaque board on the side of the aquarium that functioned as a window. After a 10-min acclimation period, we removed the removal part of the opaque partition. Subsequently, through the window, we recorded the behavior of the males around the algae by using a digital video camera (DCR TRV-30, Sony, Japan) for 20 min. The males placed in one compartment (A in Fig. 1) could not see the algae at the bottom of the opposite compartment. To motivate the test males to forage for food, they were not fed from the day prior to the algal-supply trial. After the 20-min trial period, we removed the algae from the aquarium. In general, aggressive behaviors such as attack and threat display among the test males were not observed during the algal-supply trials. We conducted the algal-supply trial once every 3 to 4 days. In total, we performed 25 trials for the males in each aquarium.

Based on the digital video recordings, we measured the foraging behavior of each male during the algal-supply trials. We measured the time (s) required by each male to peck the algae after the removable part of the opaque partition was removed.

If the test males did not peck algae during the algal-supply trial, their score of the time required to peck algae was considered as 1200 s. The time required by the males to peck the algae appeared to represent their algal-searching ability. The previous study (Karino *et al.*, 2005) has demonstrated that tested males could learn the location of algae in the experimental aquarium during the trials. Therefore, this algal-searching ability of the males may indicate not only their exploration ability and the sensitivity to the chemical cue from the algae but also their learning ability. In addition, we measured the frequency of the algal-pecking behavior of each male (algal-pecking frequency) during the 20-min trial period. We calculated the average values of both the time required to peck algae and the algal-pecking frequency during the 25 trials for each male as the score of its algal-foraging ability.

Measurement of morphological traits

At the end of the 90-day experimental period, we measured the sexually selected traits of the test males. To evaluate the sexually selected traits, we recorded the right and left sides of the males by using a digital camera (Coolpix 950, Nikon, Japan). During the recordings, the males were anesthetized using a 2-phenoxyethanol solution. A fixed distance of 9.5 cm was maintained between the lens of the camera and the male. Two lights (23 watt, Tokin, Japan) were maintained at a fixed distance and angle from the male. For calibration, scale and color plates were displayed in the background of the fish.

The recorded images of the males were uploaded into a computer (PowerMac G4, Apple, USA) and analyzed using Photoshop 6.0 (Adobe, USA). We measured the areas of both the body and tail of each male. The body and tail areas were averaged

between the right and left sides of the male. In this study, the body and tail areas were used as the indicators of the body and tail sizes, respectively. The body and tail areas of the males at the beginning of the experiment individually varied (body area: 33.5-64.2 mm², mean±SD=47.8±6.9 mm²; tail area: 12.5-24.7 mm², 17.7±2.5 mm², n=32), although they had similar ages. In order to quantify the growth during the experiment, we calculated the increment values of body and tail areas of the males between the areas at the beginning and the end of the experiment. For further analysis, we used the body and tail area increments of the males as parameters of their growth during the experimental period.

From the digital images, we also measured the areas of all the orange spots on both the body and tail of each male and averaged the orange spot areas between the right and left sides. In addition, to obtain the relative area of orange spots (%), the orange spot area was divided by the total area of body and tail of the male. In order to quantify the coloration of the orange spots of males, we measured the hue (°), saturation (%), and brightness (%) of six points of each spot, and we calculated the average of the six points as the score of the spot. We used the average value of all the orange spots on both the right and left sides as the score of orange spot coloration of the male (see Karino and Haijima, 2001, 2004 for the detailed method).

Statistical analysis

Among the 34 test males, one individual died during the experiment. In addition, another male in a different aquarium was infected by a disease during the experiment. These two males were excluded from the analysis.

For the statistical analysis, values of the time required by the males to peck

algae were transformed to natural logarithms because the distribution of these data was skewed; most individuals (75%) pecked algae within 300 s; however, the remaining males required more time up to 1100 s to peck algae. Percentage data such as the relative orange spot area, and the saturation and brightness of the orange spot coloration were arcsine transformed (Zar, 1999). All data including these transformed data showed normal distributions (Kolmogorov-Smirnov one-sample test, $P>0.6$). To examine the relationship between the algal-foraging ability and expression of the secondary sexual traits in the males, we calculated Pearson's correlation coefficients.

RESULTS

The two aspects of the algal-foraging ability that were measured in this study, *i.e.*, the time required by the males to peck algae and the algal-pecking frequency, showed a highly significant correlation ($r=-0.78$, $n=32$, $P<0.001$). This result indicates that males possessing a higher algal-searching ability forage algae at a higher frequency.

The body area increment of the males showed a negative correlation with the time required to peck algae and a positive correlation with the algal-pecking frequency (Table 1, Fig. 2). Although the time required to peck algae did not significantly correlate with the tail area increment of the males, the latter increased significantly with the algal-pecking frequency (Table 1).

The time required by the males to peck algae and both the absolute and relative areas of their orange spots showed significant negative correlation (Table 1, Fig. 3). Both the absolute and relative areas of orange spots in the males showed a positive correlation with the algal-pecking frequency (Table 1, Fig. 3).

No significant correlation was observed between the hue of the orange spot coloration of the males and both the time required to peck algae and the algal-pecking frequency (Table 1). The relationships between the color saturation of the orange spots of the males and both the time required to peck algae and the algal-pecking frequency were not significant (Table 1). Similarly, neither the time required to peck algae nor the algal-pecking frequency showed significant correlation with the brightness of the orange spot coloration of the males (Table 1).

DISCUSSION

The results of this study demonstrated that the male guppies that exhibited a better performance in terms of the algal-foraging behavior grew larger by the end of the experiment. It is known that algal intake enhances the growth of guppies (Karino and Haijima, 2004). Carotenoids and other substances such as carbohydrates within algae may contribute to some physiological systems of the males and consequently enhance their growth (Krinsky *et al.*, 1989; Olson and Owens, 1998; Hemre *et al.*, 2002). In addition, it is possible that males exhibiting a better performance in terms of the algal-foraging behavior also possessed a higher foraging ability for foods other than algae. Kolluru *et al.* (2006) have documented that carotenoid intake did not influence the regrowth of the tail in male guppies. However, the result of this study indicated that males with a higher algal-foraging frequency exhibited larger tails. It is still unclear whether the algal diet directly contributed to the tail growth of the males or the total foraging success involving not only the algal intake but also that of other foods enhanced their tail sizes. The large tails of the males may contribute in increasing

their sexual attractiveness to females, because the female guppies often exhibit mate preferences for males with a large total length (Reynolds and Gross, 1992; Karino and Matsunaga, 2002).

In contrast to our prediction, the males that exhibited a better performance in terms of both the algal-searching ability and algal-pecking frequency had larger orange spots. In addition, the orange spot coloration was not influenced by the algal-foraging ability. These results were inconsistent with the data acquired from previous studies that in male guppies, the size of the orange spots is primarily determined by genetic factors (Houde, 1992; Brooks and Endler, 2001), and that the coloration of the orange spots is influenced by carotenoid intake (Kodric-Brown, 1989; Grether, 2000; Karino and Haijima, 2004). One possible explanation for the results of this study is that the males could not completely express the intrinsic size of their orange spots due to the shortage of carotenoids. In the present study, the males were fed food (CE-2) that contained almost no carotenoids, except when they were fed algae for a short duration during the algal-supply trials. Due to the shortage of carotenoid pigments within the body, the males might have been unable to completely develop their orange spots to the sizes that were genetically determined. A previous study (Karino and Shinjo, 2004) has also demonstrated the smaller size of the orange spots in male guppies that were fed the same food (CE-2) as compared with their sibling males that were fed carotenoid-rich food. In addition, the average size of the relative orange spot area of the males in this study (7.7 %) was 70-85 % smaller than those of males that were fed algae and had similar ages with the males in this study (cf. Karino and Haijima, 2004; Karino and Shinjo, 2004). These findings may support the possibility that the males in this study could not completely expand their orange spots due to the shortage of carotenoids. In

this study, the positive contribution of the algal-foraging performance of the males to their orange spot sizes suggests that under a carotenoid-limited situation, male guppies use carotenoids primarily to enlarge their orange spots up to the intrinsic size. When the males completely expand their orange spots, they may subsequently use the carotenoids to enhance the coloration of their orange spots. Further studies are required to examine the trade-off regarding the carotenoid allocation between the expansion of the orange spot size and the enhancement of the orange spot coloration in male guppies under a carotenoid-limited situation. Since both the size and coloration of the orange spots in male guppies are known as criteria for female mate choice (Houde, 1987; Kodric-Brown, 1989; Endler and Houde, 1995; Grether, 2000), a trade-off in terms of the carotenoid allocation may imply the relative importance of the size and coloration of the orange spots in the males for sexual selection.

The other possible explanation for the positive correlation between the algal-foraging ability and the size of the orange spots observed in this study can be considered to be that the males with larger intrinsic size of their orange spots possessed greater algal-foraging ability. It has been suggested that in guppies, the size of the orange spots of males correlates with their genetic qualities with respect to anti-predator behavior (Evans *et al.*, 2004) and sperm quality (Locatello *et al.*, 2006). If the size of the orange spots indicates the overall genetic quality of the males, it is likely that it may also indicate their foraging ability.

The results of this study demonstrated that males possessing high algal-foraging ability have a large body and can produce large orange spots. Since the large body as well as the large orange spots of male guppies may increase their sexual attractiveness to females (Houde, 1987; Reynolds and Gross, 1992; Endler and Houde,

1995), males possessing high algal-foraging ability may be able to obtain high mating success. Moreover, an aspect of the algal-foraging ability is a heritable component (Karino *et al.*, 2005). In general, the conspicuousness of the orange spot coloration in male guppies is determined by the algal intake (Grether *et al.*, 1999; Karino and Haijima, 2004). Hence, females may be able to obtain indirect (genetic) benefits such as production of offspring exhibiting a higher algal-foraging ability and consequently possessing considerable sexual attractiveness via their mate preferences based on the orange spot patterns that signal algal-foraging ability of the males.

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Table 1. Mean±SD of morphological traits of males (n=32) at the end of the experiment and Pearson's correlation coefficients (r) between these traits and the scores of the algal-foraging ability. Pearson's correlation coefficients of both the body and tail areas were calculated from the increment values between the areas at the beginning and the end of the experiment. Values of time required to peck algae were log-transformed, and those of the relative orange spot area, and the saturation and brightness of the orange spot coloration were arcsine transformed prior to the analysis.

Traits	mean±SD	Time required to peck algae r (P)	Algal-pecking frequency r (P)
Body area (mm ²)	58.4±8.2	-0.43 (0.01)	0.44 (0.01)
Tail area (mm ²)	23.8±3.7	-0.15 (0.42)	0.41 (0.02)
Absolute area of orange spots (mm ²)	6.4±3.0	-0.56 (<0.001)	0.57 (<0.001)
Relative area of orange spots (%)	7.7±3.3	-0.49 (0.004)	0.43 (0.02)
Orange spot coloration			
Hue (°)	32.5±3.6	-0.01 (0.98)	0.09 (0.63)
Saturation (%)	82.6±8.9	0.23 (0.20)	-0.23 (0.21)
Brightness (%)	62.3±9.0	-0.18 (0.32)	-0.04 (0.81)

Figure legends

Fig. 1. Vertical (upper) and three-dimensional (lower) diagrams of apparatus used for the experiment. The broken line represents the removable part of the opaque partition.

Fig. 2. Relationships between the body area increment of the males and **(a)** the time required to peck algae and **(b)** the algal-pecking frequency. Values of the time required to peck algae were log-transformed.

Fig. 3. Relationships between the relative orange spot area of the males and **(a)** the time required to peck algae and **(b)** the algal-pecking frequency. Values of the relative orange spot area were arcsine transformed, and those of the time required to peck algae were log-transformed.

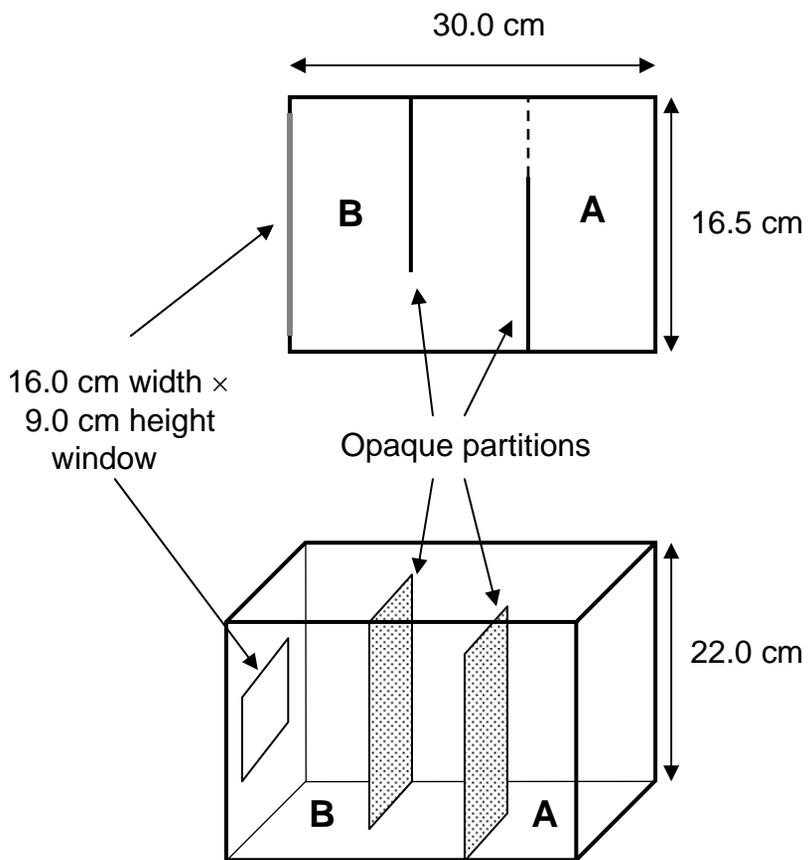


Fig. 1. (Karino and Shinjo)

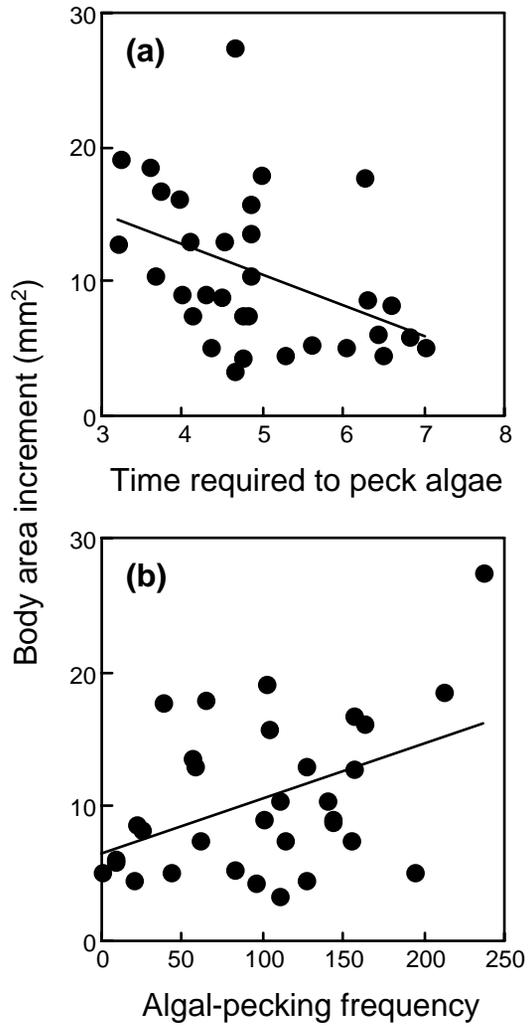


Fig. 2. (Karino and Shinjo)

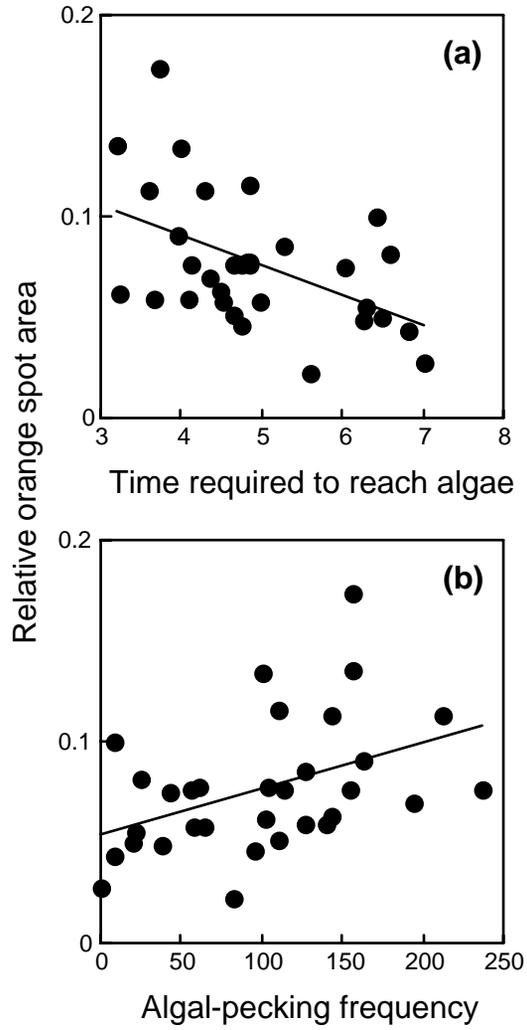


Fig. 3. (Karino and Shinjo)