

## THE EFFECT OF TEMPERATURE MANIPULATION ON THE GROWTH OF AXOLOTL (*Ambystoma mexicanum*)

### Pengaruh Manipulasi Suhu yang Berbeda Terhadap Pertumbuhan Axolotl (*Ambystoma mexicanum*)

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#### ABSTRACT

Axolotl (*Ambystoma mexicanum*) is a unique animal whose population is threatened with extinction. Axolotl is an unique animal, one of its unique features is that they can regenerate almost all parts of its body. In Indonesia itself, there are still very few people who cultivate this animal. In Indonesia, axolotl cultivation activities encounter many obstacles, especially due to environmental factors that are very different from their natural habitat. One of the main obstacles to breeding axolotls is temperature. To create a suitable environment for axolotls to grow and develop, tools are needed. One of the tools used is the ultra recirculate chiller system (URCS). This research aims to determine the effect of different temperature manipulations on axolotl growth. This research used an experimental method using three different temperature treatments (18°C, 22°C and 26°C). The main parameters tested were survival rate (SR), specific growth rate (SGR) and feed conversion ratio (FCR). The research results showed that temperature had an effect on survival rate, specific growth rate and feed conversion ratio. The best treatment was obtained at a temperature of 18°C with SR 100%, SGR 0.49%.

Keywords: Axolotl, Growth, Temperature, URCS

#### ABSTRAK

Axolotl (*Ambystoma mexicanum*) merupakan hewan unik yang populasinya sudah terancam punah. Axolotl merupakan yang unik, salah satu keunikannya adalah dapat meregenerasi hampir seluruh bagian tubuhnya. Di Indonesia sendiri masih sedikit sekali yang membudidayakan hewan satu ini. Di Indonesia kegiatan budidaya axolotl banyak menemui hambatan terutama karena faktor lingkungan yang sangat berbeda dengan habitat aslinya. Salah satu kendala utama untuk membudidayakan axolotl adalah suhu. Untuk membuat lingkungan yang sesuai untuk axolotl tumbuh dan berkembang diperlukan bantuan alat. Salah satu alat yang digunakan adalah ultra recirculate chiller system (URCS). Penelitian ini bertujuan untuk mengetahui pengaruh manipulasi suhu yang berbeda terhadap pertumbuhan axolotl. Penelitian ini menggunakan metode eksperimen dengan penggunaan tiga perlakuan suhu yang berbeda (18°C, 22°C dan 26°C). Parameter utama yang diuji adalah tingkat kelulushidupan (SR), laju

pertumbuhan spesifik (SGR) dan rasio konversi pakan (FCR). Hasil penelitian menunjukkan suhu berpengaruh terhadap tingkat kelulushidupan, laju pertumbuhan spesifik dan rasio konversi pakan. Perlakuan terbaik didapat pada perlakuan suhu 18°C dengan SR 100%, SGR 0,49%.

Kata Kunci: Axolotl, Pertumbuhan, Suhu, URCS

## INTRODUCTION

Axolotl is one of the unique ornamental fish and has great potential to be developed. Axolotl is a commodity that has many benefits. Axolotl is a native fauna of Mexico, and is known as a water salamander. Axolotl can be found in the Transmexican Volcanic Belt, one of the areas known for its highest biodiversity in Mexico. Among the 16 species of *Ambystoma*, Axolotl (*Ambystoma mexicanum*) is the most interesting and unique to study (Caballero-Pérez *et al.*, 2018).

Axolotl is a unique amphibian because it has the ability to regenerate severed body parts (Menger *et al.*, 2010). Currently, most axolotls are widely used for research in several scientific fields and are starting to be widely used as pets. Based on the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (2022), Axolotl is currently in Appendix II status, namely fauna that can be traded but with strict conditions. Trade, especially exports for appendix II commodities, must have a legal permit from CITES. Trade is permitted if it does not harm the survival of the Axolotl itself.

Efforts to prevent the extinction of the axolotl (*A. mexicanum*) can be done through cultivation and enlargement. To cultivate the axolotl (*A. mexicanum*) itself, water conditions are needed that are in accordance with its natural environment. Considering that the axolotl is an animal that comes from a cold environment, temperature manipulation is needed with a tool to adjust the parameters needed for the axolotl to live. The most important main parameter in axolotl cultivation is temperature. One of the tools that can manipulate water temperature is a chiller. The chiller itself is a tool used to lower the water temperature (Kuncoro *et al.*, 2018). With the Ultra recirculate chiller system (URCS) which contains a chiller, it is expected that axolotls can be preserved even though they are not in their natural habitat. Therefore, research is needed on the optimal temperature for cultivating axolotls. This research is expected to be useful as an effort to preserve axolotls (*A. mexicanum*). The purpose of this study was to determine the effect of different temperature manipulations on the growth of axolotls.

## METHODS

### Time and Place

This research was conducted for 2 months with Axolotl maintenance time of 30 days. This research was conducted at Axotic Farm in Batu City, with the address Ngaglik Village, Batu District, Batu City, East Java.

### Research Tools and Materials

The tools used in this research were aquariums, URCS system water filters, fishing nets, crystal bio, ginger clams, resun pumps, pH meter sample bottles, water test kits, test tubes, seser, scales, latex gloves, heaters, thermometers. The materials used in this research were Axolotl (*A. mexicanum*), distilled water, label paper, crystal bio, ginger clams, water conditioner, *Tubifex* sp.

### Research Design

The research method used was the experimental method. The experimental design in this research was a completely randomized design (CRD) with 3 different treatments and 3

replications. This research used an aquarium container measuring 60 cm x 35 cm x 35 cm. The treatment design given is as follows:

- Cold : Temperature treatment 18°C
- Normal : Temperature treatment 22°C
- Warm : Temperature treatment 26°C

The selection of temperature parameters 18°C, 22°C, and 26°C in this study was based on a literature review regarding optimal thermal conditions for axolotls (*Ambystoma mexicanum*). According to Ramos *et al.*, (2021), the natural habitat of axolotls has a temperature range of less than 16°C, while a study by Figiel, (2023) stated that axolotls can survive in a temperature range of 10-28°C as a temperature range that can be tolerated by axolotls. Meanwhile, maintaining axolotls at low temperatures requires special efforts to be able to maintain the temperature within the ideal temperature range, especially in Indonesia which has a tropical climate. Therefore, this study was conducted at a temperature of 18°C, which is the lowest temperature that can be maintained using a chiller system or URCS. While a temperature of 22°C is a normal temperature condition that can be achieved in mountainous areas such as Batu City, and a temperature of 26°C is the maximum temperature that can still be used by axolotls to grow.

### Specific Growth Rate

The specific growth rate is the percentage of daily fish weight gain during the maintenance period. Measurement of the specific growth rate can be obtained through the formula used by Fauzi *et al.*, (2012):

$$\text{SGR (\%/day)} = \frac{(\text{Ln } \Delta W_t - \text{Ln } \Delta W_o)}{t}$$

Description:

SGR = Specific growth rate (%/day)  
 $\Delta W_t$  = Final average weight (g)  
 $\Delta W_o$  = Initial average weight (g)  
t = maintenance period

### Feed Conversion Ratio

Feed conversion ratio is the comparison of the weight of feed given to fish with the increase in fish weight at the end of maintenance. According to Afrianto & Liviawaty (2005) the feed conversion ratio can be calculated using the following formula:

$$\text{FCR} = \frac{F}{W_t - W_0} \times 100\%$$

Description:

FCR = Feed conversion ratio  
F = Total feed given (g)  
 $W_t$  = Average final weight (g)  
 $W_0$  = Average initial weight (g)

### Survival Rate

The survival rate of axolotls (*A. mexicanum*) is calculated by comparing the number of axolotls at the beginning of the study with the number of axolotls at the end of the study. The survival rate value can be obtained through the formula used by Damayanti *et al.*, (2023):

$$\text{SR} = \frac{N_t}{N_0} \times 100\%$$

#### Description:

SR = Survival rate  
Nt = Initial number of axolotls  
N0 = Final number of axolotls

#### Water Quality

The supporting parameters measured in this study were temperature, pH, nitrate and level. Temperature measurement using a thermometer. pH measurement using a pH meter. Temperature and pH measurements were carried out every day at 12:00 WIB. Nitrate and level measurements using a test kit were carried out once a week.

#### Data Analysis

Data analysis from this thesis research was tested statistically using the analysis of variance (ANOVA) test. If there was data that was not normally distributed, a non-parametric Kruskal Wallis test was carried out. If the results of the ANOVA test and the non-parametric test showed a different effect, the test would be continued with the BNJ test to determine the average difference in each treatment. Furthermore, an orthogonal polynomial test was carried out to determine the value of the relationship between treatments and test parameters and to determine the optimal treatment. Data analysis was carried out at a confidence level of 95% ( $\alpha = 0.05$ ).

### RESULTS

Temperature plays a very important role in the life of all organisms including axolotls (*A. mexicanum*). All metabolic processes in cells can only take place properly when the temperature is at its optimum. One effort that can be made to maintain the stability of the water temperature for axolotl maintenance is by using URCS which is able to maintain the water temperature at a cold state. The results of this study indicate the effect of temperature on survival, growth and feed conversion ratio. The results of observations of specific growth rates, feed conversion ratios and survival rates are presented in Table 1.

Table 1. Average Results of Growth Test Parameters at Different Temperature Treatments

Parameter	Temperature Treatments		
	Cold	Normal	Warm
	18°C	22°C	26°C
Specific Growth Rate (%/day)	0.476 ± 0.010	0.201 ± 0.051	0.107 ± 0.004
Feed Conversion Ratio	1.48 ± 0.048	2.83 ± 0.004	4.12 ± 0.198
Survival Rate (%)	100 ± 0	80 ± 8.164	40 ± 8.164

#### Growth and Specific Growth Rate

Axolotl growth is greatly influenced by the temperature of the maintenance water. The higher the maintenance temperature, the lower the growth. At a maintenance temperature of 18°C, the highest weight growth value reached 18.33 grams/tail with a specific growth rate of 0.476% ± 0.010. The growth data obtained were normally distributed and homogeneous based on normality and homogeneity tests. The results of the ANOVA test showed a significant effect of temperature treatment on axolotl growth (sig <0.05). This is reinforced by the results of the Turkey HSD post-hoc test which showed significant differences in each treatment.

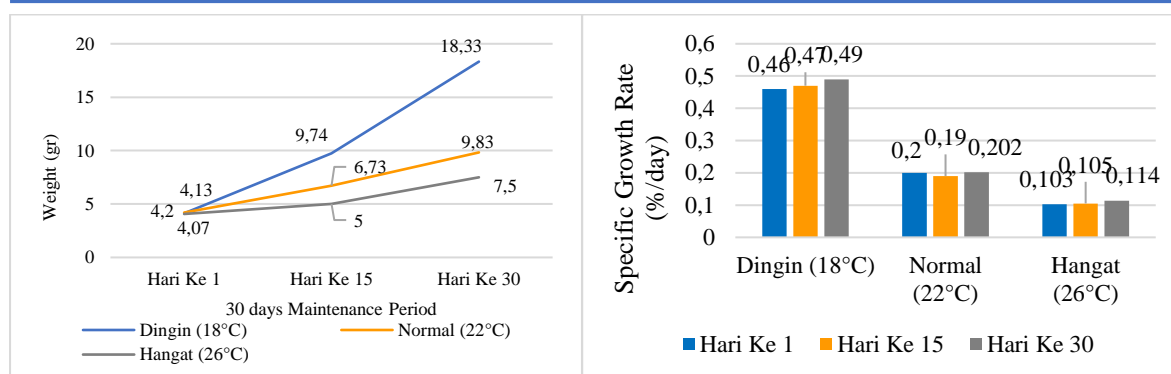


Figure 1. Growth of Axolotls Maintained at Different Temperatures. a. Weight Growth, b. Specific Growth Rate of Axolotls

The relationship between temperature and specific growth rate shows a quadratic regression pattern with the equation  $Y = 149.85 - 11.403x + 0.2279x^2$  and coefficient  $R^2 = 0.995$  (Figure 2). The coefficient value shows that the difference in temperature has an effect on the specific growth rate of axolotls. Treatment of temperatures from 18°C to 26°C appears to decrease the specific growth rate. The highest specific growth rate point on the quadratic curve is at  $x = 18^\circ\text{C}$  and  $y = 0.49\%$ .

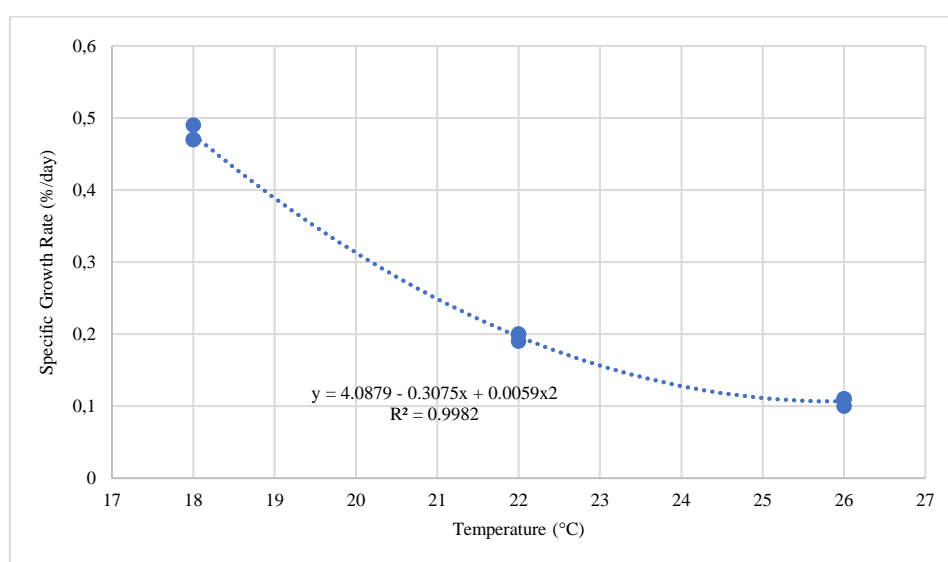


Figure 2. Graph of the Relationship Between Temperature and Specific Growth Rate of Axolotl

### Feed Conversion Ratio

The results of this study indicate that the higher the temperature of the maintenance water, the greater the feed conversion ratio value. Data on the feed conversion ratio in axolotl maintenance can be seen in Figure 3. The best feed conversion ratio value is in the cold temperature treatment which reaches an average of 1.48 and the worst value is in the warm temperature treatment (26°C) with an average of 4.12. This means that at cold temperatures, axolotls need 1.48 grams of feed to gain 1 gram of weight. While at warm temperatures (26°C), 4.12 grams of feed are needed.

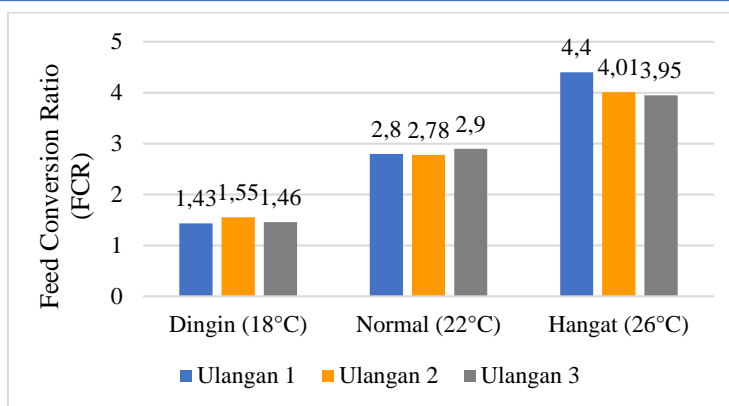


Figure 3. Axolotl Feed Conversion Ratio at Different Temperature Conditions

The results of the ANOVA test illustrated a significant effect of temperature treatment on the feed conversion ratio with a significance value below 0.05. Furthermore, the Turkey HSD post-hoc test showed a significant difference between each treatment.

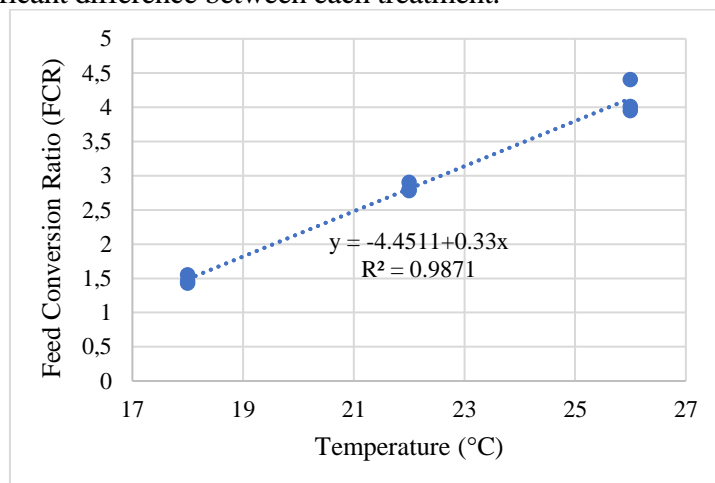


Figure 4. Graph of the Relationship Between Temperature and Axolotl Feed Conversion Ratio

Based on the graph in Figure 3, it is found that the relationship between temperature and feed conversion ratio shows a linear regression pattern with the equation  $Y = -4.145 + 0.33x$  and coefficient  $R^2 = 0.9871$ . The coefficient value shows that the difference in temperature has an effect on the specific growth rate of axolotls. The temperature treatment of 18°C to 26°C appears to decrease the specific growth rate. The highest feed conversion ratio point on the quadratic curve is at  $x = 26^\circ\text{C}$  and  $y = 4.4$ .

### Survival Rate

The highest survival rate is in the aquarium with a cold temperature with a survival rate of 100%. The lowest survival rate is in the aquarium with a warm temperature, with an average of 30%. Data on the axolotl survival rate can be seen in Figure 5.

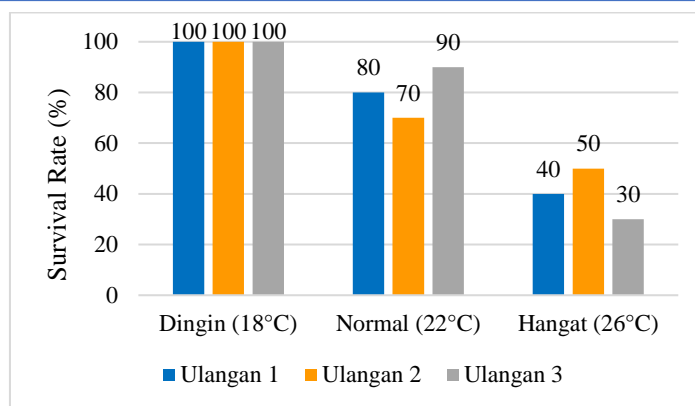


Figure 5. Axolotl Survival at Different Media Temperature Treatments

The results of the ANOVA test showed that there were differences in the average for each treatment. Based on the graph in Figure 6, it was found that the relationship between temperature and survival rate showed a linear regression pattern with the equation  $Y = 238.33 - 7.50x$  and the coefficient  $R^2 = 0.949$ . The coefficient value shows that the difference in temperature has an effect on the survival rate of axolotls. The temperature treatment of 18°C to 26°C showed a decrease in the specific growth rate. The highest specific growth rate point on the quadratic curve was at  $x = 18^\circ\text{C}$  and  $y = 0.100\%$ .

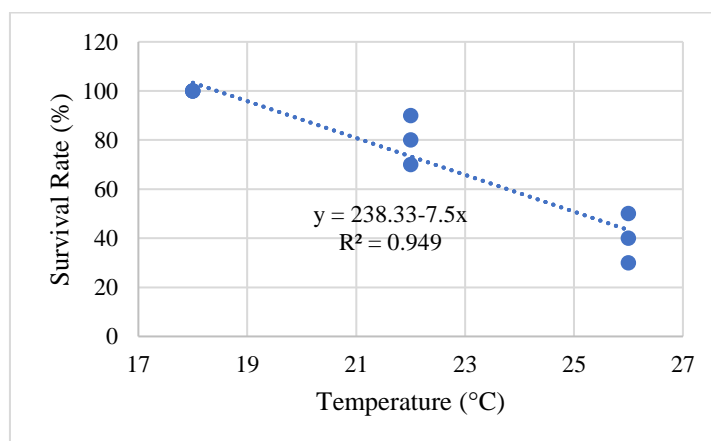


Figure 6. Graph of the Relationship Between Temperature and Axolotl Survival Rate

## Water Quality

The water quality of axolotl maintenance is always controlled to maintain the health and growth of axolotls. Several water quality parameters measured during the research period are temperature, pH, nitrate and ammonia. The treatment temperature is always kept stable according to the design that has been given. There is an insignificant increase and decrease in temperature with a range of between 0.1 - 0.7°C. The pH parameters in the axolotl maintenance water media are in the optimal pH range, which is between 7.5 and 8.4.

The concentration of nitrate in the maintenance media is in a range that can still be accommodated by axolotls, which is between 0 mg/L to 25 mg/L. Although there are differences in each treatment, the differences are not too significant. The highest nitrate levels are found in the normal and warm temperature treatment aquariums. The increase and decrease in nitrate levels are influenced by the siphon process and the routine water change process. The results of nitrate level measurements carried out during the maintenance period showed normal results. These levels can still be tolerated by axolotls. These levels are still optimal for axolotl growth.

The concentration of ammonia in the experimental aquarium showed a value between 0 - 0.25

mg/L. The increase in the amount of ammonia in the media is due to dirt and uneaten food residue. In all treatments, there was a change in ammonia levels caused by a decrease in the ability of the filter that had decreased.

## DISCUSSION

### Growth and Specific Growth Rate

The highest specific growth rate value was obtained in the cold temperature treatment (18°C) with an average of  $\pm 0.473\%$ , followed by the normal treatment (22°C) with an average of  $\pm 0.201\%$ , and the lowest in the warm temperature treatment (26°C) with an average of  $\pm 0.107\%$  (Figure 1). These results are in line with research conducted by Irwin *et al.*, (1998), where axolotls raised at cold temperatures ( $<18^{\circ}\text{C}$ ) grew faster than axolotls raised at warm temperatures ( $>25^{\circ}\text{C}$ ). The specific growth rate in axolotls raised at cold temperatures was 0.06%, with an initial weight of 6 grams and a final weight of 2 grams. Meanwhile, the specific growth rate of axolotls raised at warm temperatures is 0.03%, with an initial weight of 4 grams and a final weight of 5 grams (Irwin *et al.*, 1998).

The original habitat of axolotls is located on the American continent where the temperature changes each season are not too large and with relatively colder temperatures. Other species of the same genus are also widespread in cold polar regions. Temperature greatly affects various chemical reactions in water bodies, including the solubility of oxygen in water and the metabolism of fish, so that it will affect fish growth. According to Kelabora (2010) temperature is a factor that greatly influences the growth of aquatic organisms, including axolotls. Fish have a certain optimum temperature for their growth. Metabolism in the axolotl's body will be optimal at cold temperatures, so that the metabolism in the axolotl's body will be used for growth.

Temperature can affect biological processes in the body such as metabolism, growth and reproduction (Catenazzi, 2016). Just like other Caudata orders, axolotls are very sensitive to temperature changes. Water temperature greatly affects the growth and development of axolotls. Axolotls are cold-blooded animals that cannot adjust their body temperature to the temperature of the water where they live. Axolotls are very dependent on water temperature, if axolotls live at low water temperatures, their metabolism will decrease and they cannot digest food perfectly. Conversely, if they live at higher temperatures, axolotls will increase their appetite and cause a wasteful energy burning process. High temperatures can cause axolotls to become stressed quickly and shorten their lifespan (C. R. J. Figiel, 2023).

### Feed Conversion Ratio

Quality feed will directly have a positive effect on axolotl growth. The feed conversion ratio value is one of the parameters of feed quality and the ability of fish to digest the feed given during the maintenance period. The lower the feed conversion ratio indicates the better the growth rate of the axolotl. The ability of axolotls to digest and absorb nutrients from the feed given is highly dependent on the metabolic process. Meanwhile, cell metabolism is influenced by temperature factors.

In cold temperature treatment, a relatively low feed conversion ratio value was obtained, this is because the growth rate of axolotls at certain temperatures is not optimal. So that axolotl feeding will be more effective at low temperatures. Media temperature is an important factor for fish in absorbing feed into biomass. Feed will be digested in the fish's body. Nutrients from feed will be used to build tissue and flesh, so that fish growth will be maximized (Ridwantara *et al.*, 2019).

### Survival Rate

Increasing the temperature of the maintenance media causes a decrease in the survival rate of axolotls. At warm temperatures, there are cases of death which are suspected to be caused by axolotls not being able to survive at relatively warm temperatures. These results are

in accordance with observations made by (C. R. J. Figiel, 2023) axolotls kept at warm temperatures (27°C) become stressed and underweight. At this temperature, several axolotls die. This is because the metabolism that occurs in the axolotl's body increases while the available food is limited, so that the axolotl is stressed and can cause death in the axolotl.

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### **Water Quality**

According to Khattak *et al.* (2014), axolotl growth will be optimal at relatively cold temperatures (18-20°C). The results of pH measurements show that the acidity of the water still meets the requirements that are suitable for axolotls ranging from 7-8 (Khattak *et al.*, 2014). The optimal nitrate levels in axolotl rearing activities are below 100 mg/l (Khattak *et al.*, 2014). According to Djokosetiyanto *et al.*, (2006), limited water can experience a decrease in water quality. To maintain water quality, a recirculation system can be used. Waste water from the maintenance process can be reused after going through the filtration stage, namely, mechanical and biological, the results of ammonia level measurements carried out during the maintenance period showed high results. These levels exceed normal limits. Ammonia levels in axolotl rearing activities must be maintained at levels below 0.25 mg/l (Khattak *et al.*, 2014).

### **CONCLUSION**

The conclusion that can be drawn based on the results of this study is that low temperatures can support the growth of axolotls to be more effective. A good temperature for axolotl enlargement at a temperature treatment of 18°C. The highest specific growth is found in cold temperature treatment. The use of URCS can increase the growth rate of axolotls, due to the temperature manipulation system used by URCS.

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