

## Stages of Gurami (*Osphronemus gouramy*) Embryogenesis at Different Temperature for 48 Hours

### Tahapan Embriogenesis Telur Ikan Gurami (*Osphronemus gouramy*) Pada Suhu Yang Berbeda Selama 48 Jam

Samsul Hadi, Muhammad Junaidi\*, Dewi Putri Lestari

Aquaculture Study Program, Mataram University

Pendidikan Street umber. 37 Mataram. West Nusa Tenggara

\*Corresponding author : m.junaidi@unram.ac.id

#### ABSTRACT

Gouramy (*Osphronemus gouramy*) is a type of freshwater fish that has high economic value and is much loved by the wider community. In aquaculture activities, carp hatchery plays an important role in meeting the needs of seeds, especially in the process of hatching eggs. The problems that are often encountered in hatching carp are low egg hatchability and low larval survival rates. In the larval phase, carp are still vulnerable to environmental changes, one of which is temperature, so it needs to be considered in the process of hatching carp eggs. Temperature is something that needs to be considered in hatching carp eggs, therefore it is necessary to manipulate the temperature in the container so that the temperature is more constant. The purpose of this study was to determine the effect of temperature on egg hatching and growth of carp (*Osphronemus gourami*) larvae. The test material used was carp (*Osphronemus gourami*) eggs with a total of 900 eggs used. This study used an experimental method with a completely randomized design with 5 treatments and 3 replications. Treatments in this study: P1 (28°C), P2 (29°C), P3 (30°C), P4 (31°C) and P5 (32°C). The observed data included survival rate, hatching rate, length of hatching, absolute length, and specific growth rate. The results showed that temperature differences had a significant effect on egg hatching time, absolute length, survival and specific growth rate, while no significant effect on carp (*O. gouramy*) egg hatchability. The E 32oC treatment was the best treatment which produced 22 hours of hatching, 96.67% hatchability, 91.35% survival rate, 1.47 cm absolute length growth and 4.42% SGR.

#### ABSTRAK

Ikan gurame (*Osphronemus gouramy*) merupakan salah satu jenis ikan air tawar yang memiliki nilai ekonomis tinggi dan banyak digemari oleh masyarakat luas. Dalam kegiatan budidaya, pembenihan ikan gurame sangat berperan penting terhadap kebutuhan benih, terutama dalam proses penetasan telur. Permasalahan yang sering dihadapi dalam melakukan penetasan ikan gurame yaitu daya tetas telur dan tingkat kelulushidupan larva

yang rendah. Pada fase larva, ikan gurame kondisinya masih rentan pada perubahan lingkungan salah satunya adalah suhu, sehingga perlu diperhatikan dalam proses penetasan telur ikan gurame. Suhu adalah hal yang perlu diperhatikan dalam penetasan telur ikan gurame, oleh karena itu perlu dilakukan manipulasi suhu dalam wadah agar suhu lebih konstan. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh suhu terhadap penetasan telur dan pertumbuhan larva ikan gurame (*Osphronemus gourami*). Bahan uji yang digunakan adalah telur ikan gurame (*Osphronemus gourami*) dengan total telur yang digunakan sebanyak 900 butir. Penelitian ini menggunakan metode eksperimental dengan Rancangan Acak Lengkap 5 perlakuan 3 ulangan. Perlakuan dalam penelitian ini: perlakuan suhu P1 (28°C), P2 (29°C), P3 (30°C), P4 (31°C) dan P5 (32)°C. Data yang diamati meliputi survival rate, hatching rate, lama penetasan, panjang mutlak, dan spesifik growt rate. Hasil penelitian menunjukkan bahwa perbedaan suhu berpengaruh nyata terhadap lama penetasan telur, panjang mutlak, kelulushidupan dan spesifik growt rate, sedangkan tidak berpengaruh nyata terhadap daya tetas telur ikan gurame (*O. gouramy*). Perlakuan E 32°C adalah perlakuan yang terbaik yang menghasilkan penetasan 22 jam, daya tetas telur 96,67% kelulushidupan 91,35%, pertumbuhan panjang mutlak 1,47 cm dan SGR 4,42%.

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<b>Kata Kunci</b>	<i>Ikan gurami, Lama Penetasan, Daya Tetas Telur, Kelulushidupan</i>
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## INTRODUCTION

Gourami fish (*Osphronemus gouramy*) is a type of freshwater fish native to Indonesia which has long been cultivated and consumed by the public because of the delicious taste of its meat, so it has high economic value (Sari, 2019). This is proven by the price of gourami fish which is relatively expensive and relatively stable. According to (Kholifah, 2015) the selling price of gourami fish reaches IDR 36,000 - IDR 40,000 / kg, in terms of this price, farmers are increasingly developing their cultivation businesses, so the need for seeds is also increasing.

Gourami fish hatcheries play a very important role in the need for seeds, especially in the egg hatching process. The problems often faced in hatching gourami fish are the low hatchability of eggs and the low survival rate of larvae. Temperature is the most important thing to pay attention to when hatching gourami fish eggs, therefore it is necessary to manipulate the temperature in the control container so that the temperature is more constant. When hatching gourami fish eggs, it is necessary to check the water quality because it will affect the hatchability of the eggs. According to (Aer et al., 2015) temperature can influence various life activities and affect the oxygen dissolved in water, the higher the temperature the lower the solubility of oxygen in water. This research continues research (Hardaningsih et al., 2016) on the influence of water temperature fluctuations on the length

of egg hatching and survival of gourami larvae. And research (Pratama et al., 2018) on the effect of temperature on egg hatching time, egg hatchability, survival and growth of gourami fish (*O. gouramy*) research conducted with temperature treatments of 32°C, 30°C and 28°C.

The aim of this research was to determine the effect of temperature on egg hatching and growth of gourami fish larvae (*O. gouramy*). The results of the research are expected to be able to provide information about the best temperature that can influence egg hatching time, hatching rate, absolute length, SGR and survival rate of gourami fish (*O. gouramy*) to fish farmers in general and to students in particular.

## **METHODS**

### **Research sites**

This research was conducted in July - August 2021 at the Aquaculture Laboratory, Aquaculture Study Program, Faculty of Agriculture, Mataram University.

### **Research Materials**

Bahan yang digunakan pada penelitian ini adalah toples ukuran 25L, heater, aerator dan selang aerator, alat tulis, kamera, thermometer, mikroskop, hand tally counter, sendok, baskom, serok, telur ikan gurame dan cacing sutra.

### **Research design**

The research design used was a completely randomized design consisting of 5 treatments and 3 replications, so that 15 experimental units were obtained. The test material used was 900 gourami fish eggs (*O. gouramy*). Total egg density 60 eggs/treatment. Determination of treatment is based on temperature differences in each treatment, namely treatment (A) 28°C, treatment (B) 29°C, treatment (C) 30°C, treatment (D) 31°C, treatment (E) 32°C by installing a water heater in each container used. The container used in the research was a jar with a volume of 25 liters. Quality checks are carried out every 10 days, namely DO and pH, while temperature checks are carried out every day.

### **Container Preparation**

The jar used is cleaned first using detergent, rinsed until clean, then dried. Water is put into a fiber tub and equipped with aeration. The water medium is first settled for 1 day before being used as a test medium with the aim of settling the particles in the water. Next, the containers are filled with 10 liters of water each. After that, aeration is introduced as an oxygen supplier and supporter in the process of hatching gourami fish eggs. Next, arrange other tools such as a heater to control the temperature. Next, adjust or regulate the water temperature with treatment (P1 28°C, P2 29°C, P3 30°C, P4 31°C, and P5 32°C).

### **Test Fish Preparation**

The eggs used in this research came from one parent obtained from the Batu Kumbung Fish Seed Center (BBI), Lingsar District, West Lombok Regency. The eggs used in this research were 900 eggs. The eggs used are counted using a spoon. While transferring, good quality gourami eggs are separated from bad quality eggs. The two can be differentiated by looking at their color. Good gourami eggs are dark yellow, while bad gourami eggs are white. Once counted, put it in a plastic bag. Eggs are transported in the afternoon to avoid high temperature fluctuations so that the eggs can survive. Eggs are packaged using plastic bags filled with oxygen with a water to oxygen ratio of 1/2:1/2. The egg bag was put in styrofoam and then taken by motorbike to the research location with a travel time of around 25 minutes. The initial stage that was carried out after arriving at the research location was to acclimatize

the eggs by floating a plastic container above a 30L volume basin for approximately 20 minutes and adjusting the temperature of the water in the plastic bag to the temperature of the water at the research location so that the eggs could adapt well.

### **Research Implementation**

Fish rearing is carried out for 30 days to see the growth and survival rate of gourami fish. During maintenance, waste and remaining fish food are removed and the water is replaced by 30% (depending on water conditions), then the water is refilled in proportion to the water released. Siphoning and changing the water is done once every two days.

### **Research Parameters**

#### **Hatching Time**

To obtain egg hatching time, it is known by recording the time after fertilization until the egg hatches into the earliest larva ( $t_0$ ) and the egg hatches completely ( $t_n$ ).  $t_0$  is the time required for the first larva to emerge, while  $t_n$  is the time required for the eggs to completely hatch.

#### **Egg Hatching Data**

According to Efrizal in (Arunde et al., 2016) to calculate the hatchability of eggs, the following formula is used:

$$Hr (\%) = \frac{\sum \text{telur yang menetas}}{\sum \text{telur yang ditebar (sampel)}} \times 100\%$$

### **Observation of Embryo and Larva Development**

The development phase of the embryo is observed under a microscope by taking the embryo using tweezers and then placing it on a concave glass slide. Time observations were made at each phase transition. Embryo development is recorded and documented, especially at certain phases such as cell division, morula, blastula, gastrula, organogenesis and hatching.

Observation of larval development was carried out macroscopically by observing morphological characteristics, larval behavior, growth in total length on days 0, 10, 20 and 30 after hatching. The type of feed given during rearing uses natural food and rearing is carried out using the same temperature at the time of egg hatching (Herjayanto, 2017).

### **Growth**

Seed growth analyzed was absolute growth (mm), relative growth (%), and daily specific growth rate (% per day). Absolute growth is determined using the formula according to (Effendi, 1997):

$$\alpha = L_t - L_0$$

Note:  $\alpha$  : Absolute length growth (mm)

$L_t$  : Length at the end of the experiment (mm)

$L_0$  : Length at the start of the experiment (mm)

The specific growth rate is calculated using the formula from (Zooneveld et al., 1991), namely:

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\%$$

Note :

SGR = Daily growth rate (%)

Wo = Test animal weight at the start of the study (g)

Wt = Test animal weight at the end of the study (g)

t = Research time (day)

### Survival Rate (SR)

The survival rate of the gourami fish was observed every day until the end of the treatment. Survival calculations were carried out at the end of treatment, namely day 30. SR calculations use the following formula (Effendi, 1997) :

$$SR = \frac{N_t}{N_o} \times 100\%$$

Note :

SR : Survival rate (%)

No : number of cultivars at the start of the study.

Nt : number of cultivars at the end of the study.

## RESULT AND DISCUSSION

### Hatching Time

Based on the results of research that has been carried out, according to statistical tests, the fastest egg hatching time is in P5, namely 31 hours, followed by P4 35 hours, then P3 39 hours, then P2 39 hours, and the longest egg hatching is in P1 with 41 hours. The statistical test results showed that all treatments were significantly different ( $P < 0.05$ ) except P2 and P3 ( $P > 0.05$ ).

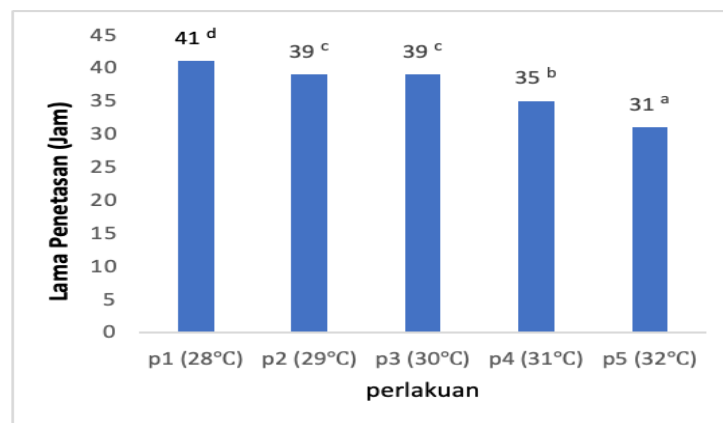


Figure 3. Hatching Time

Egg hatching is the stages of egg development until the egg hatches. Gourami fish eggs will hatch in around 22 - 48 hours at a natural temperature of around 28°C - 32°C. In this study, the fastest hatching time was at P5, namely 31 hours. It is thought that the faster the hatching rate at P5, the higher the temperature, the faster the development of the gourami egg phase. Based on research results (Renita, 2016), the fastest average egg hatching time is at a temperature of 32°C. This occurs when there is a striking change in temperature which can affect the metabolic process because at high temperatures the metabolic speed will decrease in accordance with the working mechanism of enzymes. According to (Hardiningsih

et al., 2008) a change in temperature of 10°C can affect changes in metabolic reactions in the body by 10%. Furthermore (Putri et al., 2013) said that at high temperatures the egg hatching process is faster so that the metabolic process can occur more quickly which means that embryo development will also be faster and the development of the embryo in the shell will be more intensive so that hatching will be faster. According to (Olivia et al., 2013) enzyme activity can influence the egg hatching process. Enzymes are able to work at optimal temperatures and have temperature limits. Temperatures that are too high will cause denaturation and enzyme activity will be disrupted so that the concentration and speed of the enzyme are reduced. Likewise, vice versa when at low temperatures. Based on the results of research that has been carried out, P5 (32°C) is the fastest egg to hatch, namely 31 hours.

### **Hatching Rate (HR)**

Based on the results of research that has been carried out, statistically the highest value is P3 at 96.67%, followed by P4 at 93.89%, then P5 at 93.33%, then P2 at 88.89% and the lowest value is at P1 amounting to 78.89%. P3 is not significantly different from P4 and P5 but is significantly different from P2 and P1.

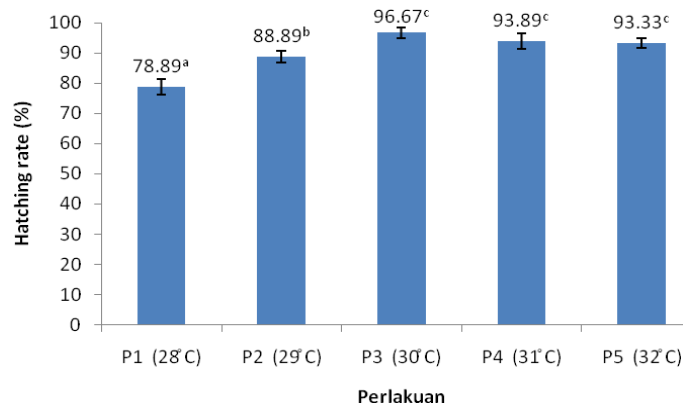


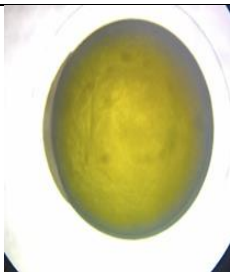
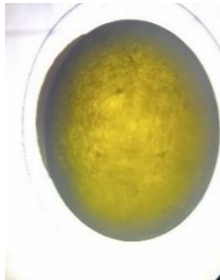
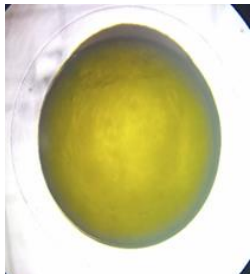
Figure 4. Hatching Rate

Based on the research results, the highest hatchability value was found at P3 at 96.67% and the lowest value was at P1 at 78.89%. The high hatching rate value at P3 is thought to be because the higher the temperature given, the higher the hatchability of the eggs with a temperature range of 30°C-32°C. According to research conducted by (Sugihartono, 2017), the highest hatchability value for gourami fish eggs was found at a temperature of 30°C at 98.05%. Furthermore, research results (Alia et al., 2019) show that the highest hatchability of eggs is at a temperature of 32°C at 98.83%. However, the research results showed that the temperature of 30°C-32°C was not significantly different between treatments. In this research, treatment using high temperatures (32°C) can speed up the incubation period so that eggs can hatch quickly, while low temperatures (28°C) will slow down the egg hatching period. According to (Hutagalung et al., 2016) the hatchability of eggs is influenced by internal and external factors. Internal factors are caused by the parent source used, resulting in incomplete fertilization. Meanwhile, external factors are caused by water quality that is not suitable for hatching fish eggs. Apart from that, egg death can also occur because the embryo is unable to carry out metabolism to develop. According to (Sugihartono, 2010) water temperature is the main factor in the success of hatching gourami fish eggs.

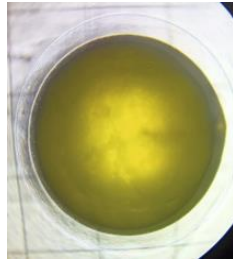
From the results of this research that has been carried out, the temperature factor has a real influence on the hatchability of eggs. This condition is in accordance with the results of research that has been carried out that the temperature considered better for hatching eggs is between 30°C - 32°C. If the temperature drops below this limit, the success rate of egg hatching will be low. At very low temperatures, namely below 28°C, egg development does not run smoothly and can cause the eggs to be attacked by fungus. Research results (Sugihartono, 2010) show that at a media temperature of 26°C, a low egg hatching rate of 72.22% was obtained. At very low temperatures, the process of embryonic development in eggs is less than perfect and the embryos cannot adapt any longer, causing the embryos to die, especially when they are ready to hatch. (Juniardi, 1994) states that at the beginning of egg development what is important is that the temperature should not be too low or too high.

### Observation of Gourami Fish Egg Embryos

Table 1. Observations of gourami fish embryos and larvae

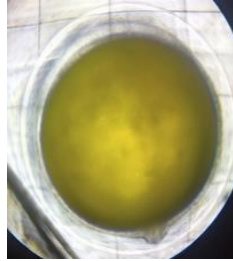
No	Time	Figure	Note
1	1 hour		Division of 2 cells
2	2 hour		Morula phase
3	3 hour		Blastula phase

4 12 hour



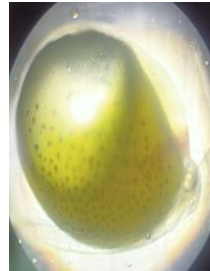
Gastrula phase

5 21 hour



Organogenesis phase

6 31 hour



Hatching phase

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Based on Figure 1, it can be seen that egg cell division begins in the first hour. According to (Hutagalung, 2016). Eggs generally undergo a process of embryogenesis, namely the process of egg development until it becomes a definite larva. Embryogenesis will take place during incubation starting from the process of cell division, morula, blastula, gastrula and continued with organogenesis which then hatches. Cell division (cleavage) is the process of cell division during embryonic development, the size of the cells becomes smaller and smaller over time or becomes small units called blastomeres.

Furthermore, at the 2nd hour after fertilization, the egg enters the morula phase, this phase is marked by the fusion of the blastomeres at the animal pole (Alawi, 1994). Morula is cell division that occurs after the cell has 32 cells and ends when the cell has produced a number of blastomeres of the same size but smaller in size. The morula stage ends when blastomeres have been produced.

Blastula is the process of changing the cells attached to the egg yolk by forming a plasma extension to the inside so that it is like a layer under an inverted bowl. This layer is called periblast or trophoblast which is related to egg yolk. The cavity inside which is shaped like this is called blastocoels. Blastula is composed of a mixture of blastomere cells in a cavity filled with fluid (Hutagalung et al., 2016). Based on the picture, the process of blastula formation occurs in the 3rd hour after the fertilization process takes place.

In this study, the gastrula phase occurred at the 13th hour. According to Sukra (1989) in (Hutagalung, 2016) the gastrula stage in fish begins with thickening at the outer edge of



the blastodisk, so that it is shaped like a circle or like a ring, usually called a sprout ring. The thicker posterior sprout ring is called the sprout ring shield.

The next phase is the Organogenesis phase which occurs 21 hours after organogenesis is the formation of organs. In line with the process of embryo formation or embryogenesis, the process of forming the embryo's body organs occurs, which is called organogenesis. In the process of organogenesis, organ buds are formed successively, including nerves, notochord, eyes, somites, kuffer cavity, olfactory sac, kidney cavity, intestine, subnotchord bone, linear litralis, aorta, gills, infundibulum, and fin folds (Tang and Ridan, 2004).

Hatching at 31-41 hours after fertilization according to (Blaxter, 1969) apart from being caused by softening of the chorion by enzymes, hatching can also be caused by movements due to increased temperature, light intensity or absorption of oxygen pressure. Hatching can occur due to two things, namely mechanical work and enzymatic work. Mechanical work, namely the embryo often changes its position due to lack of space in the shell or because the embryo is longer than its shell environment, while enzymatic work, namely enzymes and other chemical elements released by the endodermal glands in the pharynx area of the embryo.

### Absolute Length

Based on the results of research that has been carried out, statistically the highest value for absolute length is P5 at 1.47 cm, followed by P4 at 1.42 cm, then P3 at 1.33 cm, then P2 at 1.32 cm and the lowest value found at P1 1.27 cm. The results of further tests showed that P5 was significantly different from P3, P2, and P1 ( $P < 0.05$ ) but not significantly different from P4 ( $P > 0.05$ ), P4 was significantly different from P1 but not significantly different from P2, P3 and P5.

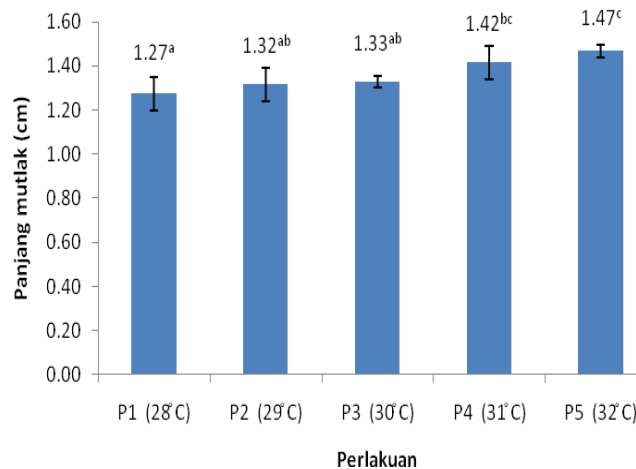


Figure 5. Absolute Length

Growth is a change in length, weight and volume in a certain period individually. Several factors that can influence fish growth include internal factors including heredity, gender and age. Meanwhile, external factors include temperature, cultivation media and fish food (Effendi, 1997). Measurements were carried out at the beginning of hatching using SRC (sedgewick rafter cell) and measurements were carried out 4 times for 30 days, namely days 0, 10, 20 and 30. From the age of 10 days measurements were made using millimeter blocks

until the 30th day. Based on the research results, the highest absolute length was at P5 at 1.47 cm and the lowest was at P1 at 1.27 cm. It is assumed that the metabolic process occurs more quickly at a temperature of 32°C compared to a temperature of 28°C. So that the consumption of nutrients by the larvae is faster in meeting the needs of the metabolic process. These results show that temperature affects fish growth, because temperature affects fish metabolism so that each certain temperature range produces a different absolute length. According to (Pratama, 2018) high temperatures cause the work of enzymes in the body to increase so that the body's metabolism and appetite also increase. According to (Andrianto, 2013) cold temperatures can slow down cell activity, thereby inhibiting growth.

### Spesifik Growth Rate (SGR)

Based on the results of research that has been carried out, statistically the highest value is P4 at 4.42%, followed by P5 at 3.86%, then P2 at 3.81%, then P3 at 3.57%, and the lowest value is at P1 it is 3.47%. The results of statistical tests with further tests showed that P4 was significantly different from all treatments.

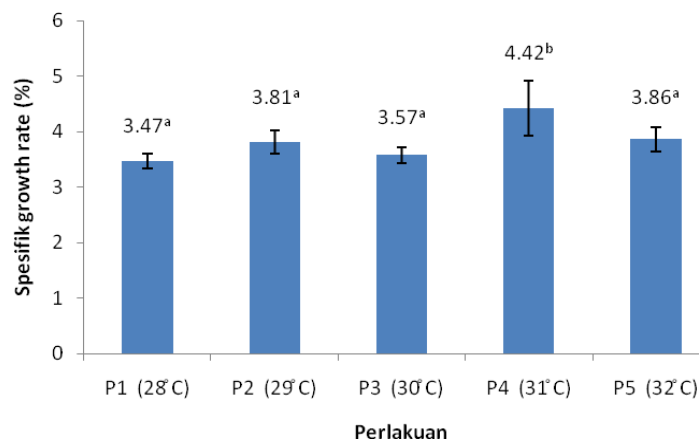


Figure 6. Spesifik Growth rate

Based on the research results, the highest SGR value was found in P4 at 4.42%/day and was significantly different between treatments. The high value of P4 is thought to be the higher the temperature, the higher the SGR value. However, temperatures that are too high will inhibit growth so that in this study the P5 treatment saw a decrease in the SGR value. Apart from that, during the research they were given natural food in the form of silk worms (*Tubifex* sp). Silk worms are given when the egg yolk runs out, namely on the 7th day after hatching. According to (Abidin, 2009) the decrease in fish weight is thought to be caused by disruption of physiological processes and fish behavior. The decrease in growth rate and absolute length is due to the transfer of energy. In general, the energy from the feed consumed will be used for maintenance energy and the remainder for growth energy. Furthermore (Pratama et al, 2018) temperature affects fish metabolism so that each certain temperature range produces a different SGR. High temperatures cause the work of enzymes in the body to increase so that metabolism in the body and appetite also increase. Apart from that, research results (Hardiningsih et al, 2008) show that increasing temperature causes enzyme work to increase and slowing down when water temperature decreases. The

metabolic process runs faster when the temperature is 32°C compared to the temperature of 30°C so that the larvae consume more nutrients to meet the needs for the metabolic process.

### Survival Rate

Based on the results of research that has been carried out, statistically the highest value is found in P3 at 91.35%. followed by P2 at 90.65%, then P4 at 89.94%, then P5 at 89.83%, and the lowest value is P1 at 81.81%. The results of statistical tests with further tests show that P3 is significantly different from P1 but not significantly different from P2, P4 and P5.

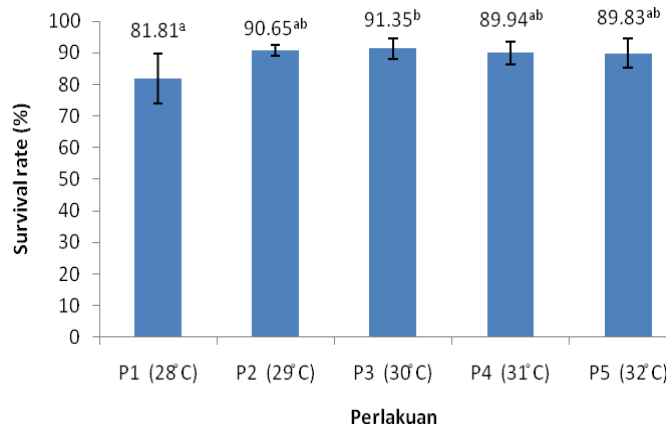


Figure 4. Survival Rate

SR observations were carried out from the time the eggs hatched until they were 30 days old by checking every day and looking at dead fish and recording the number of dead fish. Based on the research results, the highest SR value is in P3 at 91.35% and the lowest value is at P1 at 81.81%. The high P3 value is thought to be the temperature used which is the optimal temperature range for the survival of gourami larvae seeds. According to (Ardianty et al., 2013) optimal for the growth of gourami fish is 29°C – 32°C. According to (Landsmen et al., 2011) high mortality of eggs and larvae is related to decreasing temperature and too high temperature, which is thought to be closely related to a high metabolic rate so that the energy required is higher. The results of research conducted by (Adriana et al., 2013) showed that the highest survival percentage was at a temperature of 30°C at 89.87%. Another factor that causes high survival is determined by the availability of food. According to (Renita 2016) the food used will increase survival and growth. Apart from that, environmental conditions also affect the survival of fish. Because fish are cold-blooded animals (poikilothermal) where body temperature is influenced by the environment so that their metabolism and immunity depend on environmental temperature. The fish's immune system can also influence survival rates. According to (Hernawati and Suantika 2007) stated that a fish's weakened immune system will cause stress and disease, causing death.

### CONCLUSION

Based on the research that has been carried out, it can be concluded that the effect of temperature differences on egg hatching and growth of gourami (*O. gouramy*) larvae has a

significantly different effect on egg hatchability (HR), hatching time, growth (SGR), survival (SR), and absolute length.

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