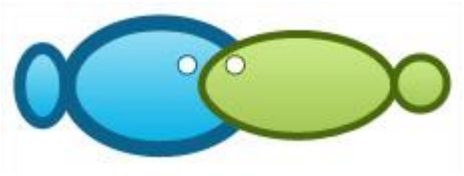

β-carotene effect on golden rabbitfish (*Siganus guttatus*) larvae

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Abstract. Siganids are important for the fishing industry, but overfishing can make their harvesting unsustainable. Production from hatcheries is required, but it is being constrained by high larval mortality considered to occur because of the limitations of the larvae in detecting feed. This study aims to make it easier for larvae to detect feed, thus improving their growth and survival. The study found that a β-carotene dose of 0.075 g L^{-10} was best for obtaining better growth and predation. However, it did not show a significant effect on eye diameter and survival of *Siganus guttatus* larvae. Meanwhile, excessive doses of β-carotene did not have a better effect on the eye development, growth, amount of feed predation, and survival of *S. guttatus* larvae. It was concluded that a dose of 0.075 g L^{-10} of enrichment media was the best for increasing growth and predation. Therefore, it is recommended to use this dose in enriching rotifers as larvae feed. It is further recommended that future studies examine the period of use of β-carotene as an enrichment material for rotifers in order to provide maximum effect on *S. guttatus* larvae, leading to more efficient use of β-carotene.
Key Words: eye diameter, feed predation, herbivorous fish.

Introduction. Fish are classified as white meat animal protein sources, often considered better for health. Prameswari (2018) mentioned that high protein in fish is due to the numerous essential amino acids and omega-3 fatty acids that are excellent for brain development, also vitamins and minerals. Further, fish is better for protein absorption compared to beef, chicken, and other types of meat, because its protein fiber is shorter.

Siganids are represented by many species, one of which is the golden rabbitfish (*Siganus guttatus*). Siganids are highly favored by consumers, causing a sharp increase in the demand while the supply still resorts to wild fisheries (Gonzales et al 2018). This situation turns the fish to be a valuable fishing target for fishermen (Latuconsina et al 2020b), possibly leading to overfishing. One solution to this problem are fish hatcheries. However, hatchery effort is still constrained by high larval mortality (Juario et al 1985; Hara et al 1986; Kamaruddin et al 2019).

To have a certain quality and sustainable seeds in adequate quantity, an approach to the case through feeding application containing adequate nutrition for the larval development is needed. Larvae have limited abilities to detect feed because their organs of vision are not fully developed (Juario et al 1985), obstructing predation activities. The number of preys is positively correlated with the growth and survival (Stuart 2013). One of the nutrients that can be added to the larvae feeds to help their eye development is β-carotene.

Based on the above explanation, studies on β-carotene addition to larval feeds is required, to examine the effect of β-carotene addition on the development of larval eyes which in turn improve the number preying, growth, and survival. The research results are

expected to be a reference for efforts in Siganid hatcheries. This research aims to help the larvae detect feed; therefore, better growth and survival can be achieved.

Material and Method

Place and time of the research. The research was conducted at the Barru Tiger Shrimp Hatchery Installation (IPUW), South Sulawesi from 14 June 2021 to 25 September 2021. Golden rabbitfish (*Siganus guttatus*) larvae observation was conducted in the Biotechnology Laboratory of IPUW. Samples for water quality parameters observation were analyzed in the water quality laboratory of Hasanuddin University, Makassar. To test the β-carotene content, samples of rotifers were sent to PT. Saraswanti Indo Genetech laboratory, Bogor to be tested.

Experimental design. This study employed a completely randomized design (CRD) with 5 treatments and 3 replications, hence consisting of 15 experimental units. The experimental treatment was that different doses of β-carotene were added to *Brachionus rotundiformis* before being administered to the larvae. The dosage levels are as follows (referring to Ridwan 2002):

- Treatment A is the control (without β-carotene addition)
- Treatment B (a dose of 0.025 g L^{-10} β-carotene)
- Treatment C (a dose of 0.050 g L^{-10} β-carotene)
- Treatment D (a dose of 0.075 g L^{-10} β-carotene)
- Treatment E (a dose of 0.100 g L^{-10} β-carotene)

Feed preparation. *B. rotundiformis* as the natural feed was harvested from a mass culture tank with density of 500-1000 individuals/ml (Ridwan 2002; Jusadi et al 2015) and was enriched before fed to the larvae. The enrichment procedure follows the following protocol:

1. For every 10 L of media, 0.5 mL of fish oil (A1 DHA Selco) was added, 0.1 g egg yolk, 0.25 g bread yeast, and β-carotene were also added as per treatment concentration (doses of 0, 05, 50, 75 and 100 mg). Then they were filled to a receptacle filled with 200 ml of water to be emulsified by a blender for three to five minutes.
2. The enriched media was poured to the receptacle of enriched rotifers.
3. The rotifers were incubated for 2 hours and were filtered with 100 µL plankton nets which were then fed to the *Siganus guttatus* larvae.

Preparation and rearing of tested animals. Natural feed experiment (*B. rotundiformis*) incorporating β-carotene was conducted for ten days (during larvae feeding with rotifers as their natural feed). The enriched *B. rotundiformis* were fed to larvae with a density of 10-20 individuals/mL (according to the technical guidance by Juario et al 1985 and Duray 1998). The larvae density of 20 individuals/L (Duray & Kohno 1988; Lante & Muslimin 2012) reared in a 6000 L tank was filled with 3500 L of seawater at a salinity of 20-25 ppt. Water replacements were done according to Duray and Kohno (1988). Larvae observations were carried out using a microscope with magnification of 4x. Larval samples were taken from each tank at 3-10 days after hatching (DAH). Total harvesting was conducted at the end of the experiment to obtain the survival data.

Observed parameters. Parameters observed in this experiment include eye diameter, absolute growth, number of feedings, survival, and water quality. Thirty individuals of larvae were extracted from each rearing tanks to observe their growth. This observation was made using Olympus 40 microscope, with 4x magnification. The microscope was equipped with a measuring scale and was connected to a computer to store pictures (documentation). Eye diameter was measured by dragging lines until the eyes were

completely observable under the microscope and measured with the help of the measuring scale. Absolute growth was determined by the differences of the larvae length at the end and the beginning of the experiment. The observation of the stomach was done by pushing the larvae stomach until the entrails were scattered, and then counting the number of rotifers within their intestinal system. Survival was quantified by comparing the number of individuals at the start of the experiment with those at the end (10 DAH). Water quality was measured as the supporting data, with ammonia concentration being sampled at day 5 and day 10. The samples were then transported to the laboratory. Other water quality parameters (temperature, pH, salinity, and dissolved oxygen) were measured in the morning (07:00 a.m.) and in the afternoon (5:00 p.m.).

Data analysis. Data retrieved from the experiment were analyzed using ANOVA. In cases where ANOVA was significant, the post-hoc test was employed using the W-Tukey (Steel & Torrie 1991). Data analysis was carried out using SPSS 16.0. The water quality parameters were analyzed descriptively based on the life expectancy of *S. guttatus*.

Results

Eye diameter. The average larval eye diameter growth of *Siganus guttatus* fed with rotifers enriched with β-carotene is described in Table 1.

Table 1

Average growth of eye diameter of *S. guttatus* larvae fed with β-carotene under different doses

Treatment	*Doses of β-carotene (g L^{-10})*	*Eye diameter average (μm±SD)*
A	0	53.41±9.170^{a}
B	0.025	49.02±7.743^{ab}
C	0.050	42.31±15.720^{ab}
D	0.075	51.73±4.526^{a}
E	0.100	23.61±7.198^{b}

Note: Different letters above the numbers indicate significant differences between treatments at the level of 5% ($p<0.05$).

ANOVA analysis showed that rotifers enriched with β-carotene under different doses affected larval eyes growth significantly ($p<0.05$). The post-hoc test results show that the dose of 0 g β-carotene (treatment A) was significantly different ($p<0.05$) from treatment E, but was not significantly different ($p>0.01$) from treatment B, C and D.

Absolute growth. Growth is defined as changes in size (length, width, and weight) of the fish body under particular period of time. This study only tested the length and width, since obtaining data on their weight requires large number of larvae samples for scaling to obtain a detected weight.

Total length. The average larval total length is presented in Table 2.

Table 2

Total length of *S. guttatus* larvae with β-carotene under different doses

Treatment	*Doses β-carotene (g L^{-10})*	*Absolute length (mm±SD)*
A	0	0.300±0.135^{a}
B	0.025	0.520±0.146^{ab}
C	0.050	0.671±0.051^{b}
D	0.075	0.707±0.490^{b}
E	0.100	0.453±0.175^{ab}

Note: Different letters above the numbers indicate significant differences between treatments at the level of 5% ($p<0.05$).

ANOVA results revealed that rotifers enriched with β-carotene under different doses significantly affected the total length increment ($p<0.01$). The post-hoc test found that β-carotene of 0.050 gr (treatment C) and 0.075 g (treatment D) are significantly different from treatment A (0 g), but not significantly different ($p>0.05$) from treatment B (0.025 g) and E (0.100 g). Table 2 shows that the highest average of the length increment was gained by treatment D (β-carotene dose of 0.075 g) with 0.707+0.490 mm, followed by treatment C, B, E and A respectively.

Body width. The average value of absolute growth of body width of larvae is presented in Table 3.

Table 3

Growth of the larvae body width of *S. guttatus* with various doses of β-carotene

Treatment	*Doses of β-carotene (g L^{-10})*	*Absolute body width (μm±SD)*
A	0	$84.534±13.567^{a}$
B	0.025	$109.365±4.754^{ab}$
C	0.050	$104.385±6.956^{ab}$
D	0.075	$132.630±10.217^{bc}$
E	0.100	$144.189±20.750^{c}$

Note: Different letters above the numbers indicate significant differences between treatments at the level of 5% ($p<0.05$).

ANOVA results found that different doses of β-carotene enrichment to the rotifers had significant effects ($p<0.01$) on the absolute body width growth of the larvae. Results from the post hoc Tukey test showed that 0 g β-carotene (treatment A) was significantly different from treatment D (0.075 g) and E (0.100 g). Treatment E was not statistically different ($p>0.05$) from treatment D but was significantly different from ($p<0.05$) from B (0.025 g) and C (0.050 g). Table 3 illustrates that the highest average of absolute width growth of was obtained from treatment E (0.075 g) which was 144.189±20.750 μm, followed by treatment D (132.630±10.217 μm), B (109.365±4.754 μm), C (104.385±6.956 μm), whereas the lowest growth was gained from treatment A (84.534±13.567 μm).

The amount of feed consumption. The number of preying is one of the indicators of feeding success. The number can be increased through signal management of external factors, such as adding colors to rotifers. In this study, rotifers were enriched using β-carotene to enhance the contrast of colored rotifers, expected to be more visible to the larvae of *S. guttatus* and thus improve the number of preying. The average of *S. guttatus* larvae feeding can be seen in Table 4.

Table 4

Average feed predation of *S. guttatus* larvae under different doses of β-carotene

Treatment	*Doses of β-carotene (g L^{-10})*	*Average feed predation (individual)*
A	0	14^{a}
B	0.025	22^{b}
C	0.050	18^{ab}
D	0.075	23^{b}
E	0.100	19^{ab}

Note: Different letters above the numbers indicate significant differences between treatments at the level of 5% ($p<0.05$).

ANOVA revealed that rotifer enrichment with different doses of β-carotene was significant ($p<0.01$) to the number of preying by the *S. guttatus* larvae at 10 DAH. W-Tukey post-hoc test showed that β-carotene at dose of 0 g (treatment A) was significantly different

from treatment B (a dose of 0.025 g) and treatment D (0.075 g), but was not significantly different from treatment C and E. Treatment C was not significantly different from treatment A, B, D and E.

Survival rate (SR). The *S. guttatus* were reared for 10 days. To obtain the survival rate data, the *S. guttatus* were harvested by total harvesting method. The survival rate of *S. guttatus* larvae fed with rotifers enriched with β-carotene is described in Table 5, while the test of β-carotene contained in rotifers after enrichment is shown in Table 6.

Table 5

Survival rate of *S. guttatus* larvae with different doses of β-carotene

Treatment	*Doses of β-carotene (g L^{-10})*	*Survival rate (%±SD)*
A	0	2.531±2.961
B	0.025	2.318±2.108
C	0.050	1.7835±0.857
D	0.075	1.8935±1.693
E	0.100	2.188±0.720

The ANOVA showed that rotifers enriched with β-carotene under different doses did not significantly affect the survival of *S. guttatus*. As the data in Table 5 illustrates, the highest survival rate was found in treatment A with the value of 2.531%, followed by treatment B (2.318%), E (2.188%), D (1.8935%), and treatment C (1.7835%) as the lowest.

Table 6

Level of β-carotene content in rotifera *Brachionus rotundiformis* after β-carotene enrichment (incubated for 2 hours)

Treatment	*Doses of β-carotene (g L^{-10})*	*β-carotene content in Brachionus rotundiformis (µg/g)*
A	0	4.76-4.80
B	0.025	716.47-721.50
C	0.050	261.45-272.84
D	0.075	457.04-460.32
E	0.100	425.32-439.24

Water quality. The water quality parameters measured during larval rearing are presented in Table 7.

Table 7

Water quality parameters measured during rearing *S. guttatus* larvae

Water quality parameters	*Measured range*
DO (mg L^{-1})	2.9-5.9
Salinity (ppt)	20-25
pH	6.29-8.20
Temperature (°C)	27.2-29.9
Ammonia (mg L^{-1})	0.0005-0.0128

As shown in Table 7 above, dissolved oxygen measured during the research was between 2.9-5.90 mg L^{-1}. Salinity was between 20-25 ppt. pH was within the range of 6.29-8.20. Temperature fell between 27.2-29.9°C, and ammonia level was between 0.0005-0.0128 mg L^{-1}.

Discussion

Eye diameter. Table 1 shows that the highest average of eye diameter was found in group A (0 g) 53.41±9.170 µm followed by group D, B, C and E (0.100 g). This highlights that excessive β-carotene had no preferred effects on the eye development. In fact, the eye development was inhibited by the highest dose of β-carotene in this experiment, namely treatment E (0.100 g). This finding illustrates that additional β-carotene cannot be used optimally by the fish because each individual has limited ability to absorb and accumulate the β-carotene in its body (Ridwan 2002; Malide et al 2018; Heruwanto et al 2020). The best diameter size was found in group with zero β-carotene; rotifers naturally contain β-carotene. From the β-carotene content analysis (Table 6), it was found that rotifers without β-carotene enrichment contained 4.76-4.80 mg/Kg of β-carotene. However, the addition of β-carotene still needs to be done, because β-carotene can increase the size of the eye diameter, produce better larval growth, and obtain a higher amount of forage (Table 4). The best dose to obtain the highest growth and predation amount was 0.075 g (treatment D).

The eye development was an indicator of the larvae eyes adapting to improve their sensitivity to light especially in deep sea fish (Afitah et al 2020). The larger the eye diameter, the bigger the size of each component that constitutes the eyes including the lens coverage and the thickness of retina. Hence, it supports the eyes in catching the light (Fiolita et al 2015). The light received by the eyes can stimulate growth, growth is triggered by hormones as growth hormones are strongly associated with the amount of energy that came through the pupils and retina in fish eyes (Fuad et al 2020). Apart from that, the duration of illumination can also stimulate growth, where the right range of illumination can improve *Siganus guttatus* larvae growth (Duray & Kohno 1988). However, the utilization of the light by eyes is limited (Stuart 2013; Widomska & Subczynsli 2018). Each fish species possesses different response ability to light as some fish prefer bright light, and some do not (Nurdin et al 2015).

Absolute growth (body length and width). The result of this experiment showed that the best dose of rotifers enrichment with β-carotene to support the growth (length and width) of the *S. guttatus* larvae was 0.75 g (treatment D). The growth of the *S. guttatus* larvae which was given natural feed (rotifers) enriched with β-carotene showed better growth performance compared to the ones without the enrichment (control = treatment A). This was due to the additional nutrition which was administered to the natural feed rotifers before they were fed to the larvae of *S. guttatus*. Additional nutrition to the rotifers is highly crucial since rotifers tend to be vitamin A deficient compared to green algae and copepods (Parma & Bonaldo 2013). Malnutrition in living organisms (including rotifers) is thought to be caused by the body's use of a number of nutrients to meet survival needs (Darsiani et al 2017), thus the nutrients balance in the rotifers' bodies becomes unstable, and often experience deficiencies (Li et al 2017). β-carotene is a carotenoid compound (Cvekl & Wang 2009) that supports growth, metabolism and larval health in fish (Widomska & Subcyaski 2018; Warastuti et al 2022). The addition of β-carotene to the maru fish (*Channa marulioides*) feed improved growth performance (Warastuti et al 2022), and in cases where β-carotene was applied to other ornamental fish such as goldfish (*Carassius auratus*), in addition to having positive effect on growth, also improved the color brightness of the fish (Madiara et al 2019).

Albeit the positive effect of β-carotene for the growth of *S. guttatus* larvae, excessive dose did not yield a better impact. This was illustrated by the finding where treatment E (0.100 g) that did not lead to better growth compared to treatment D (0.075 g). This highlights that larvae have limitations in utilizing nutrition from feed (β-carotene in our case). Enrichment of β-carotene to a different species (milkfish *Chanos chanos*) was experimented by Ridwan (2002), and he reported similar effect that the 60 mg L^{-10} dose of β-carotene was the best for fish growth. When the dose was increased, it did not lead to better growth, survival, and health performance of the eyes of the milkfish. This was expected because the milkfish larvae have limited ability in accumulating β-carotene in their bodies, hence the physiological function of vitamin A was declining. Similar result

was also reported by Hendra et al (2014), Malide et al (2018) and Heruwanto et al (2020), that each individual fish has limited ability in absorbing and accumulating β-carotene in its body. In fact, β-carotene overdose can disrupt the performance of LH (luteineizing hormone) in fish (Malide et al 2018), even lead to fish mortality (Heruwanto et al 2020).

The growth (length and width) of the larvae is slow, yet it is possible that the larvae to grow quickly after 10 DAH. For instance, the larvae of Kurumoi rainbow (*Melanotaenia parva*) (Kadarini et al 2013) grew relatively slow at 1-16 DAH yet the growth rate highly increased after 21 DAH.

The number of preying. The number of preying is the number of rotifers present in the fish intestinal system. As Table 4 shows, the highest average of preying was found in group D, followed by group B, E, C and A. Therefore, it can be concluded that the addition of β-carotene can increase the number of preying by the *S. guttatus* larvae. The best dose to improve the number of preying is 0.075 g (treatment D). The preying intensity increased in group fed with enriched rotifers. This result indicated that β-carotene enrichment can enhance the contrast of rotifer color. Indeed, β-carotene is a nutrient that increases color intensity of the body of organisms consuming it (Effendi et al 2004; Chavarria & Flores 2013; Hekimoglu et al 2017; Madiara et al 2019).

Naturally, rotifers tend to be transparent and mimic their environment. It is highly advisable that coloring is done to contrast rotifers color from the environment to ease the larvae in detecting and preying them (Hendra et al 2014). Rotifers are filter feeders; hence they could function as a biocapsule for fish larvae (Salsabila et al 2019). Therefore, β-carotene used as enrichment in this experiment was easily transferred to the larvae through the rotifers.

The daily number of feed preying shows that it positively correlated with age increment. This indicates that the older the larvae, the better the eyes at detecting prey. The capability of the larvae to detect feed will greatly affect the success of preying (Yufera et al 2014). If preying is successful, growth can be achieved (Stuart 2013).

Survival rate (SR). The survival percentages obtained from each treatment showed that rotifers enriched with β-carotene cannot support survival of *S. guttatus*. However, compared to the survival rate from the 2019 field study (Darsiani et al 2022) yielding only 1%, this experiment yielded better result and it was promising for the success of *S. guttatus* hatchery.

The best survival was obtained from treatment A, possibly due to the use of other enrichment ingredients alongside the β-carotene. In this experiment, the enrichment used additional ingredients such as fish oil (A1 DHA Selco), bread yeast, and egg yolk. These ingredients increase nutrients for the rotifers in terms of protein, fat, carbohydrate, water, and minerals (Oktaviani et al 2012). Darosman et al (2019) mentioned that several factors must be considered in preparation of larvae feed; they need to be easy to digest, appropriate in size, slow in motion, and can be sustainably obtained.

β-carotene contain test in each treatment showed that all treatments have β-carotene content, including treatment A. β-carotene content in treatment A was 4.78 mg/kg (Table 6). Rotifers naturally contain β-carotene. Despite the small amount, it was highly possible that it had significant effect on the larvae development including the eye's, affecting the larvae capability in detecting objects (including feed). A good survival can also be affected by other external factors such as the environment and the cultivation media in a stable condition, and the water quality necessary for the niche of the organism (Warastuti et al 2022).

Water quality. The ranges of water quality parameters measured during the experiment (Table 7) were still in the normal range of tolerance of the *S. guttatus* larvae (Darsiani et al 2022). *S. guttatus* can live naturally in the water with the oxygen concentration between 4.0 – 7.7 mg L^{-1} (Suci et al 2020; Latuconsina et al 2020a). The salinity tolerated by *S. guttatus* is between 10-36 ppt (Sarisma et al 2017; Kamaruddin et al

2019; Suci et al 2020; Latuconsina et al 2020a). The range of the pH and temperatures measured in this study were also suitable for the life of *S. guttatus* (Sarisma et al 2017; Kamaruddin et al 2019; Suci et al 2020; Latuconsina et al 2020a; Salampessy & Irawati 2021). Measured ammonia content was between 0.0005-0.0128 mg L^{-1}, still appropriate for *S. guttatus*. Generally, aquatic organism growth will be obstructed when they live in media with ammonia concentration > 0.2 mg L^{-1} (Zahra et al 2019), the acceptable limit being only 0.025 mg L^{-1} (Wahyuningsih & Gitarama 2020). Toxic ammonia concentrations can induce stress and lead to mortality (Firmansyah et al 2021).

Conclusions. It is concluded that rotifer enrichment using β-carotene must be given under appropriate doses. The best dose to obtain better growth and increase number of preying is 0.075 g L^{-10}.

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Conflict of interest. The authors declare that there is no conflict of interest.

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