Growth and survival of juvenile Banggai cardinalfish (Pterapogon kauderni) reared under different salinities in recirculating aquaria equipped with protein skimmers

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Abstract. The Banggai cardinalfish (Pterapogon kauderni) is a small marine ornamental fish endemic to the Banggai Archipelago in Indonesia, listed as Endangered in the IUCN (International Union for the Conservation of Nature) Red List, and twice proposed for CITES (Convention on International Trade in Endangered Wild Species of Fauna and Flora) Appendix II listing. In addition to in-situ conservation, the Indonesian Action Plan (2017-2021) calls for ex-situ P. kauderni conservation, including the development of captive breeding. The accumulation of metabolic waste was suspected as a causal factor in high levels of mortality under all treatments in preliminary captive rearing trials. Protein skimmers can minimise the accumulation of potentially toxic nutrients, organic compounds, and pathogens. This research evaluated the effect of salinity on the growth and survival of juvenile P. kauderni in recirculating systems equipped with protein skimmers and periodical siphoning of waste with partial water change. Juvenile P. kauderni were obtained from the introduced population at Mamboro, Palu Bay, Central Sulawesi Province, Indonesia. Initial size range was 1-3 cm standard length (SL) and 0.105-0.329 g weight. Four salinity treatments (27 ppt, 30 ppt, 33 ppt, 36 ppt) with 5 replicates were applied, with 3 fish per experimental unit (aquarium, 20×30×20 cm, 7 litre capacity). Each fish was measured and weighed at the beginning and end of the 30 day experimental period. The fish were fed with mosquito (Culex sp.) larvae twice a day. The protein skimmers maintained high water quality and survival was high under all treatments, Survival was 100% at 27 ppt and 30 ppt. The 27 ppt treatment yielded the highest average growth (0.35 g in weight and 1.15 cm in SL). Our results show that juvenile P. kauderni grow faster at salinity levels lower than the average in endemic or introduced habitats of this species, and indicate that the use of protein skimmers can effective in promoting the survival of captive juvenile P. kauderni.

1. Introduction
The Banggai cardinalfish (Pterapogon kauderni) is a marine ornamental fish of international concern, listed as Endangered in the IUCN (International Union for Conservation of Nature) Red List [1]. The Ministry of Marine Affairs and Fisheries (MMAF) of the Republic of Indonesia has declared 20 aquatic species or species groups as conservation priorities for the period 2015-2019, one of which is the Banggai cardinalfish [2], endemic to the Banggai Islands, Central Sulawesi, Indonesia [3,4].

While the English common name of Pterapogon kauderni is Banggai cardinalfish, often shortened to BCF in the ornamental fish trade [5,6], local names include Bebesa tayung (Bajo language) which means little sea urchin fish [6], while other local or trade names include capungan Banggai [6], capungan layar, capungan Ambon [7], and high-fin cardinalfish.
In line with development in this era of modernization, both scientific advances and exploitation are increasing. The need for efforts to preserve both Banggai cardinalfish habitat and populations is widely recognised [1,2,3,5,6]. Culture outside the original habitat (ex-situ culture), can also support Banggai cardinalfish conservation and sustainable use [4,8], however culture conditions do not always support the survival and growth of Banggai cardinalfish. The development of aquaculture science and technology can support the breeding and husbandry of marine organisms outside of their natural habitat by manipulating the environment, e.g. by adjusting temperature, salinity and DO.

In this context, equipment such as protein skimmers can be used to maintain water quality and reduce the volume and frequency of water replacement needed. Protein skimmers work on the principle of removing various types of organic matter and compounds derived from aquatic organisms by producing smooth bubbles or foam to raise and trap this nitrogen-rich matter [9]. Commercial protein skimmers are quite expensive and not always very effective.

The objective of this study was to evaluate the growth and survival of juvenile Banggai cardinalfish (Pterapogon kauderni) maintained at different salinities using specially designed and constructed low-cost protein skimmers. It is hoped that this research will be useful as a reference for domestication and cultivation (maintenance) of Banggai cardinalfish and other marine species.

2. Materials and Methods

2.1. Experimental design

The research was conducted from November to December 2017 at the Aquaculture Laboratory, Faculty of Animal Husbandry and Fisheries, Tadulako University, Palu, Central Sulawesi, Indonesia. The experimental animals were 60 wild-caught juvenile Banggai cardinalfish (P. kauderni) with standard length (SL) of 1-3 cm and weight of 0.1050-0.3298 grams, sourced from the Mamboro introduced P. kauderni population [10] in Palu Bay, Palu City, Central Sulawesi Province, Indonesia.

The fish were kept in aquaria (20 units, 20×30×20 cm), each filled with 7 litres of salinity-controlled seawater, with three fish per unit. The water (media) used (sea water and fresh water) was filtered to remove dirt and potential pathogens. During the 30 day trial there were no complete water changes, but waste was siphoned and water replaced with saline water according to the salinity treatment.

Protein skimmers were designed and constructed, and were then used throughout the study to prevent a build-up of impurities (especially ammonia) in the media (saline water) which was pumped through the skimmer and returned to the aquaria. The protein skimmers rely on the processes which take place when fine air bubbles are streamed through a column of water containing dissolved organic matter, especially ammonia. These impurities become concentrated in the foam at the surface of the water column, which becomes progressively darker in colour, from yellowish to blackish brown, as the nitrogen-rich proteins accumulate in the foam layer. Throughout the study the foam was controlled and the skimmers cleaned as necessary, at least 3 times a week, to prevent this foam from falling back down and re-contaminating the cleaned media.

Throughout the trial, the fish were fed twice a day (morning and afternoon) with live mosquito larvae (Culex sp.), at a rate of 6-8 larvae per unit (aquarium). The fish were acclimated to ensure they had adapted to the feed and other conditions in the laboratory before the 30 days trial began.

The salinity treatments followed a complete randomized design (CRD) consisting of four treatments and five replicates giving a total of 20 experimental units. The four salinity treatments (A,B,C,D) were 27 ppt, 30 ppt, 33 ppt, and 36 ppt, respectively. The desired salinity for each treatment was obtained through dilution, by adding fresh water to the sea water according to the following formula [11,12]:

\[
S_2 = \frac{(V \cdot S_1)}{(n+V)}
\]

where:

- \( S_2 \) = desired salinity (ppt)
S = salinity of sea water to be diluted (ppt)
V = volume of sea water to be diluted (L)
n = volume of fresh water to be added (L)

Each fish was weighed at the beginning and end of the study using digital scales (precision 0.01g) and standard length was measured using digital callipers (precision 0.1 mm). The absolute growth in weight (W) and standard length (L) of the juvenile Banggai cardinalfish in each unit were calculated based on initial and final measurement as follows:

\[ W = W_t - W_0 \]  \hspace{1cm} (2) [13]
\[ L = L_t - L_0 \]  \hspace{1cm} (3) [14]

where:
W = absolute growth in weight (biomass)
Wt = mean juvenile weight at the end of the study (g)
Wo = mean juvenile weight at the start of the study (g)
L = absolute growth in length (cm)
Lt = juvenile length at end of study (cm)
Lo = juvenile length at begin of study (cm)

Survival (SR) was calculated for each unit using the following equation:

\[ SR = \frac{S_t}{S_0} \times 100\% \]  \hspace{1cm} (4) [15]

where:
SR = Survival rate (%)
St = number of live fish at end of study
S0 = initial number of fish

Salinity was monitored daily to ensure treatment consistency (refractometer, ppt). The other water quality parameters measured were temperature (thermometer, °C), dissolved oxygen (DO meter, mg/L), pH (pH meter) and ammonia (spectrophotometer, ppm). Temperature was measured daily, while dissolved oxygen (DO) and pH were measured 6 times: on day 0, 7, 14, 21, 28, and 30. Ammonia was measured twice, at the beginning and end of the research.

2.2. Data analysis

An analysis of variance (ANOVA) was performed on the absolute growth data in weight and standard length in Microsoft Excel 2007. Where a significant difference (p < 0.05, confidence level 95%) was found, a post-hoc Honestly Significant Difference test (Tukey test) was performed (at 95% confidence level) in MINITAB 16.0. Survival data (SR) and water quality were analysed descriptively using graphs, tables and literature comparisons.

3. Results and Discussion

3.1. Growth and survival of juvenile P. kauderni

The data on absolute growth of juvenile Banggai cardinalfish (P. kauderni) in terms of weight (Figure 1) and standard length (Figure 2) indicate an inverse correlation between salinity and growth over the range (27-36 ppt) in this study. The highest growth under the 27 ppt salinity treatment (average 0.35 g, 1.15 cm) was around 30% higher in length and 40% higher in weight than the lowest growth (average 0.25 g, 0.88 cm) under the 36 ppt salinity treatment. The analysis of variance (ANOVA) on absolute weight gain and on absolute increase in length both showed a highly significant (p <0.01) difference between salinity treatments.
Figure 1. Absolute growth in weight of juvenile *P. kauderni* by salinity treatment

Figure 2. Absolute growth in standard length of juvenile *P. kauderni* by salinity treatment

The data on juvenile Banggai cardinalfish (*P. kauderni*) survival rate (SR) (Figure 3) show no mortality (SR 100%) under salinity treatments 27 ppt and 30 ppt. At the higher salinities in this study mortality was still relatively low (around 6.7%) at 33 ppt, but doubled to around 13.3% under the highest salinity treatment of 36 ppt.

Figure 3. Survival (SR) of juvenile *P. kauderni* by salinity treatment.

3.2. Water quality
The recorded values of water quality parameters during the study (Table 1) are within suitable ranges for a tropical marine fish. This indicates the effectiveness of the protein skimmers in maintaining water quality. Furthermore, the skimmers were able to maintain water clarity, even when leftover feed was floating around the periphery of the aquaria.
3.3. Discussion

3.3.1. Salinity. The salinity of the media in which they live will affect the growth of aquatic animals. As in the wild, in a controlled environment an organism will endeavour to maintain an appropriate salinity level within its body through osmoregulation or ion adjustment [16]. The reported salinity in the natural habitat of *P. kauderni* ranges from 30-35 ppt in the Banggai Archipelago [5,6,17,18] and 29-34 at the introduced site of Mamboro [5]. The results shown in Figures 1 and 2 indicate that juvenile *P. kauderni* can benefit from salinity levels lower than those typical of their natural environment. In the 27 ppt salinity treatment, the juvenile *P. kauderni* exhibited normal active swimming behaviour, with a higher level of activity and appetite compared to other treatments. When feeding, these fish immediately grabbed and swallowed the feed. It has been suggested [8] that higher growth under low salinity (27 ppt) might be due to a lower energy demand for osmoregulation, so that more energy is available to actively feed and to convert feed into growth.

Osmoregulation enables aquatic biota to continuously control the balance of ions in their body, and is greatly influenced by salinity, with increasing energetic costs at salinity levels above or below the optimal salinity range of an organism [21], thus reducing the energy available for physical activity as well as for mechanisms promoting growth and survival. It is likely that increased costs of osmoregulation are one reason for the comparatively low activity level and slow growth of juvenile Banggai cardinalfish under the 36 ppt treatment. These fish became less mobile and tended to stay at the bottom of the aquarium, with a decrease over time in feed consumption, as evidenced by higher levels of left-over feed seen floating around the aquaria undergoing this treatment.

3.3.2. Banggai cardinalfish (*P. kauderni*) survival rate. The results of this study are similar to, albeit with a slightly higher minimum than, the range of 80% to 100% previously reported for salinities ranging from 27 ppt to 35 ppt under a different husbandry regime [22]. By the end of the study, the survival rates of juvenile Banggai cardinalfish (*P. kauderni*) were high under all treatments, ranging from 86.66% to 100.00%. However the decline at higher salinity levels indicates that salinity levels in excess of 35 ppt may not be conducive to the survival of this species, at least in the juvenile stage. In contrast the results also indicate that a salinity of 27 ppt, lower than the salinity range commonly reported from native habitat, is suited to the needs of *P. kauderni*. Each aquatic organism will have an optimal salinity range within its envelope of tolerance. While *P. kauderni* has been shown to have a wide tolerance envelope, at least in the short term, grown and survival rate have been reported as declining not only at high salinity but also at salinities below 24 ppt [23].

3.3.3. Water quality. While temperatures below the optimum range will tend to have a negative effect on the metabolism of aquatic organisms, inhibiting movement, growth and development[21], higher temperatures can increase the virulence of and host susceptibility to pests and diseases [3], and will eventually exceed metabolic limits [21]. Water temperature (26-28 °C) remained within the range (25-30 °C) widely considered suitable for tropical aquatic organisms [22], and in particular for the Banggai cardinalfish [3,8,11].

Table 1. Results of water quality measurements during the study.

| treatment (salinity) | Parameter Range | Temperature (°C) | Dissolved Oxygen (mg/L) | pH | Ammonia (mg L⁻¹) |
|---------------------|-----------------|------------------|-------------------------|----|------------------|
| 27 ppt              |                 | 26 - 28          | 5.8 – 6.9               | 8.3 – 8.5 | 0.014  | 0.013 |
| 30 ppt              |                 | 27 - 28          | 5.7 – 6.7               | 8.3 – 8.5 | 0.015  | 0.013 |
| 33 ppt              |                 | 26 - 28          | 5.8 – 6.6               | 8.3 – 8.6 | 0.015  | 0.012 |
| 36 ppt              |                 | 26 - 28          | 5.7 – 7.1               | 8.3 – 8.5 | 0.014  | 0.011 |

- **Dissolved Oxygen**: The dissolved oxygen levels were maintained within the range of 5.7 to 8.3 mg/L, suitable for aquatic life.
- **pH**: The pH levels were within the range of 8.3 to 8.6, indicating a neutral to slightly alkaline environment.
- **Ammonia (mg L⁻¹)**: The ammonia levels were maintained below 0.015 mg L⁻¹, indicating a healthy water quality.

Despite the high survival rates, the juvenile cardinalfish showed decreased activity levels and growth at salinities above or below the optimal range of 27 ppt.
According to Clark (1996) in [24], a reduced concentration of dissolved oxygen can suppress respiration, lead to depression of appetite and decreased feed intake, and result in fish mortality. Dissolved oxygen (DO) levels measured during the study ranged from 5.8-7.1 mg/L, and were consistently above levels which have enabled successful Banggai cardinalfish reproduction (4.79-4.99 mg/L, [25]; 4.3-5.8 mg/L, [26]). Furthermore, the DO range was comparable to the recommended water quality standard for the maintenance of juvenile *P. kauderni* in aquaria (5.6-6.1 mg/L, [7]), and for captive reared *P. kauderni* growth and survival (7.62-7.86 mg/L [8]).

The pH values recorded for all treatment media were around pH 8, a value which is not uncommon for tropical seawater. This is within or close to pH ranges reported as suitable for the growth and survival of the Banggai cardinalfish in other studies including pH 8.3-8.7 [7], and pH 8.0-8.1 [26]. There is some evidence that *P. kauderni* may have a relatively broad tolerance envelope for pH, as high survival has been reported with pH values fluctuating from 6.58-8.16, indicating that (at least for limited periods of time) *P. kauderni* can withstand acidic conditions below pH 7 [8], which may occur in the shallow water coastal habitat of this species due to natural or anthropogenic factors.

Ammonia (NH3) is toxic to most aquatic animals and readily diffuses through the gill membrane. Ammonia toxicity is influenced by pH, temperature and salinity. Elevated ammonia concentrations can result in slower growth, decreased appetite, and tissue damage, and indeed the accumulation of ammonia in water can be a major cause of functional and structural disruption in aquatic biota [27]. Banggai cardinalfish are reported as tolerant of ammonia concentrations in the range 0.1-0.5 mg L⁻¹ [8, 25]. Ammonia concentrations in all experimental units were low (0.011-0.015 mg L⁻¹). This range is an order of magnitude lower than the values reported from some other *ex-situ* studies on Banggai cardinalfish, indicating that the protein skimmers were effective in maintaining water quality.

### 3.3.4. Live feed suitability

Feed is one of the factors supporting the growth of fish. During the trials, the juvenile Banggai cardinalfish were given both mosquito larvae (*Culex* sp.) and *Artemia* sp. to increase feed variety and nutritional content. The Banggai cardinalfish appeared strongly attracted to the larval mosquitos (*Culex* sp.) as well as the larval stage brine shrimp (*Artemia* sp.). Research has indicated that, although juvenile Banggai cardinals fed a diet of *Artemia* sp. may grow well, mortality can be high due to the low polyunsaturated fatty acid (PUFA) content of unenriched *Artemia* [19]. Furthermore, in addition to enrichment, other Crustacea have been found may suitable as feed, including *Euphausia superba* and *E. pacifica* [19], and mysid shrimps [7]. One reason for the acceptability of mosquito larvae may be a superficial resemblance to some small crustaceans, while the nutritional content of mosquito larvae, which require high PUFA levels for development [20], may make them a good source of essential nutrients for juvenile Banggai cardinalfish.

### 3.3.5. Protein skimmer function

Protein skimmers can minimise the build-up of an oil layer on the water surface [28], substantially reduce the build-up of residual organic waste matter and bacteria resulting from metabolism and decomposition, and in particular they can decrease the total concentration of total ammonia nitrogen (TAN) [29]. The use of protein skimmers relies on the continuous functioning of a controlled water and air working system, so that a froth of fine bubbles can be produced continuously. Any interruption in this process will at best impede or stop the cleaning function of the skimmer, and at worst may allow impurities previously removed to re-enter the culture media. When the foam is dark in colour (blackish brown) due to the build-up of impurities (especially ammonia), it is not only important to remove the foam but vital to prevent the foam being returned (falling back into) the cleaned media passing through the skimmer. One challenge encountered was the risk of power failures, both total power cuts and brown-outs when the electricity voltage was inadequate to enable proper functioning of the skimmer. Steps to ensure 24 hour efficient skimmer function are extremely important to reduce the risk of mortality. During power cuts or brown-outs, as the impurities collecting in the skimmer come back down and mix with the clean media, the fish tend to become aggressive and loose appetite, not eating the feed provided. After such incidents both water
quality and fish behaviour appeared to return to normal around 1 to 2 hours after the restoration of an adequate electrical power supply.

4. Conclusion

Based on our results, we can draw several conclusions. Firstly, salinity does affect the growth of juvenile Banggai cardinalfish (P. kauderni). Secondly, growth (length and weight) and survival rate are higher at salinities (27-30 ppt) below the salinity range common in the P. kauderni natural habitat, while not only growth but also survival is negatively affected if salinity is higher than this range. Furthermore, the use of low-cost protein skimmers such as those designed, constructed and used during this study can help maintain water quality and keep ammonia concentrations low. We suggest further research on the effectiveness of protein skimmers for optimising growth and survival of Banggai cardinalfish (P. kauderni) at different stocking densities and with different feed regimes, as well as for the ex-situ husbandry of other marine organisms.

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