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Growth Performance of Clown Anemonefish (*Amphiprion ocellaris*) in Maluku at Optimum Salinity

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ABSTRACT

Clown anemonefish (*Amphiprion ocellaris*) is one of the most popular ornamental coral reefs. The marine ornamental fish trade has increased fishing pressure. It has a considerable impact on the clown anemonefish's declining population. The ability of each fish species to tolerate the salinity is a vital consideration in aquaculture marine organisms as it gives information on primary environmental necessities. This study aimed to analyze the clown anemonefish growth performance by modifying different salinity levels through survival rate, specific growth rate, and feed conversion ratio parameters. The experiment was conducted with a Completely Randomized Design (CRD) to investigate the effect of salinity on clown anemonefish growth performance with six treatments (24, 26, 28, 30, 32, and 35 ppt) and three replications. Water quality parameters in this study observed include pH, temperature, and dissolved oxygen. The clown anemonefish reared with six different salinity treatments for 30 days showed that there was a significantly different effect on SGR (p value = 0.007) but no significant differences in FCR (p value = 0.189) and survival rates (p value = 0.458). The considerable effect of salinity on specific growth rates explains that the amount of energy used in the osmoregulation process disrupts a portion of growth energy. The optimal growth performance of clown anemonefish was in a 24 ppt salinity treatment, accompanied by other water quality parameters such as temperature, dissolved oxygen, and pH, which ranged from 26.5 – 32 ^{o}C; 4.9 - 5.6 ppm; and 7.9 - 8.2, respectively.

Keywords: *Amphiprion ocellaris*, clown anemonefish, growth performance, salinity

ABSTRAK

Ikan badut (*Amphiprion ocellaris*) merupakan salah satu jenis ikan hias laut yang cukup popoler. Perdagangan ikan hias telah meningkatkan tekanan penangkapan. Hal ini memiliki dampak besar terhadap penurunan populasi ikan badut. Kapasitas spesies dalam toleransi salinitas merupakan pertimbangan penting dalam upaya budi daya biota laut karena dapat memberikan informasi terkait parameter lingkungan yang paling sesuai dalam hidupnya. Penelitian ini bertujuan untuk menganalisis performa pertumbuhan ikan badut dengan tingkat salinitas yang berbeda melalui parameter tingkat pertumbuhan spesifik harian, sintasan, dan rasio konversi pakan. Penelitian dilakukan dengan menggunakan Rancangan Acak Lengkap (RAL) untuk mengetahui adanya pengaruh salinitas terhadap performa pertumbuhan ikan dengan menggunakan enam perlakuan salinitas yang berbeda (24, 26, 28, 30, 32, dan 35 ppt) dan tiga ulangan pada masing-masing perlakuan. Parameter kualitas air yang juga diamati dalam penelitian ini meliputi pH, suhu, dan oksigen terlarut. Ikan badut yang dipelihara dengan enam perlakuan salinitas yang berbeda selama 30 hari menunjukkan bahwa terdapat pengaruh yang berbeda nyata terhadap pertumbuhan spesifik harian (p value = 0,007) namun tidak berbeda nyata terhadap sintasan (p value = 0,458), dan rasio konversi pakan (p value = 0,189). Pengaruh salinitas yang signifikan menjelaskan bahwa besarnya energi yang digunakan untuk proses osmoregulasi menyebabkan terganggunya porsi energi untuk pertumbuhan. Performa pertumbuhan ikan badut yang optimal terdapat pada salinitas 24 ppt yang diikuti dengan parameter kualitas air lainnya seperti suhu, oksigen terlarut, dan pH dengan nilai masing-masing berkisar antara 26,5 – 32 ^{o}C; 4,9 – 5,6 ppm; dan 7,9 – 8,2.

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Kata kunci: *Amphiprion ocellaris*, ikan badut, performa pertumbuhan, salinitas.

1. Introduction

Clown anemonefish (*Amphiprion ocellaris*) has recently begun to experience an alarming population decline that raises concerns for the survival of this species. This species is an attractive ornamental fish kept as a pet in a confined space as an object of visual interest. The clown anemonefish is a tropical marine fish that impresses many people because of its color and beauty. The coral reef is a habitat for the clown anemonefish and is a fragile ecosystem. Improper fishing methods will destroy the ecosystem, and species over-harvesting as demand is the critical problem associated with the trade (Uthaya et al., 2014). The clown anemonefish trade has increased the fishing pressure, which has indirectly caused the rapidly declining population of clown anemonefish (Madduppa et al., 2014); (Pirarat et al., 2011).

Cultivation is one method to prevent the declining population of clownfish in Indonesia by considering the variables that affect it (Biondo, 2017). Domestication is the early step in raising clown anemonefish that previously lived wild in their natural habitat (uncontrolled), and they can live and grow in controlled conditions (Diansyah et al., 2016). Improving the production of the species can be achieved by controlling variables that affect their growth performance in a closed culture system that minimizes external influences (Teletchea, 2021). A variable that significantly impacts the success of marine fish farming is salinity.

Salinity is one of the environmental factors that cause significant physiological effects and growth performance of clown anemonefish and other marine fish (Dhaneesh et al., 2012; Fitrinawati & Utami, 2023). Most coral organisms indicate limited salinity tolerance that affects mortality (Chiu et al., 2023; Nybakken, 1988). Tolerance of salinity species capacity is a vital consideration in aquaculture marine organisms as it gives information on primary environmental necessities (Dhaneesh et al., 2012). This study aims to analyze the feasibility of clown anemonefish (*Amphiprion ocellaris*) by modifying different salinity levels through Specific Growth Rate (SGR), Survival Rate (SR), and Feeding Conversion Ratio (FCR) parameters. The expected results of this study are to be a reference in efforts to preserve clown anemonefish through an appropriate cultivation system.

2. Material and Methods

2.1. Fish Collection

The capturing of the clown anemonefish (5±1.5 g) used a cash net on the shore of Sathean, East Maluku. Clown anemonefish were kept in a 60-litre marvelous box filled 30-litre of seawater and then moved to the hatchery laboratory of Tual State Fisheries Polytechnic for domestication. Fish were acclimated in 2-tonne capacity tanks at 20 liters per fish density for two weeks. Before being released in the experimental media, clown anemonefish were first acclimated to different salinity according to the treatment for 30 minutes, then slowly released until the fish themself came out of the transfer tank. The rearing tanks during the study used plastic tanks with 80-liter capacity, totaling 18 cylindrical units. Then, four artificial anemones and four clown anemonefish with lengths ranging from 4 to 7 cm filled each experimental tank. Feeding was applied at 08:00, 12:00, and 17:00 at satiation with 1.5 mm pellets (30% protein). During maintenance, water was changed every day by 50 % of the volume with the same salinity using a refractometer to measure it (Atago Master-53 PM).

2.2. The Experiment and Media Preparation

The experimental design was a completely randomized design (CRD) with six treatments and three replications (Gomez & Gomez, 1983). The six treatments were media with different salinity levels, which consisted of 24, 26, 28, 30, 32, and 35 ppt.

Diluting seawater with fresh water was conducted to prepare the experimental media of low salinity (Prananingtyas et al., 2019; Shaughnessy et al., 2022). An electric heating element was a device to evaporate the seawater artificially to acquire media with a higher salinity than the standard (Tuwo et al., 2021). Fish weight was collected using digital scales at an accuracy level of 0.01 g. Before being weighed, fish were calmed by the chemo-anesthesia method using MS-222 at 25 ppm (Ross & Ross, 2008; Shaughnessy et al., 2022). Weight and length measurements of previously calmed fish were observed weekly for one month.

2.3. Water Quality Observation

The water quality components observed in this study were salinity, temperature, dissolved oxygen (DO), and pH. Water samples were taken daily with the insitu measurement method (Azaza & Dhraief, 2020). Water quality of pH and salinity were measured using a pH meter and refractometer (Atago Master-53 PM), respectively, whereas temperature and dissolved oxygen (DO) were measured using a DO meter (Lutron DO-5510). Water quality components were analyzed using MS Excel 2010 and then descriptively explained.

2.4. Growth Performance

The influence of different salinity was observed based on the Survival Rate (SR), Specific Growth Rate (SGR), and Feed Conversion Ratio (FCR). SGR was determined based on the exponential weight gain of the fish using the formula as follows (Hopkins, 1992; Shaughnessy et al., 2022):

$$SGR = \frac{Ln\,Wt - Ln\,Wo}{t} x\ 100\%$$

Where, SGR = Specific Growth Rate (%)/day; W_t = Final weight (g); W_o= Initial weight (g) and t= days

SR can describe the level of the fish's ability to survive by comparing the final and initial counts during the observation (Effendie, 2002). SR was computed with the formula as follows (Venkatachalam et al., 2018):

$$SR = \frac{N_t}{N_0} x\ 100\%$$

Where, SR= Survival Rate (%); N_t = Number of fish harvested and N_o= Number of fish stocked

FCR expresses the ratio between feed intake and fish body weight gain at a particular time. The lower FCR value illustrates the higher feed efficiency (Elvy et al., 2023; Vandeputte et al., 2022; Young et al., 2023). FCR was calculated with the formula as follows (Castel & Tiews, 1990; Elvy et al., 2023; Schwebel et al., 2018) :

$$FCR = \frac{total\ feed\ (g)}{weight\ gain\ (g)}$$

2.5. Data Analysis

The analysis of variance was performed to investigate the effect of salinity on fish performance with a significance p value<0.05 using SPSS 25 software. A Duncan's Multiple Range Test (DMRT) was used to compare the various parameters among examined treatments (Duncan, 1955).

3. Result and Discussion

3.1. Fish Growth Performance

Fisheries management approaches for optimizing harvest efficiency include balancing individual growth, population biomass, and survival rates (Isely & Grabowski, 2006). Growth is the biomass increase in a population or individual. Clown anemonefish growth expression could be evaluated based on a specific growth rate. A previous study revealed that compared to other water quality variables such as temperature, acidity, and dissolved oxygen, salinity was the variable that significantly influenced the growth of marine fishes (Fitrinawati & Utami, 2023).

Clown anemonefish reared with six different salinity treatments for 30 days showed a significantly different effect (p value = 0,007) on specific growth rates (Figure 1). Salinity treatment that led to a significantly different impact on the clown anemonefish SGR was at 24 and 26 ppt salinity (Duncan's test).

Media with 24 and 26 ppt salinity are still in the isotonic range and are good enough for clown anemonefish growth. The swimming activity and color of the clown anemonefish also looked quite good in this salinity range but began to decline in media with salinity below 24 ppt of salinity (Noh et al., 2013; Soewandhi et al., 2015). It explains that salinity below 24 ppt is a hypertonic media that describes that fish fluids are denser than their environment such that body fluids move out osmotically through the digestive system, gills, and skin. The isotonic water transfers the osmotic pressure energy in the fish body so it will grow more optimally. Fish will expend lower osmoregulatory energy in isosmotic media because it will minimize the gradient between blood and water so that fish will store more energy as an essential resource for growth (Boeuf & Payan, 2001).

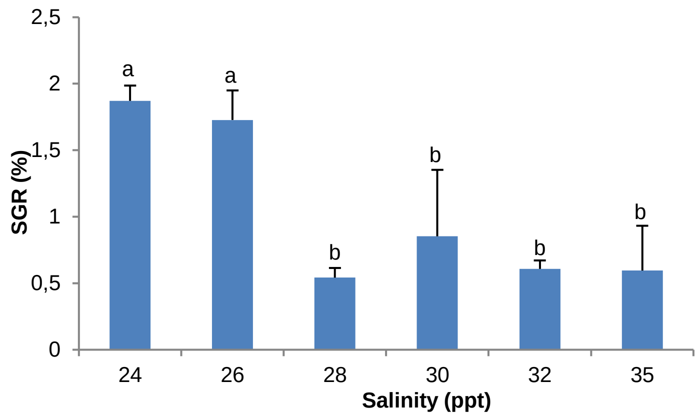

Figure 1. Specific Growth Rate of Amphiprion ocellaris in different salinity. Different small letters above the bars represented significant differences (P < 0.05)

Hormones are a crucial component in the process of osmoregulation in fish. These hormones regulate the ions and water intake into the fish's body. Clown anemonefish acclimatized to several different salinities showed that fish reared 90 days at 25 ppt had higher length and weight growth than at 35 ppt salinity. Clown anemonefish reached the highest level of prolactin production at a salinity of 24 ppt. (Noh et al., 2013). Marine fish reared on media with salinities lower than 28 ppt and above also have higher specific growth levels (Lambert et al., 2011; Mabrouk, 2011). The results of the study conducted by Partridge & Jenkins (2002) explained that fish reared at 24 ppt of salinity have maximum weight and specific growth rates. The significant SGR in clown anemonefish in this study might be because the fish will produce more crude protein in water with a salinity of 26 ppt compared to a salinity above 30 ppt. (Nassar et al., 2021).

3.2. Survival Rate (SR)

The saltiness of water is one of the influential factors contributing to the survival rate and fish metabolism (Amornsakun et al., 2019). Fish would have a favorable survival rate (above 90%) if they were maintained in health and water quality during preservation (Susanti & Mukti, 2020). Clown anemonefish reared for 30 days with different salinity treatments did not show significant differences in survival rates (p value = 0,458). In the study, six different salinity treatments revealed that the clown anemonefish exhibited a high survival rate (SR = 100%) except at 30 ppt salinity, where the survival rate was slightly lower (SR = 98.5%). This condition may explain why the salinity difference in clown

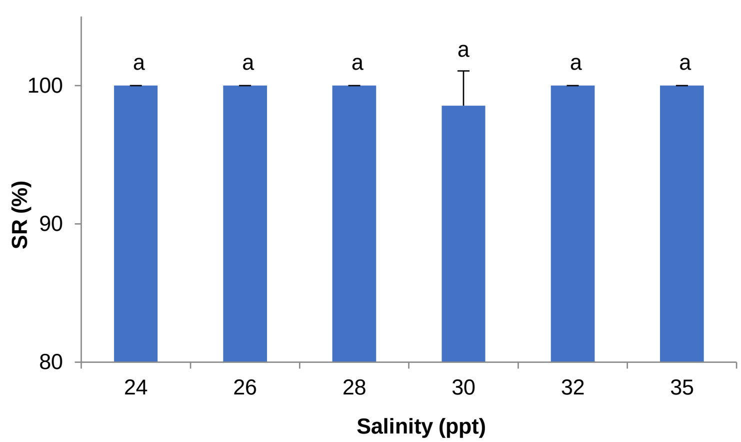

Figure 2. Survival Rate of Amphiprion ocellaris in different salinity. Same small letters above the bars represented insignificant differences (P > 0.05)

anemonefish reared for 30 days was not enough to interfere with the survival rate (Figure 2).

Marine fish would have a slightly favorable survival rate at 24 over 32 ppt salinity (Mabrouk, 2011). SR levels performed best at a salinity of 26 ppt as opposed to salinities below 20 and above 30 ppt (Nassar et al., 2021). Condition factor (K) in marine fish is slightly favorable in the salinity range of 20 over 35 ppt (Azab et al., 2015). It interprets fish's biological status, such as the environmental suitability (salinity) that promotes their metabolism (Le Cren, 2012; Ridanovic et al., 2015). A high condition factor (K) indicates a better suitable environment for fish and increases the survival rates (Le Cren, 2012).

3.3. Feed Conversion Rate (FCR)

Feed consumption is an essential variable for fish growth. It is crucial to determine the optimal food consumption for the success of reared fish (Barani et al., 2019). Feed consumption divided by fish body weight indicates the efficiency of fish in converting feed into body mass. The effect of different salinities on FCR in clown anemonefish reared for 30 days was quite similar to its influence on SGR with inversely proportional values (Figure 3).

Different salinity treatments on clown anemonefish reared for 30 days gave an influence that was not significantly different from the feed conversion rate (p value = 0,189). This study explains that 24 ppt salinity had the lowest average FCR of the six differences of salinities. This condition was consistent with the SGR and SR having a value of 24 ppt salinity. The low FCR value of clown anemonefish at 24 and 26 ppt salinity describes that the media with this salinity was isotonic since energy for osmoregulation was low and transferred to digestion and growth. This fact confirms that at 24 and 26 ppt salinity, the clown anemonefish reared for 30 days also had the highest growth. A low FCR explains that the feed will be more effective for fish growth (Setyowati et al., 2022). As well as the fish's internal condition, the factor that affects food ingredient digestibility is the suitability of its environment, including its live media salinity level (Affandi & Tang, 2017).

The feed conversion ratio is determined by dividing the total dry weight of feed consumed by the wet weight of the fish (Hopkins, 1992). It explains that an FCR of 0.66 at 24 ppt salinity would mean that every 0.66 kg dry feed will generate 1 kg of wet flesh fish. FCR with a value of less than 1 is possible because the fish is composed of water in its flesh, while the feed does not contain much water. When the fish converts dry food into moist flesh using a highly efficient diet, it can produce more moist flesh than the weight of dry feed used. FCR of less than 1 is impossible if we dry the fish at the same rate as the feed drying rate before weighing it. (HARVEST. Helping Address Rural Vulnerabilities and Ecosystem Stability, 2011). Some fish (trout, salmon, and tilapia) also exhibited FCR values of less than 1 (Craig, 2017). Three strains of tilapia NS, GIFT, and FaST reared 42 days in rectangular fiberglass tanks had FCR values of 0.89, 0.8, and 0.83, respectively. This study concluded that the best tilapia strains were GIFT and FaST due to their

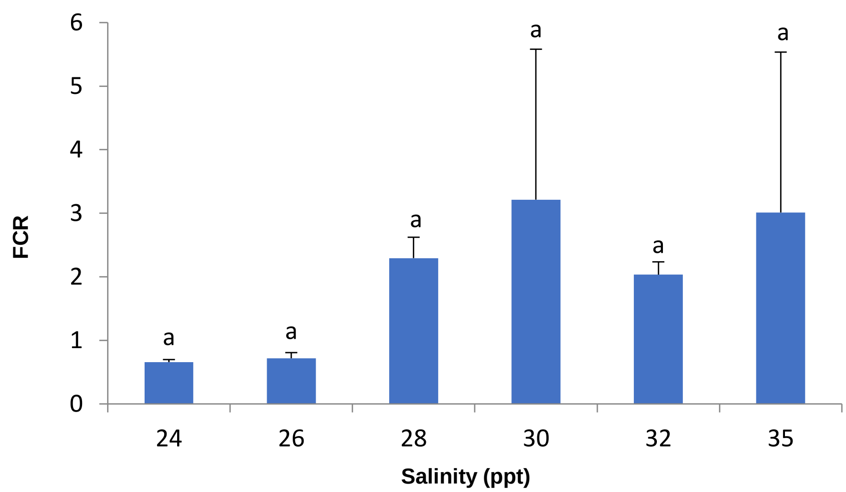

Figure 3. Feed Conversion Ratio of Amphiprion ocellaris in different salinity. Same small letters above the bars represented insignificant differences (P > 0.05)

low FCR values (Ridha, 2006). Gourami reared in containers for 60 days also exhibited FCR values less than 1, ranging 0.4 - 0.9 (Setyowati et al., 2022).

3.4. Water Quality

Environmental factors greatly influence fish's biological functions, including feeding conversion ratio and growth (Bethke et al., 2013). Salinity, pH, temperature, and dissolved oxygen will influence the clown anemonefish growth, survival rate, and feeding conversion rate (Bethke et al., 2013; Rao et al., 2014; Velasco-Blanco et al., 2019). Generally, the water quality of clown anemonefish media reared for 30 days at different salinities had relatively similar ranges (Tabel 1). The range of water quality for pH, dissolved oxygen, and temperature in this study is generally still within the range of Indonesian national standards for ornamental fish farming. The most considered crucial water quality parameters in mariculture management explain that favorable salinity is generally in the 10 – 30 ppt range (Loka, 2015).

Table 1. Range of water quality

Salinity (ppt)	pH	DO (ppm)	T (ºC)
24	7.9 – 8.2	4.9 – 5.6	26.5 - 32
26	6.6 – 8.3	5 – 5.6	26.6 - 30
28	8 – 8.3	4.7 – 5.5	26.5 - 31
30	6.9 – 8.2	5 – 5.3	27.6 - 32
32	7 – 8.1	5 – 5.3	27 - 31
35	8 – 8.3	5 – 5.5	28 - 32
30-32 ppt*	7 – 8*	>5*	28 – 32*
10 – 30 ppt**	7 – 8**	>4**	26 – 32 **

* Indonesian National Standard. No.8228.3/2015
** Winter School on Technological Advances in Mariculture (Loka, 2015)

The highest pH range during the observation was at 35 ppt salinity, which reached 8 - 8.3. The increasing salinity may cause a rise in water's pH, which then causes biological changes in the biota that live in it (Saraswat et al., 2015). Clown anemonefish had the best specific growth, survival rate, and feeding conversion ratio during the study found at salinities of 24 ppt, which also had pH with a higher range (7.9 - 8.2). It was in contrast to conditions at 35 ppt salinity, which had shown a high pH range (8 - 8.3) that indicated clown anemonefish had low specific growth and a high feeding conversion ratio. Marine fish would experience a decrease in SGR and an increase in FCR with an elevation in water pH (Shuang-Yao et al., 2018).

Clown anemonefish have optimal physiological performance in the temperature range between 26 – 32 ºC (Velasco-Blanco et al., 2019; Loka, 2015). This temperature range was consistent with the observations in the study, which showed that the most favorable SGR and FCR were in the range of 26.5 – 32 ºC, and it is precisely at 24 ppt salinity treatment (Table 1). *Amphiprion ocellaris* had the highest survival rate (75% ± 0,125) and specific growth rate (2,45 ± 0.034) at 33 °C (Rao et al., 2014). The ideal temperature for clown anemonfish growth and survival rate is 29 ºC (Syazili et al., 2022; Ye et al., 2011). The higher temperature will decrease growth and survival rate but increase the brightness of the clown anemonefish color (Syazili et al., 2022).

The solubility of oxygen in water would be affected by the salt concentration (salinity) as higher salinity would lead to a higher oxygen mass transfer coefficient in water, which would reduce the oxygen diffusion coefficient (Zannotti & Giovannetti, 2015). Clown anemonefish rearing within 30 days in this study showed the highest DO level (5.6 ppm) found in the 24 and 26 ppt salinities accompanied by the best SGR, SR, and FCR (Table 1). The range was within that referred to as normoxia. A previous study found that dissolved oxygen affected the stress level of fish, and hypoxic conditions would decrease its growth and survival rate (Lakani et al., 2013).

The higher water salinity would have led to a linear increase in the oxygen consumption rate of fish (Rao, 1968). It confirmed the explanation that the SGR, SR, and FCR of clown anemonefish reared for 30 days in this study recorded in the low salinity treatment, which was at 24 ppt. Growth performance, antioxidant enzyme enhancement, and decreased liver damage in fish improved at dissolved oxygen levels of 5 ppm (Li et al., 2020). Aside from affecting growth performance, the dissolved oxygen level also greatly influences fish feed intake (Abdel-Tawwab et al., 2015).

4. Conclusions

Salinity differences significantly affect the specific growth rate but are not enough for the feed conversion ratio and survival rate. Clown anemonefish reared for 30 days had optimal specific growth rate, feed conversion ratio, and survival rate at 24 ppt salinity. The growth performance of clown anemone fish will be optimized when accompanied by other water qualities, such as temperature, DO, and pH, which range 26.5 - 32 ^{o}C; 4.9 - 5.6 ppm, and 7.9 - 8.2, respectively.

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