

Biological Evaluation of *Brachionus rotundiformis* and *Nitzschia* sp. as Initial Feed for *Siganus guttatus* Larvae

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Abstract - The availability of quality exogenous feed suitable for mouth opening is very decisive survival of the larvae of *Siganus guttatus*. This study evaluates the application of two live feeds for survival, growth, and the choice of feeding index for *S. guttatus* larvae. Three treatments tested different types of natural feed, namely *Brachionus rotundiformis* with a density of 7-10 ind mL⁻¹, *Nitzschia* sp. with a density of 5×10^5 ind mL⁻¹, and a combination of these two live feeds. Live feed was given to *S. guttatus* larvae that were one day after hatching (D-1) with a total initial length (TL) of 2.429 mm and a density of 15 individuals L⁻¹. Larvae were reared for 15 days indoor system using a tank with a salinity of 24-25 ppt as much as 2300 L. The results showed that *B. rotundiformis* is a type of feed that produces a survival rate of $0.513 \pm 0.05\%$, the relative length growth was $32.107 \pm 0.83\%$ which was significantly different ($p < 0.05$) and had an IP value of 78.69%. The *B. rotundiformis* type feed is the preferred feed for *S. guttatus* larvae, supporting growth and survival.

Keywords: Index of Preponderance, *Nitzschia* sp., *Siganus guttatus*, survival rate.

I. INTRODUCTION

Currently, *Siganus guttatus* demand is still being fulfilled from fishing in the wild. The increasing fishing activity can threaten the sustainability of *S. guttatus*, so that to meet market demand, cultivation activities need to be carried out (El-Dakar et al., 2007; Saoud et al., 2007; Saoud and Ghanawi 2010; Gonzales et al., 2018). However, the obstacle faced for cultivation activities, in general, is the availability of seeds (Duray and Juario, 1988; Tabugo et al., 2012). Therefore, hatchery activity is a strategic step to meet market needs and diversification of *S. guttatus* cultivation in general.

There has been no hatchery that can meet *S. guttatus* seeds' needs because of the low survival of the larvae, especially during the transition from endogenous to exogenous (Jaroszewska and Dąbrowski, 2011). Low larval survival can be affected by unsuitable exogenous feed, including type and size (Fourrooghifard et al., 2017).

According to Le et al. (2019), it is unknown what type of feed is suitable for the early stages (first feeding) of *S. guttatus*, the larval. One of the efforts made to find suitable food is by observing larvae that are reared naturally. Laining et al. (2019) reported several types of live feeds found in the stomach of *S. guttatus* larvae, including zooplankton such as rotifers, copepod nauplii, and phytoplankton from diatoms such as *Nitzschia* sp. Rotifera (*Brachionus rotundiformis*) feed has been widely applied to fish larvae, including *S. guttatus*, while *Nitzschia* sp. species has never been applied to *S. guttatus* larvae. According to Widianingsih et al. (2012), *Nitzschia* sp. is widely used as live feeds for larvae of marine organisms such as crustaceans and bivalves. *Nitzschia* sp. has a complete nutritional content, namely 33% protein, 21% fat, 28% crude fiber, and 31% unsaturated fatty acids (Setyabudi et al., 2013). In general, *Nitzschia* sp. is used in abalone maintenance (Priyambodo et al., 2005). This study aimed to evaluate the application of two live feeds in the form of *B. rotundiformis*, *Nitzschia* sp., and their combination on survival, growth, and index of the preponderance of *S. guttatus* larvae.

II. MATERIALS AND METHODS

A. Time and Place of Research

The research was conducted in December 2020, at the Siganid Hatchery Section of Tiger Shrimp Hatchery Station (IPUW) of Research Institute for Coastal Aquaculture and Fisheries Extension (RICAFE) Lawallu Village, Soppeng Riaja District, Barru Regency, South Sulawesi Province, Indonesia.

B. Method of collecting data

The larvae used in this experiment was one day after hatching of *S.guttatus* larvae (D-1) with a total initial length (TL) of 2.429 mm obtained from 20 different broodstock. Larvae were kept for 15 days at a density of 15 individuals / L in a closed room (indoor) using a concrete tank with a capacity of 2300 L of media water with a salinity of 24-25 ppt, equipped with an aeration system.

The design of this study was a Completely Randomized Design (CRD) consisting of three treatments which were 1) application of zooplankton *B. rotundiformis* with a density of 7-10 ind mL⁻¹, 2) diatom *Nitzschia* sp. with a density of 5 × 10⁵ ind mL⁻¹, and 3) combination of these two live feeds. Feeding was carried out on the second day (D-2) of rearing time by maintaining life feed density during maintenance.

C. Observation parameters

1. Survival Rate (SR)

Survival Rate calculated at the end of the study by following the following formula:

$$\text{Survival Rate (\%)} = \left[\frac{(\text{Number of initial larvae} - \text{Number of final larvae})}{\text{Number of initial larvae}} \right] \times 100$$

2. Relative length growth rate (RLG)

The relative length growth rate was calculated by taking ten samples for each treatment. Measurements were made on the 1st, 4th, 9th, and 15th day of larvae post-hatched by following the following formula:

$$\text{Relative length growth rate (\%)} = \frac{\text{Final length}_{(\text{day}-i)} - \text{Initial length}}{\text{initial length}} \times 100$$

3. Index of preponderance (IP)

The index of preponderance calculation was done by taking a sample of 10 larvae 1 hour after feeding. Observations were made on the 2nd, 3rd, 4th, and 5th day. Observation of stomach contents was carried out using two methods: the percentage of incidence method (Hynes, 1950) and the points method (Pillay, 1953). The index of preponderance (IP) was estimated by following the formula from Natarajan and Jhingran (1961) as follows:

$$\text{IP (\%)} = \frac{V_i \times O_i}{\sum V_i \times O_i} \times 100$$

IP - Index of Preponderance, V_i - Percentage of number of feeds, O_i - Percentage of frequency of occurrence

Data on survival rates and relative growth rates were analyzed using analysis of variance (ANOVA). The differences between treatments were determined by performing the W-Tuckey follow-up test. Index of preponderance data was analyzed descriptively.

III. RESULTS

1. Survival rate (SR)

The survival rate of *S. guttatus* larvae for 15 days of rearing with the treatment of various types of life feeds is presented in Table 1. The results of the analysis of variance showed various types. The live feeds has a significant effect ($p < 0.05$) on the SR. W-Tuckey further test showed that the highest SR value was obtained in *B. rotundiformis* live feeds, followed by combination treatment. In contrast, *Nitzschia* sp. live feeds with the lowest SR and only lasted until the 5th day of the rearing period.

Table 1. The survival rate for *S. guttatus* larvae

Treatment	Survival Rate (%)
<i>B. rotundiformis</i>	0.513 ± 0.05^c
Combination	0.206 ± 0.03^b
<i>Nitzschia</i> sp.	0

Note: Different letters in the same column indicate significant differences between treatments at the 95% confidence level ($p < 0.05$)

2. Relative length growth rate (RLG)

The relative length growth rates of *S. guttatus* larvae for 15 days of rearing with the treatment of various types of live feeds are presented in Table 2. The results of the analysis of variance showed that various types of live feeds had a significant effect ($p < 0.05$) on the RLG. The W-Tuckey results on the 4th day of the combination treatment had the highest RLG value, followed by *B. rotundiformis* and *Nitzschia* sp. treatment. In contrast, on the 9 and 15th day, the highest RLG value was obtained in the *B. rotundiformis* treatment and followed by the combination treatment. Meanwhile, larvae in *Nitzschia* sp. treatment as in SR only lasted until the 5th day of the rearing period.

Table 2. The daily relative length growth rate of *S. guttatus* larvae

Treatment	Relative growth rate (%)		
	Day 4	Day 9	Day 15
<i>B. rotundiformis</i>	6.535 ± 0.24^a	25.594 ± 0.36^c	32.107 ± 0.83^c
Combination	9.503 ± 0.26^b	20.382 ± 0.03^b	26.923 ± 0.75^b
<i>Nitzschia</i> sp.	5.016 ± 1.20^a	0.00	0.00

Note: Different letters in the same column indicate significant differences between treatments at the 95% confidence level ($P < 0.05$)

3. Index of preponderance (IP)

The preponderance (IP) index of *S. guttatus* larvae for 15 days of rearing with the treatment of various types of life feeds is presented in Table 3. The results of observing the gut content of larvae show that *B. rotundiformis* is the type preferred by *S. guttatus* larvae compared to other types of feed. *Nitzschia* sp. This can be seen from the high IP value in the *B. rotundiformis* type feed.

Table 3. Index of preponderance (IP) types of feed in the stomach of *S. guttatus* larvae based on age

Age	Food Item	Number of the item of feed (%)	Occurrence (%)	IP (%)	Grade
D-2	<i>B. rotundiformis</i>	0.002	100.00	100.00	I
	<i>Nitzschia</i> sp.	99.998	0.00	0.00	II
D-3	<i>B. rotundiformis</i>	0.017	100.00	100.00	I
	<i>Nitzschia</i> sp.	99.983	0.00	0.00	II
D-4	<i>B. rotundiformis</i>	0.087	100.00	57.14	I
	<i>Nitzschia</i> sp.	99.913	75.00	42.86	II
D-5	<i>B. rotundiformis</i>	0.169	100.00	75.00	I
	<i>Nitzschia</i> sp.	99.831	33.33	25.00	II

Average	<i>B. rotundiformis</i>	0.069	100.00	78.69	I
	<i>Nitzschia</i> sp.	99.931	27.08	21.31	II

IV. DISCUSSION

Brachionus rotundiformis is a live feed that provides the highest SR value. The ability of the *S. guttatus* larvae to exploit *B. rotundiformis* cannot be separated from the characteristics they have. *B. rotundiformis* has slow movement, so that fish larvae can easily take advantage of the *B. rotundiformis*. According to Rumengan (1997), *B. rotundiformis* is also considered a suitable biocapsule for fish larvae because it can transfer nutrients from the environment to larvae without the effect of relative pollutants. According to Makridis and Oslen (1999), the rotifers protein content is 63% and 28% fat in general. These nutrients are higher than *Nitzschia* sp. which only contains 33% protein and 21% fat (Setyabudi et al., 2013). Treatment *Nitzschia* sp. could not support the survival rate of *S. guttatus* larvae. The immature digestive organs of larvae result in *Nitzschia* sp. classified as phytoplankton, unable to be digested, proven by the condition of *Nitzschia* sp., which is still intact in the stomach of the larva.

Another possible reason for applying *Nitzschia* sp. is that the application of *Nitzschia* sp. in a rearing tank produced mucus in the water and on the tank walls, which allowed the larvae to be trapped and leads to death. This condition also results in the SR value in the combination treatment is lower than the *B. rotundiformis* treatment. Larvae which were unable to consume feed will lack energy for body metabolism, which further can cause death. According to Stroband and Dabrowski (1981), fish larvae have very simple, relatively short, and undifferentiated digestive tools. This development occurs morphologically and anatomically, and physiologically, namely developing digestive enzymes and their activities. So the simple morphological structure of the digestive tract correlates with the low production of digestive enzymes (Lauf and Hofer, 1984) to reduce digestibility. It ultimately affects the quality of the seeds produced, and this is a significant problem in feeding larvae in the early stage (Kawai and Ikeda, 1973). The high mortality rate was probably also caused by stress resulting from transferring larvae to the rearing tank. The SR value obtained in this study is still relatively low, while reports on the SR of *S. guttatus* larvae are still scarcely available. Research on *S. guttatus* larvae was carried out by Duray and Kohno (1988), who obtained a survival rate of 17.10% for 7 days. Ayson (1989) received a survival rate of 17.9% for 5 days. Lante and Palinggi (2009) obtain a survival rate of 0.4% for 50 days. Meanwhile, Laining et al. (2019), in the development of *S. guttatus* cultivation, received a survival rate of 0.04-0.26%.

Siganus guttatus growth is determined by the availability and nutritional content of the feed consumed. According to Duray et al. (1996), larval growth will occur if the larvae can consume the feed given. The feed consumed will be used as a source of energy for body metabolism and growth. The rearing of larvae for 15 days showed that *B. rotundiformis* treatment had a higher RLG value than other treatments. This indicates that the larvae of *S. guttatus* can take advantage of *B. rotundiformis* as energy sources. Optimal larval growth can occur if there is increased contact and greater prey catch efficiency. The lower RLG value of combination treatment than *B. rotundiformis* is thought to be due to the accumulation of feed due to combining two types of feed. Dewi et al. (2019) suggest that feed must be given in an appropriate amount because the fish will not grow optimally if the feed is given too little or too much. To determine the effect of live feeds dose on the growth of *S. guttatus* larvae, it is necessary to carry out further research without neglecting the increase in feed size every day.

The *B. rotundiformis* type feed was the preferred feed for *S. guttatus* larvae based on the IP value. According to Muchlisin (2011), IP values > 40% include main feed, IP = 4-40% as additional feed, and IP <4% as complementary feed. The mouth of the larvae was open on the second day, with the water temperature of the rearing tank ranging from 27.4-18.6 0C. The mouth only functions when the jaw is movable. The results of observations on day 2 showed that there was feed in the stomach of the larvae. According to Luchavez and Carumbana (1982), the larvae are learning to eat in this condition. The frequency of feeding tends to

increase with the age of the larvae. Larvae begin to actively prey on feed at the age of D-4 by discovering 1-10 *B. rotundiformis* in one larva's stomach. The same thing was also found by Duray (1986). Although the stomach of the larvae has been found in the type of feed on D-2, However, based on the SR value of feed applied, especially *B. rotundiformis*, it has not reduced the high mortality rate. It is suspected that the size of life feeds plays an important role in supporting the growth and survival of larvae. The size of the *B. rotundiformis* used in this study ranged from 106.3 to 215.1 μm . Type *Nitzschia* sp. found in the stomach of the larva on D-4. The fact there is *Nitzschia* sp. in the stomach of the larva is inconclusive that this type can be used as feed by the larvae, but the presence of *Nitzschia* sp. in the stomach of the larva is an accident. This is because the small size of *Nitzschia* sp. allows the larvae to eat accidentally, besides the high density of *Nitzschia* sp. in the rearing container but the disproportionate frequency of occurrence is evidence the larvae eat *Nitzschia* sp. accidentally. According to Pavlov and Kasumyan (2002), each type of fish has specifications for the feed to be consumed. From these observations, it is possible that *S. guttatus* can choose its feed at the larval stage.

V. CONCLUSION

The *B. rotundiformis* type feed is the preferred feed for *S. guttatus* larvae, supporting growth and survival.

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REFERENCES

- [1] Ayson, F. G., 1989. The Effect of Stress on Spawning of Brood Fish and Survival of Larvae of the Rabbitfish, *Siganus guttatus* (Bloch). *Aquaculture*, 80(3-4): 241–246.
- [2] Dewi, A.T., Suminto & R.A. Nugroho, 2019. Pengaruh Pemberian Pakan Alami *Moina* sp. dengan Dosis yang Berbeda dalam *Feeding Regime* Terhadap Pertumbuhan dan Kelulushidupan Larva Ikan Baung (*Hemibagrus nemurus*). *Jurnal Sains Akuakultur Tropis*, 3(1):17-26.
- [3] Duray, M. N., C.B. Estudillo, & L.G. Alpasan, 1996. Larval Rearing of the Grouper *Epinephelus suillus* Under Laboratory Conditions. Elsevier. *Aquaculture*, 150: 63-76.
- [4] Duray, M. N., 1986. Biological evaluation of three phytoplankton species (*Chlorella* sp., *Tetraselmis* sp., *Isochrysis galbana*) and two zooplankton species (*Crassostrea iredalei*, *Brachionus plicatilis*) as food for the first-feeding *Siganus guttatus* larvae. *The Philippine Scientist*, 23, 41-49.
- [5] Duray, M.N & H. Kohno, 1988. Effects of Continuous Lighting on Growth and Survival of First-Feeding Larval Rabbitfish, *Siganus guttatus*. *Aquaculture*, 72(1-2), 73–79.
- [6] Duray, M.N & J.V. Juario, 1988. Broodstock Management and Seed Production of the Rabbitfish *Siganus guttatus* (Bloch) and the Sea Bass *Lates Calcarifer* (Bloch). Proceedings of the Seminar on Aquaculture Development in Sout East Asia, Iloilo City, Philippines, pp. 195-210.
- [7] El-Dakar, A.Y., S.M Shalaby & I.P. Saoud, 2007. Assessing the Use of a Dietary Probiotic/Prebiotic as an Enhancer of Spinefoot Rabbitfish *Siganus rivulatus* Survival and Growth. *Aquaculture Nutrition*, 13(6), 407–412.
- [8] Fouroughifard, H., A. Matinfar, G.K . Roohani, M. Moezzi, E. Abdolalian, M.R. Zahedi, & J.S. Tamadoni, , 2017. Effect of Salinity, Light Intensity, and Tank Size on Larval Survival Rate of Shoemaker Rabbitfish *Siganus sutor* (Valenciennes, 1835). *Iranian Journal of Fisheries Sciences*, pp. 1-12.
- [9] Gonzalez, R.D., S.S. Parreno, R.S. Abalos, L.A. Santos, C.C. Salayog, P.J.B. Ramirez & S.I. Celino, 2018. Comparative Analysis of Siganid (*Siganus guttatus*) Value Chains from Aquaculture in Regions 1 And 2, Philippines. *International Journal of Scientific and Technology Research* 7(7): 145-140.
- [10] Hynes, H.B.N., 1950. On the food of the freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review of the methods used in the study of food fishes. *J. Anim. Ecol.*, 19(1) 36–58.
- [11] Jaroszweska, M., & K. Dabrowski, , 2011. *Utilization of Yolk: Transition from Endogenous to Exogenous Nutrition in Fish*. In: Holt GJ (Ed.). *Larval Fish Nutrition*. Wiley-Blackwell, Oxford.

- [12] Kawai, S. & S. Ikeda, 1973. Studies on Digestive Enzymes of Fishes - III. Development of Digestive Enzymes of Rainbow Trout After Hatching and the Effect of Dietary Change on the Activities of Digestive Enzymes in the Juvenile Stage. *Bull. Jpn. Soc. Sci. Fish.*, 39(7): 819-823.
- [13] Laining, A., I. Trismawanti, M.C. Undu, S.H.M. Redjeki, B.R. Tampangallo, Usman, Rachmansyah, Ramadhan & Rosni., 2019. Laporan Teknis Akhir Kegiatan Tahun 2019, Pengembangan Budidaya Ikan Baronang, Accelerating the Development of Finfish Mariculture in Cambodia Through South-South Research Cooperation with Indonesia (ACIAR FISH/2016/130). Kementerian Kelautan dan Perikanan. BRPBAP3.
- [14] Lante, S & N.N. Palinggi, 2009. Pemeliharaan Larva Ikan Beronang (*Siganus Guttatus*) Dengan Nuansa Warna Wadah yang Berbeda. *Prosiding Forum Inovasi teknologi Akuakultur*
- [15] Lauff, M. & R. Hofer. 1984. Proteolytic Enzymes in Fish Development and the Importance of Dietary Enzymes. *Aquaculture*, 37: 335-346.
- [16] Le, D., P. Nguyen, D. Nguyen, K. Dierckens, N. Boon, T. Lacoere, F.M. Kerckhof, J.D. Vrieze, O. Vadstein, & P. Bossier, 2019. Gut Microbiota of Migrating Wild Rabbit Fish (*Siganus guttatus*) Larvae Have Low Spatial and Temporal Variability. *Microbial Ecology*. doi:10.1007/s00248-019-01436-1
- [17] Luchavez, J.A. & E.E. Carumbana, 1982. Observation on the spawning, larval development, and larval rearing of *Siganus argenteus* (Quoy and Gaimard) under laboratory conditions. *Silliman J.* 29 (1 & 2): 24-34.
- [18] Makridis, P., & Y. Olsen, 1999. Protein depletion of the rotifer *Brachionus plicatilis* during starvation. *Aquaculture* 174(3-4):343-353
- [19] Muchlisin, Z.A., 2011. *Buku Ajar Ikhtiologi*. Koordinator Kelautan dan Perikanan Universitas Syiah Kuala, Banda Aceh.
- [20] Natarajan, A.V. & A.G. Jhingran, , 1961. Index of preponderance a method of grading the food elements in the stomach analysis of fishes, *Indian J. Fish.*, 8(1)54-59.
- [21] Pavlov, D.S & A.O.Kaumyan, 2002. Feeding Diversity in Fishes: Trophic Classification of Fish. *Journal of Ichthyology*, 42(2): S137-S159.
- [22] Pillay, T.V.R., 1953. Studies on the food, feeding habits, and alimentary tract of the grey mullet, *Mugil tade* (Forsskal), *Proc. Natl. Inst. Sci. India*, 19(6) 777- 827.
- [23] Priyambodo, B., Y. Sofyan, I.B.M. Swastika. 2005. Produksi benih kerang abalon *Haliotis asinina* di lokasi budidaya laut lombok. *Prosiding Seminar Nasional Hasil Penelitian Perikanan dan Kelautan. Jurusan BDP. UGM.* 144-148 hal.
- [24] Rumengan, I.F.M., V. Warouw & A. Hagiwara, 1998. Morphometry and resting egg production potential of the tropical ultra-minute rotifer *Brachionus rutundiformis* (Manado strain) fed different algae. *Bull. Fac.Fish. Nagasaki Univ.*, 5(79): 31-36.
- [25] Saoud, I.P & J. Ghanawi, 2010. Effect of Size Sorting on Growth Performance of Juvenile Spinefoot Rabbitfish, *Siganus rivulatus*. *Journal of The World Aquaculture Society*, 41(4): 565-573.
- [26] Saoud, I.P., J. Ghanawi & N. Lebbos, 2007. Effects of Stocking Density on the Survival, Growth, Size Variation and Condition Index of Juvenile Rabbitfish *Siganus rivulatus*. *Aquaculture International*, 16(2): 109-116.
- [27] Setyabudi H., G. Garnawansyah, A. Supriyanto, M. Imanuddin, Adeyana. 2013. Petunjuk Teknis Produksi Benih Abalon Hibrid (Ninamata). Balai Budidaya Laut Lombok, Direktorat Jenderal Perikanan Budidaya, Kementerian Kelautan dan Perikanan. Lombok. 9 hal.
- [28] Stroband, H. W. J. & K. R. Dabrowski. 1981. Morphological and Physiological Aspects of the Digestive System and Feeding in Fresh-Water Fish Larvae. In, M. Fontain (Ed.) *La Nutrition des Poissons. CNERNA, Paris.* pp. 355-376.
- [29] Tabugo, S.R.M., J.P., Sendaydiego, E.A, Requieron & M.D. Dimalen, 2012. Embryonic Developmental Stages in Cultured Rabbitfish (*Siganus guttatus*, Bloch 1787). *Int. Res. J. Biological Sci.*, 1(8): 65-70.
- [30] Widianingsing, W., R. Hartati, H. Enrawati & M. Hilal, 2012. Kajian kasar lipid dan kepadatan *Nitzschia* sp. yang dikultur dengan salinitas yang berbeda. *METANA* 7(1): 29-37.