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Interaction of salinity and natural feed on growth and survival rate of the Banggai cardinalfish (*Pterapogon kauderni*) for ex-situ conservation development

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ABSTRACT

The Banggai cardinalfish (*Pterapogon kauderni*) is an endemic and protected fish. However, its exploitative use and damage to its microhabitat ecosystem have led the fish to be endangered. This study aims at determining the effect of salinity and type of natural food on the growth and survival rate of Banggai cardinalfish. The method employed is experiment with a factorial completely randomized design (CRD) encompassing salinity factor (26 ppt, 20 ppt, 14 ppt, and 8 ppt) and food factor (rebon shrimp, Artemia sp. and mosquito larvae). This study uses 36 aquariums as containers with a volume of 10 liters equipped with a Recirculation Aquaculture System (RAS). The animals used in this study are 360 Banggai cardinalfish with an average length of 28.31 mm and an average weight of 0.29 g. The stocking density is 1 fish/L with a rearing period of 30 days. The observed data include growth and survival rate, in which the data are analyzed by applying analysis of variance (ANOVA). The result of the analysis reveals that the difference in salinity level does not significantly affect the length and weight gain ($F_{count} > F_{table(0.05)}$), yet it affects the survival rate significantly ($F_{count} > F_{table(0.05)}$). In addition, the different types of natural food significantly affect the length and weight gain, and survival rate ($F_{count} > F_{table(0.01)}$).

INTRODUCTION

One of the endemic fishes in Banggai Islands-Central Sulawesi Province, Indonesia is Banggai cardinalfish **(Moore *et al*., 2015; Adel *et al*., 2016; Hulu *et al*., 2020)**. In international trade, the Banggai cardinalfish is often abbreviated as BCF. The Banggai cardinalfish is endangered, as reported by several experts (**Lunn & Moreau, 2004; Moore *et al*., 2012; Ndobe *et al*., 2019; Ndobe *et al*., 2020**) as the fish are subjects to exploitative fishing, trade as an ornamental fish that is not controlled and its microhabitat is damaged. Therefore, the government encourages in-situ and ex-situ cultivation as a conservation effort.

Growth and survival rate are biological aspects of a particular organism, including endurance, growth, and development optimally. Therefore, the fish farming study often employs growth and survival rate as variables. For instance, studies conducted by several researchers **(Venkatachalam *et al*., 2018; Ul Hassan *et al*., 2021)** used growth and survival rate, which confirms that growth and survival rate variables can signify the most optimal treatment during farming.

An essential factor in the farming process is creating environmental conditions and food according to fish needs. One of the sensitive environmental factors in domestication efforts is salinity. Water salinity is the amount of salt in the water in which the salt is the content of ions dissolved in the water (**Armis, 2017**). Low salinity will endanger the growth of fish due to it can reduce oxygen level, while on the other hand, too high a salinity level will impact the fish growth too (**Hendrawati *et al*., 2008**). In general, since salinity is a limiting factor for fish, Banggai cardinalfish's ex-situ conservation efforts need this information. Besides, food availability is one factor that affects the growth of fish (**Prasetyo *et al*., 2018**). In this study, natural food feeding has been based on previous studies by **Anggraeni & Abdulgani (2013**), which stated that natural food was better than the artificial one. Natural food contains high protein and fat so that it supports the acceleration of fish growth. Therefore, in this study, a portion of different natural food is used to be one factor in obtaining the most appropriate natural food information.

Several previous researchers have carried out studies on Banggai cardinalfish, for instance, is the one by **Safir *et al.*, (2020) and Hulu *et al.*, (2020)** that compared commercial food and natural food on the growth of Banggai cardinalfish. Meanwhile, a study on salinity in Banggai cardinalfish has been carried out (**Ndobe, 2011 and Pompon *et al.*, 2019**). The study is still carried out separately by each researcher; thus, it has not discovered the effect of salinity and different natural food on the growth and survival rate of fish. Therefore, this study is designed to observe the best results from the interaction of two factors that are natural food and salinity, on the growth and survival rate of Banggai cardinalfish in controlled containers as part of developing an ex-situ conservation effort.

MATERIALS AND METHODS

This present study was conducted from November to December 2019 at the Center for Marine Conservation and Cultivation Training of BCF Lestari Community Group, Banggai Laut District, Central Sulawesi Province. The method used was an experiment with a Completely Randomized Design with two factors (S: Salinity and A: Food). In this study, the salinity factor comprises four levels (salinity: 26 ppt, 20 ppt, 14 ppt and 8 ppt) in which it refers to the previous research **(Ndobe, 2011; Pompon *et al.*, 2019**) as it aims to observe fish endurance in a lower salinity level. In the meantime, food factor comprises three levels (type of food: rebon shrimp, Artemia sp., and mosquito larvae) in which it refers to a research (**Safir *et al*., 2020;Safir *et al*., 2020)**. The following is the combination of treatment:

S1 A1: 26 ppt Salinity and Rebon Shrimp

S1 A2: Salinity 26 ppt and Artemia sp.
S1 A3: 26 ppt Salinity and Mosquito Larvae
S2 A1: 20 ppt Salinity and Rebon Shrimp
S2 A2: Salinity 20 ppt and Artemia sp.
S2 A3: 20 ppt Salinity and Mosquito Larvae
S3 A1: Salinity 14 ppt and Rebon Shrimp
S3 A2: Salinity 14 ppt and Artemia sp.
S3 A3: Salinity 14 ppt and Mosquito Larvae
S4 A1: Salinity 8 ppt and Rebon Shrimp
S4 A2: Salinity 8 ppt and Artemia sp.
S4 A3: 8 ppt salinity and mosquito larvae
Each combination was repeated 3 times so that the total experiment was 36 units.

Research procedure

The study was commenced by preparing testing media (water with different salinity: 26 ppt, 20 ppt, 14 ppt, and 8 ppt) as factor S and food as factor P (rebon shrimp, *Artemia* sp., and mosquito larvae). The fish for animal testing were reared in the aquarium with a size of 30x20x20 cm and 10 L water. In total, there were 36 aquariums equipped with 4 units of Recirculation Aquaculture System (RAS). The fish used for animal testing were obtained from fishermen with an average weight of 0.29 g and an average length of 28.31 mm with a stocking density of 1 fish/L. The size of the fish refers to research conducted by (Pompon et al., 2019). Then, seawater was diluted to obtain the expected salinity using the following equation formula proposed by **Madinawati *et al.*, (2009)**:

$$V_2 = \frac{V_1 \,.\, (S_1 - S_n)}{S_n + S_2}$$

Information :

V_1 = Volume of seawater (ml)
V_2 = Volume of seawater (ml)
S_n = Expected salinity (ppt)
S_1 = Salinity of seawater (ppt)
S_2 = Salinity of freshwater (ppt)

Initially, the fish were adapted for a week before entering the rearing container and conducting the study. The adaptation process encompassed habituating to the environment with different salinity levels and habituating to the type of food provided. Afterward, Banggai Cardinalfish were reared for 30 days. The fish were stocked in containers at a ratio of 1 fish/liter with a volume of ten liters of water per container. During the study, the fish were fed three types of food: rebon shrimp, Artemia sp., and mosquito larvae, and they were fed with ad libitum method. The feeding was carried out at 17.00, 00.00, and 06.00, where the water quality in the rearing media was maintained due to it played a pivotal role. Meanwhile, to maintain water quality during the rearing period, daily siphoning and water changes of 10-20% were carried out.

Observations were performed every ten days of rearing. The variables observed were growth, including measurement of length and weight, survival rate, and water quality. The water quality parameters observed were temperature, salinity (treatment), pH, dissolved oxygen, ammonia, nitrite, and nitrate. In the meantime, the observation towards ammonia, nitrite, and nitrate was carried out at the start and end of rearing, whereas the survival rate was observed every day in which they were presented accumulatively during the rearing period in the form of a percentage.

Data analysis

Parameters observed in this study include growth in length and absolute weight and survival. The formula for growth and survival can be expressed as follows.

Absolute length formula **(Cruz *et al*., 2021)**.

$$LG = FL - IL$$

Notes :

LG = Length gain

FL = Final length

IL = Initial (Initial length)

Absolute weight growth formula **(Mohammadi *et al*., 2020)**

$$WG = FW - IW$$

Notes:

WG = Weight gain (weight gain, gr)

FW = Final weight

IN = Initial weight

Survival Rate Formula **(Giri *et al.*, 2019)**

$$SR = \frac{FN}{IN} x100$$

Information :

SR = Survival rate (Survival,%)

FN = Final number of fish

IN = Initial number of fish

To see the effect of each factor, an analysis of variance (ANOVA) at 0.05 and 0.01 levels was performed using Microsoft Excel.

RESULTS

1. *The Water Quality*

Water quality is an essential factor in the survival and growth of fish. In this study, several quality parameters are measured and presented in the following table 1.

Table 1. Water Quality

Salinity (ppt)	Temperature (^{o}C)				pH				Dissolved oxigen (ppm)				Ammonia (ppm)		Nitrite (ppm)		Nitrate (ppm)	
	D-0	D-10	D-20	D-30	D-0	D-10	D-20	D-30	D-0	D-10	D-20	D-30	Innitial	Final	Innitial	Final	Innitial	Final
26	28.2	27.3	27.3	26.3	8.04	8.21	7.73	8.58	3	9.5	8.9	6.8	<0.05	<0.05	0	0.5	0	0
20	28.2	27.3	27.3	26.3	8.21	8.31	7.79	8.65	3.1	7.6	10.1	9.3	<0.05	<0.05	0	0.5	0	5
14	28.3	27.3	27.4	26.2	8.28	8.42	7.76	8.7	3.2	8.1	6.6	6.9	<0.05	<0.05	0	0	0	10
8	28.3	27.4	27.4	26.3	8	8.48	7.9	8.85	3.3	10.5	7.9	7.8	<0.05	<0.05	0	0	0	5

Information:
D-0 : First day observation
D-10 : Tenth day observation
D-30 :Thirtieth day observation

Table 1 signifies that the temperature and pH parameters show relatively small dynamics between treatments and each observation time. On the other hand, the dissolved oxygen data indicate a significant improvement on the first day of observation compared to the tenth day—furthermore, the dissolved oxygen data experience dynamics in the next observation. However, in general, the parameters of temperature and dissolved oxygen are in the range that suits fish needs (**Government Regulation No. 82 of 2001**). Observation on ammonia is carried out at the beginning and end of rearing and is not detected. Nitrite and nitrate are not detected at the beginning of the observation, while at the end of the observation, it detected nitrite for 0.5 ppm and nitrate for 5-10 ppm. Based on the quality standard in Government Regulation No. 82 of 2001, the salinity of 26 ppt and 20 ppt have nitrite content above the quality standard threshold.

2. *Growth Length And Weight*

The growth and survival of Banggai cardinalfish (*Pterapogon kauderni*) reared at different salinities and fed different natural feeds showed differences. The results in the study can be seen as follows (Fig. 1).

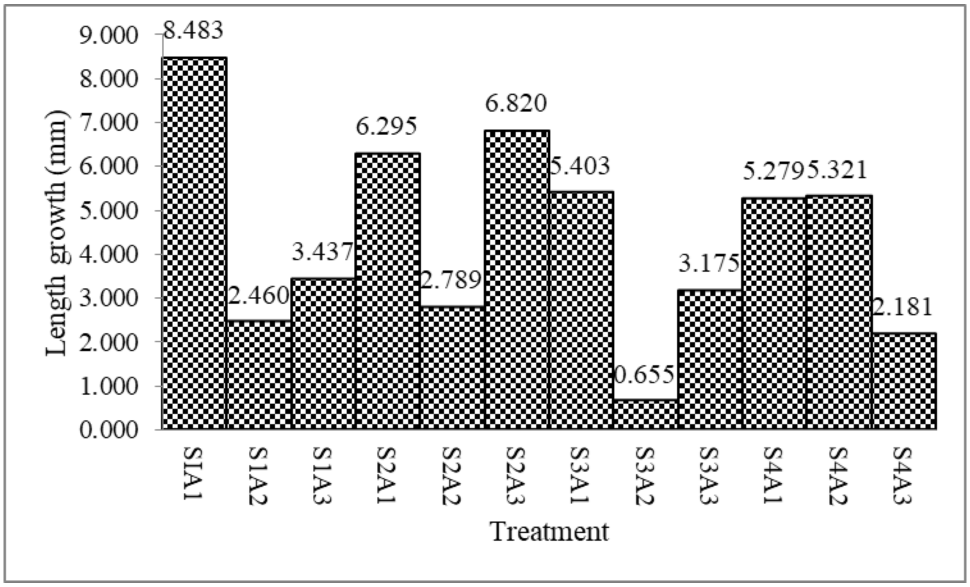

Fig. 1. Absolute length growth graph

The results of the ANOVA test analysis (P < 0.05) on the combination factor S and A showed no significant effect on absolute length growth where P = 0.05. In the treatment, the S factor showed no significant effect on the length growth where P>0.05. While factor A had a significant effect on length growth where P<0.05 (Table 2).

Table 2. ANOVA of Growth in absolute length

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	175.920[a]	11	15.993	3.525	.005
Intercept	675.567	1	675.567	148.920	.000
Faktor S	26.559	3	8.853	1.952	.148
Faktor A	82.599	2	41.299	9.104	.001
Faktor S * A	66.762	6	11.127	2.453	.05
Error	108.874	24	4.536		
Total	960.361	36			
Corrected Total	284.794	35			

Absolute weight growth showed differences in fish growth in each treatment. The best growth was shown in the S1A1 treatment, which was 0.25 gr and the lowest in S3A2, which was 0.02 gr (Fig. 2).

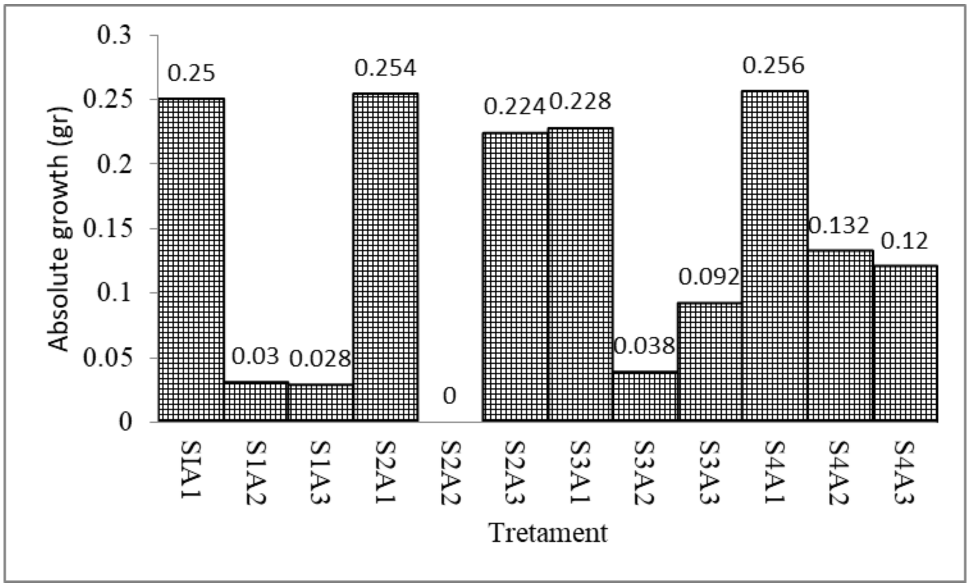

Fig. 2. Absolute weight growth

The results of the ANOVA test analysis (P<0.05) on the combination factor S and A showed no significant effect on weight growth where P>0.05. The S factor shows no significant effect where P>0.05. Factor A shows a significant effect where P <0.05 (Table 3).

Table 3 ANOVA of Weight Growth

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.227[a]	11	.021	7.762	.000
Intercept	.497	1	.497	187.164	.000
Faktor S	.011	3	.004	1.393	.269
Faktor A	.192	2	.096	36.188	.000
Faktor S * A	.023	6	.004	1.471	.230
Error	.064	24	.003		
Total	.788	36			
Corrected Total	.290	35			

3. *Survival Rate*

Survival data showed varied results in each treatment. The highest survival was shown in the S3A2 treatment, which was 100% and the lowest was S2A1 63.33% (Fig. 3).

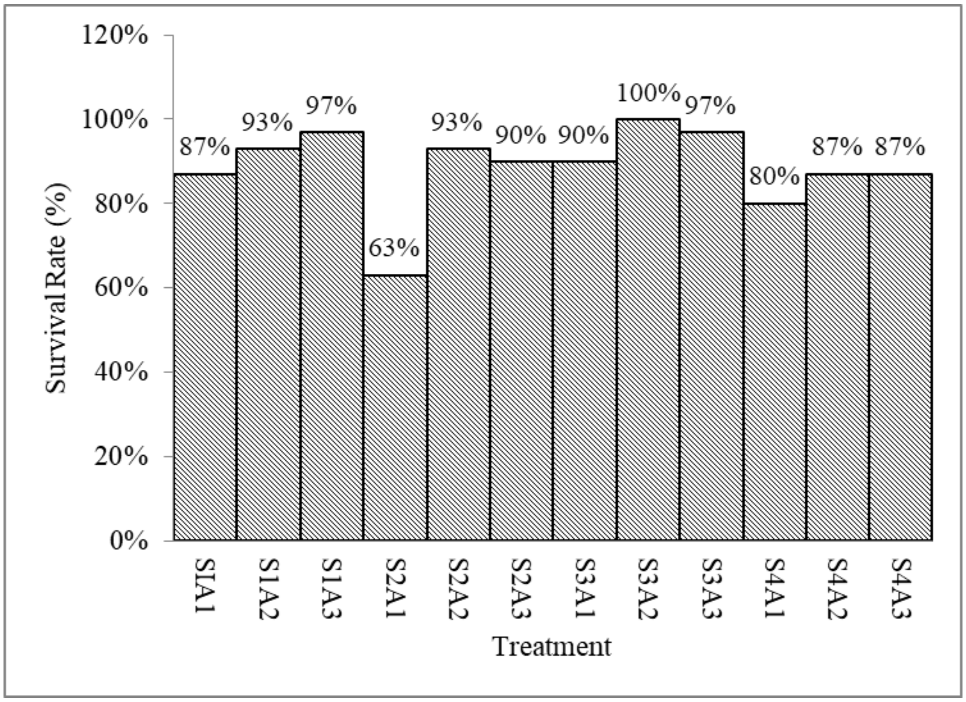

Fig. 3. The survival rate of Banggai Cardinalfish by treatment

The results of the ANOVA analysis (P<0.05) on the combination factor S and A showed no significant effect (P>0.05). On the other hand, factors S and A showed a significant effect where P<0.05.

Table 4. ANOVA of Survival rate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3097.222[a]	11	281.566	2.472	.031
Intercept	282669.444	1	282669.444	2481.976	.000
Faktor S	1075.000	3	358.333	3.146	.044
Faktor F	1338.889	2	669.444	5.878	.008
Faktor S * A	683.333	6	113.889	1.000	.448
Error	2733.333	24	113.889		
Total	288500.000	36			
Corrected Total	5830.556	35			

DISCUSSION

The difference in fish growth in each treatment is assumed to be led by particular supporting and inhibiting factors, including osmoregulation level, protein content of the food, and sexuality of the fish. Growth is a biological process that is affected by many factors and is complex **(Johan *et al.*, 2020).**

Fish need energy for biological activities, including growth, development and reproduction, as well as for physical activity. The energy needed for these activities comes from the feed consumed. The food consumed then goes through the stages of digestion, absorption, transportation, and metabolism. Energy from this metabolic process is used for basal metabolism and other activities, while the rest is used for growth **(Putra, 2015)**. In addition, protein elements in food are needed by fish for energy sources and growth **(Diana *et al.*, 2017)**. Growth occurs due to excess energy after all energy needs for survival have been met **(Setiawati *et al.*, 2008)**. In conformity with the statistical test, it is shown that different types of natural food own a significant effect on the growth and survival of Banggai cardinalfish.

Salinity affects the osmoregulation of fish, where a good salinity for the growth of Banggai cardinalfish is 27 ppt **(Madinawati *et al.*, 2009; Pompon *et al.*, 2019)**. In the meantime, **Ndobe (2011)** states that the best growth of Banggai cardinalfish is discovered in salinity of 26 ppt whereas, in this study, the best growth is in the treatment with the result of 26 ppt too. After all, the result of the statistical test denotes that the salinity does not own a significant effect on the growth of Banggai cardinalfish.

The survival rate shows various values in which, on average, the best results are indicated by salinity for 14 ppt and 26 ppt followed by 8 ppt and 20 ppt. These results denote that Banggai cardinalfish can survive in a salinity range of up to 8 ppt with an average survival rate value of 84.44%. The previous results are inversely proportional to the statement of **Ndobe, (2011)**, which states that the lower limit of rearing for Banggai Cardinalfish is around 24 ppt. The increase in survival rate compared to previous studies is predicted to be due to the carrying capacity of the food fed. **Johan *et al*., (2020)** urge that a decrease in the percentage of fish survival rate is in response to changes in salinity, so that fish need more energy to maintain a balance of salt level between the environment and body. Moreover, fish that cannot adapt and tolerate their environment will experience a symptom of stress and culminate in death. This study is in line with the statement by **Bœuf & Payan (2001)** that reef fish can tolerate moderate salinity.

Food is one of the determinant factors of fish growth and survival. During the rearing period, the Banggai cardinalfish were fed three types of natural food, including rebon shrimp, *Artemia* sp. and mosquito larvae. The nutritional content, particularly protein in these three food are considered sufficiently high where the protein content of rebon shrimp, *Artemia* sp. and mosquito larvae are 16.2% **(Pawe, 2015)**, 40-50% **(Panggabean, 1984)**, and 48.72% **(To'bungan, 2017)** respectively.

Sugama (2008) appends that dissection of the Banggai cardinalfish stomach contained mysids and milkfish larvae. The fish-related study indicates that the highest growth at all salinity levels is almost dominated by rebon shrimp food. It is strongly believed that the rebon shrimp are more suitable for the fish's mouth opening. In addition, the rebon shrimp, which tend to be able to adapt to low salinity levels and remain active, attract fish to prey.

Additionally, **Sugama (2008)** conveys that feeding Artemia sp. results in good growth and survival rate. However, in this study, the growth of Banggai Cardinalfish is lower in nearly all salinity factors compared to rebon shrimp and mosquito larvae, and it is presumably due to the size of Artemia sp. is too small so that the fish cannot view and are attracted to eat it.

Pompon *et al.*, (2019) declare that feeding mosquito larvae to Banggai cardinalfish signifies good growth and survival rate. The shape of the mosquito larvae that resemble rebon shrimp and actively moves in the water attracts the attention of fish to prey. Although it has a fairly good protein content, in this study, at S1 treatment level, fish growth is almost equivalent to the S3 and S4 treatments. The decline in growth is strongly believed due to mosquito larvae are no longer active in the high salinity range, so that fish have less interest in preying them. However, the interaction of factors of salinity and natural food does not affect the growth and survival rate of Banggai cardinalfish significantly.

CONCLUSION

Feed has a significant effect on the absolute length and absolute weight growth. On the other hand, salinity and the combination of salinity and feed did not affect. Feed has a significant effect on survival. On the other hand, the combination of feed and salinity did not affect. While salinity affects survival at the level of 5%. Development of ex situ conservation of Banggai cardinalfish by conditioning the most effective salinity and feeding natural rebon shrimp.

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