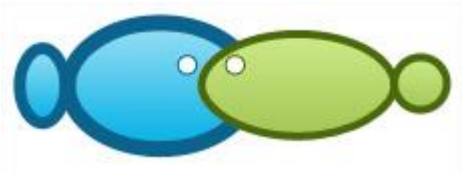

The effect of different salinity levels on the production performance and physiological response of juvenile gold-mouth snail *Turbo chrysostomus* Linnaeus, 1758

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Abstract. Nowadays, the excessive use of the gold-mouth snail (*Turbo chrysostomus* Linnaeus, 1758) is feared to interfere with its sustainability. Therefore, a proper culture technique is required to obtain a suitable condition for the survival and growth of *T. chrysostomus* and one important parameter is the salinity level. This study aimed to analyze the effect of salinity on the physiological responses and production performance of *T. chrysostomus*. A completely randomized design consisting of four different salinity treatments, i.e., 26 ppt, 29 ppt, 32 ppt, and 35 ppt with four replications in each treatment, was used in this study. The results showed that salinity significantly affected the tested parameters – i.e., survival rate, absolute shell growth, shell-specific growth, hemolymph cell fragility value, and body glucose level value of *T. chrysostomus*. Treatment with 35 ppt salinity was the optimal salinity level among tested treatments.
Key Words: cell fragility, glucose, growth, osmoregulation.

Introduction. The gold-mouth snail (*Turbo chrysostomus* Linnaeus, 1758) is an invertebrate aquatic organism from the gastropod class that can be found in the Eastern Indonesia. This species lives around the coral reef in the intertidal areas which have a rocky to muddy type of substrate (Susintowati et al 2019), similarly to *T. marmoratus*, *Trocus niloticus* (Soekendarsi 2018), and *T. bruneus* (Sanpanich & Duangdee 2013). The utilization rate of this species continues to increase because *T. chrysostomus* can be easily caught (Hamzah 2015). Excessive exploitation will negatively impact on genetic diversity and cause growth disturbance (Saleky et al 2016), leading to decreasing stock, which can threaten its sustainability in the nature. The availability of *T. chrysostomus* in nature become scarce because price of their shells is relatively high. According to the Indonesian Institute of Sciences (LIPI), the selling price of *T. chrysostomus* shells in 2019 was around 25 USD to 40 USD per kilogram. Its maintenance until harvesting period can take up to eight years, which results in a culture activity as a solution to maintain stock availability of this species, especially in Indonesia (Hamzah et al 2021).

The culture of *T. chrysostomus* is one of the efforts to preserve its resource potential. The success of the culture is highly dependent on water quality condition and substrate as a maintenance medium. These factors affect the growth and health of the cultured biota. The culture of aquatic biota is influenced by genetic factors, osmoregulation, feed quality, growth media, and water quality (Jumaisa et al 2016). Physiologically, water quality has an important role in the development of gastropods that successfully pass through the embryonic stage. The development and growth of organisms such as shrimp, clams, abalone, pearls, and other types of snails at the

juvenile stage are most susceptible to environmental influences (Baticados & Tendencia 1991; Burks et al 2011; Denic et al 2015; Garr et al 2011; Greiner et al 2018; Hamzah et al 2012). One of the water quality parameters that strongly affect the survival of cultivated biota is salinity (Ainis 2014; Cheng et al 2002). Gastropods are vertically and horizontally affected by salinity (Imamsyah et al 2020). Alternative culture business, starting from the hatchery process to the maintenance process of *T. chrysostomus* for production scale, needs to be done. For this purpose, optimal growth conditions must be known, and salinity is one of them.

Seawater salinity level depends on evaporation and rainfall (Awaluddin et al 2013). Each marine biota requires a specific salinity level. Salinity directly affects the metabolic processes of body, especially the osmoregulation (Yuliani et al 2018). Higher salinity can result in a faster growth rate of *T. chrysostomus* body weight (Hamzah 2016). A sudden decreased salinity causes animals to have difficulty in regulating their osmoregulation so that it can result in death (Rachmawati et al 2012). In mollusks, fluctuations in salinity can increase or decrease the osmoregulatory ability of the hemolymph, which causes shrinkage and swelling of cells through regulation of body fluids so that it can affect their growth, development, and survival (Amin et al 2016). A precise environmental condition will support the optimal physiological process in the cultured organisms, which will impact on the maximum production performance (Taqwa et al 2018).

Information on *T. chrysostomus* cultivation techniques at the juvenile stage has not been reported, especially regarding the effect of salinity on the physiological response and production performance of *T. chrysostomus* at the juvenile stage. Therefore, this present study objective is to analyze the effect of salinity on the cultivation of *T. chrysostomus*, especially at the juvenile stage.

Material and Method

Time and location of research. Observations of *T. chrysostomus* were carried out for 90 days, starting from November 2019 to February 2020 at the Marine Bio Industry, Indonesian Institute of Science (BBIL LIPI) Mataram, West Nusa Tenggara, Indonesia. Analysis of hemolymph cell fragility and body glucose levels were analyzed at the Fish Nutrition Laboratory, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University in March 2020.

Material. The biota used was two months old *T. chrysostomus* with an average length of 1.65±0.26 mm and a width of 1.39±0.24 mm (Figure 1a). The gold-mouth snail was obtained from the *T. chrysostomus* broodstock spawning production at the BBIL LIPI. The 800 individuals from the spawning production were distributed into different 16 boxes (50 snails/container).

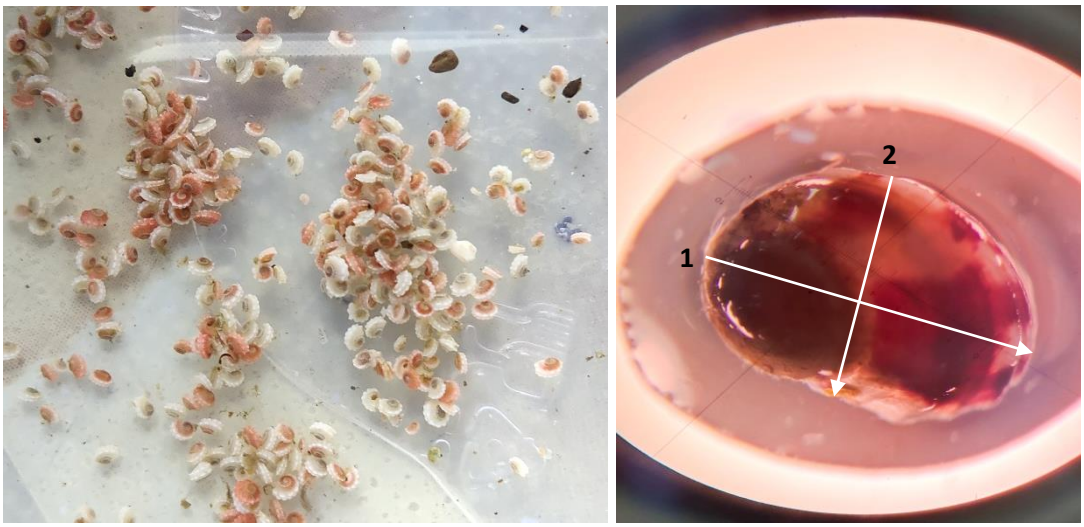
Procedures. A completely randomized design was used for this study, consisting of four treatments with four replications. Each container contained 60 L of aerated water with four different salinity levels: 26 ppt, 29 ppt, 32 ppt, and 35 ppt. Seawater was obtained from the waters around North Lombok, accommodated in a large tube and sterilized using UV before entering the reservoir, then filtered with a filter bag as physical filtration to remove particles that could interfere the snails, while freshwater was obtained from BBIL. Determination of salinity levels for treatment was carried out by the method of dilution for low salinity and the addition of salt for high salinity. The dilution was carried out according to the instructions of Jumaisa et al (2016) with the equation of:

$$V1 \times N1 = V2 \times N2 \quad \text{(Equation 1)}$$

Where, V1: seawater volume (L); V2: volume of water after dilution (L); N1: initial salinity (ppt); and N2: desired salinity (ppt)

Water was replaced when biota was fed. The water was replaced at follows: 50% of the water volume every two days and 100% on the 4th day of culture. The feed used was

natural microalgae from *Navicula sp.* (Dody 2012) as much as 300 mL per container, and when the gold-mouth snail was four months old, *Gracilaria sp.* seaweed was obtained from the seaweed culture in the BBIL LIPI laboratory. The water quality measurements were carried out every day for temperature, salinity, pH, and DO (dissolved oxygen). Survival monitoring was carried out by counting the still alive snails in the rearing container during the research period. The survival rate of *T. chrysostomus* was then calculated using the equation based on Solanki et al (2012) (Equation 2). Measurement of the length and width of the shell was carried out every 30 days to determine the specific growth rate, which is calculated using Equation 3 (Bhujel 2008). Meanwhile, to obtain the absolute growth of the shell, measurements were carried out at the beginning and end of the study using an ocular lens by taking 10 samples at random in each experimental unit (Equation 4). An example of measuring the length and width of a *T. chrysostomus* is presented in Figure 1b.



(a) (b)

Figure 1. Two-month old *T. chrysostomus* (a); view of mouth side of *T. chrysostomus* (b) with measurement: length (1) and width (2).
Source: Authors' personal archive

$$SR = \frac{N_1}{N_0} \times 100 \qquad \text{(Equation 2)}$$

Note:
SR = survival rate (%)
N_1 = number of juveniles at the end of observation
N_0 = number of juveniles at the beginning observation

$$SGR = \frac{(Ln\ L_1 - Ln\ L_0)}{T} \times 100 \qquad \text{(Equation 3)}$$

Note:
SGR = specific growth rate (%)
L_n = natural logarithm
L_1 = average length or width at the end of observation (mm)
L_0 = average length or width early observation (mm)
T = observation time (days)

$$AG = L_1 - L_0 \qquad \text{(Equation 4)}$$

Note:
AG = absolute growth (μm)
L_1 = length or width at the end of observation (mm)
L_0 = length or width early observation (mm)

The glucose level measurement was obtained from the homogenized snails with an anticoagulant at a ratio of 1:3 and centrifuged at 600 rpm to obtain 10 µl of the sample. The homogenized snail samples were moved into glacial acetic acid containing 3.5 mL of an ortho-toluidine color reagent. The homogenate was put into boiling water for 10 minutes, then cooled at room temperature, and the body's glucose concentration was measured using a spectrophotometer at a wavelength of 635 nm (Fendjalang et al 2016). Glucose levels were calculated based on (Wedemeyer & Yasutake, 1977) by converting the absorbance value in mg/100 mL (Equation 5). In addition, hemolymph analysis was also carried out to see the permeability of cell membranes by measuring the level of cell fragility using a spectrophotometer. Cells with the highest fragility are characterized by the highest optical density value and are assumed to have 100% lysis value. Hemolymph analysis was calculated by Equation 6.

$$GD = \frac{AbsSp}{AbsSt} \times GSt \qquad \text{(Equation 5)}$$

$$\text{Number of lytic hemolymph cells } (\%) = \frac{\text{Number of lytic cells}}{\text{Total cells}} \times 100 \qquad \text{(Equation 6)}$$

Note:
GD = glucose levels (mdL^{-1})
AbsSp = sample absorbance
AbsSt = standard absorbance
GSt = standard glucose level (mg dL^{-1})

Statistical analysis. The data obtained were analyzed using analysis of variance and continued with Duncan test at a 95% confidence level and a significant difference value ($p<0.05$). Statistical analyses were performed using SPSS software.

Results and Discussion

Water quality. DO, water temperature, and pH are the environmental factors that can limit the activity of sea snails (Ainis 2014). According to Gosling (2003), understanding the relationship between ecological parameters such as water temperature and salinity is very important for the management of marine biota populations in nature. This study also measured several factors that affect the water quality, namely temperature, pH, and DO. Water quality is an important factor that must be considered when culturing the aquatic organisms, including *T. chrysostomus*. Soekendarsi (2018) noted that the salinity of the gold-mouth snail habitat was 32 to 33 ppt, the pH of 7 to 8, and the temperature of 23 to 26^0C. The results of the measurement of the water quality of the cultivation media in the present study were within the normal threshold conditions, i.e., temperature was at 26.90 to 27.38^0C (Hamzah 2015), pH at 7.98 to 8.01 (Litaay et al 2017), and dissolved oxygen at 4.54 to 4.77 ppm (Hamzah et al 2021). Furthermore, the results of our study revealed that the best salinity culture media for *T. chrysostomus* s was 32 - 35 ppt, which is in line with the results Soekendarsi (2018) research, which states that the salinity range was around 32 - 33 ppt. Soekandarsi's research was based on field research in the waters of Ujung Genteng Pelabuhan Ratu where the highest salinity in the area was 33 ppt. Several types of mollusks that live in seawater have a relatively good environmental resistance to ammonia content at temperatures around 24^0-25^0C and pH of 7-8 (Protho 1989). According to Latupeirissa et al (2020), temperature is an important factor affecting gastropod metabolism. Water quality data in this study are presented in Table 1.

Table 1

Water quality of juvenile *T. chrysostomus* on 90 days of culture

	Salinity			
	A (26 ppt)	*B (29 ppt)*	*C (32 ppt)*	*D (35 ppt)*
Temperature (°C)	26.90 ± 0.15	27.01 ± 0.07	27.26 ± 0.09	27.38 ± 0.08
pH	7.98 ± 0.00	8.01 ± 0.01	8.00 ± 0.01	8.00 ± 0.01
DO (ppm)	4.63 ± 0.13	4.77 ± 0.02	4.54 ± 0.19	4.59 ± 0.16

Visual appearance. At the early cultivation stage, the shell of the *T. chrysostomus* was brown, still thin, and smooth on the surface (Figure 2a). Meanwhile, at 90 days of culture period, the color of the shell was light brown and had thicker black spots with a thorny surface (Figure 2b). In mature age (1-2 years old), the *T. chrysostomus* is light brown in color, oval in shape, and has a thorny spiral on the surface of the shell (Setyono et al 2013). These changes were seen in all treatments with similar changes. Temperature, food supply, and predation cues significantly affected the shell morphology (Clark et al 2020).

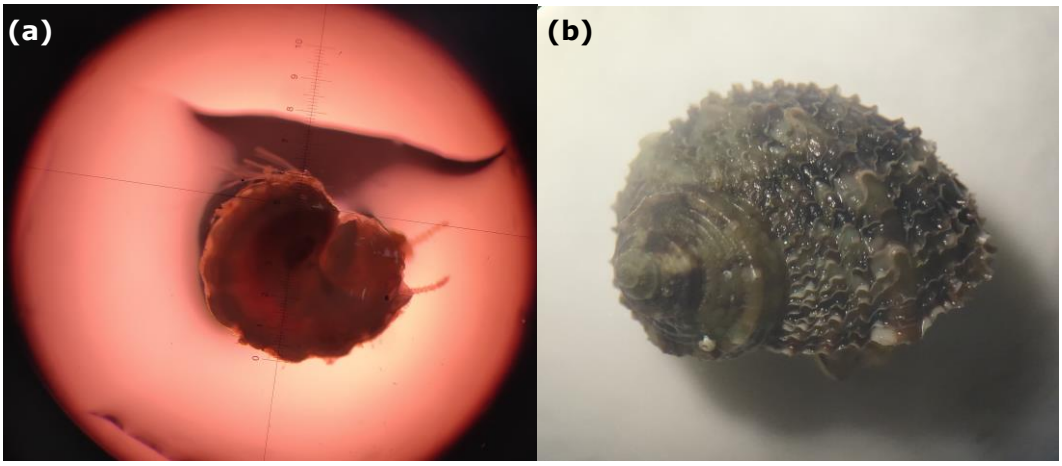


Figure 2. The visual appearance of juvenile *T. chrysostomus* before (a) and after (b) being cultured.
Source: Authors' personal archive

Survival rate. The average values of the parameters tested in this study are presented in Table 2. Survival rate is the main parameter in the cultivation activities to determine the commodity culture production success. Table 2 shows that the survival rate of *T. chrysostomus* was different in each treatment. This means that the survival rate of *T. chrysostomus* reared for 90 days is affected by different salinity levels. The 35 ppt treatment obtained the highest survival rate of *T. chrysostomus* compared to the other treatments. Based on the analysis of variance, the D treatment was not significantly different from the C treatment, but significantly different from the A and B treatments. In other words, different salinity in juvenile gold-mouth snail rearing will affect the survival rate of this species. In addition, this snail can be categorized into stenohaline because it can only live in a narrow salinity range (Davis 2000).

Table 2

The average value of the parameters tested of juvenile *T. chrysostomus* after 90 days of culture

Parameter	*Salinity*			
	A (26 ppt)	*B (29 ppt)*	*C (32 ppt)*	*D (35 ppt)*
Survival rate (%)	26.50 ± 2.22^{a}	36.00 ± 1.15^{b}	48.00 ± 0.82^{c}	55.00 ± 1.73^{c}
Absolute shell length (mm)	3.97 ± 0.09^{a}	4.53 ± 0.17^{a}	7.01 ± 0.62^{b}	7.58 ± 0.41^{b}
Absolute shell width (mm)	3.63 ± 0.05^{a}	4.36 ± 0.21^{a}	7.01 ± 0.62^{b}	7.43 ± 0.42^{b}
Hemolymph cell fragility (%)	87.55 ±1.34^{c}	69.43 ± 3.9^{b}	48.08±3.39^{a}	44.78±3.7^{a}
Body glucose level (mg dL^{-1})	33.51 ± 2.21^{b}	24.24 ± 6.05^{ab}	16.25 ± 3.04^{a}	14.04 ± 1.87^{a}

Note: Different letters in the same row showed significantly different treatment effects ($p<0.05$).

Salinity significantly influenced the survival of the *T. chrysostomus*. The results of this study showed that study, the salinity of 32-35 ppt was the most favorable condition for the life of *T. chrysostomus*. The salinity of more than 31 ppt was proved to offer stable ecosystem condition for gastropods (Normalasari et al 2019). Salinity can affect oxygen consumption (Villarreal et al 1994), biota behavior, survival, embryonic development, growth, reproduction (Cui et al 2019), energy availability, immune response, and body metabolism of aquatic biota (Tian et al 2019). Previous research also revealed that salinity had a significant effect on the survival of *Strombus gigas* (Davis 2000), *Pomacea maculate* (Martin & Valentine 2014), and *Pomacea canaliculata* (Yang et al 2017). A low survival rate indicates that the individual is experiencing stress and unsuitable salinity may be one reason (Hiebenthal et al 2012). According to Zhang et al (2014), *Nassarius festivus* and *Nassarius conoidalis* exposed with the combined effects of ocean acidification, temperature, and salinity, had lower mortality at high salinity than at low salinity. If the salinity deviates from optimal, adaptive mechanisms are activated to maintain the physiological activity of the organism (Muraeva et al 2016).

Absolute growth. The absolute growth measured in this study was absolute length growth and absolute width growth, and the values for each treatment in this study were almost the same. Based on Table 2, the average absolute length and width growth at 35 ppt salinity was the had the best levels among other treatments. This is presumably because, at high salinity level, the energy used for growth activities is used optimally. The increase in energy requirement to balance the osmotic pressure between the body of *T. chrysostomus* and its environment is caused by changes in salinity (Amin et al 2016). The difference in growth performance also indicates that salinity has an impact on the growth performance of juvenile *T. chrysostomus*, both in length and width absolute growth of the shell. Environmental factors such as competition within the population and food availability greatly affect growth (Abukena et al 2014). Proper water quality, such as salinity, can also support the growth and survival of aquatic biota (Hamzah 2015). Salinity is one of the physiological factors that affect the utilization of feed and the growth of marine biota (Anggoro 2017). Therefore, the absolute growth of the shell is strongly influenced by salinity. Amin et al (2016) further explained observed that the growth of abalone larvae at 30 ppt salinity was lower than at 33 ppt and 35 ppt salinity.

Specific growth rate. The specific growth rate was observed every 30 days so that three measurements of the specific growth rate were obtained. The specific length growth rate increased along with the increased salinity levels. The highest specific shell length rate was in the D treatment, which was not significantly different from the C treatment (Figure 3). A suitable salinity affects the growth of shell length (Davis 2000). The cumulative growth in shells and mortality are cumulatively influenced by salinity and temperature (Hiebenthal et al 2012).

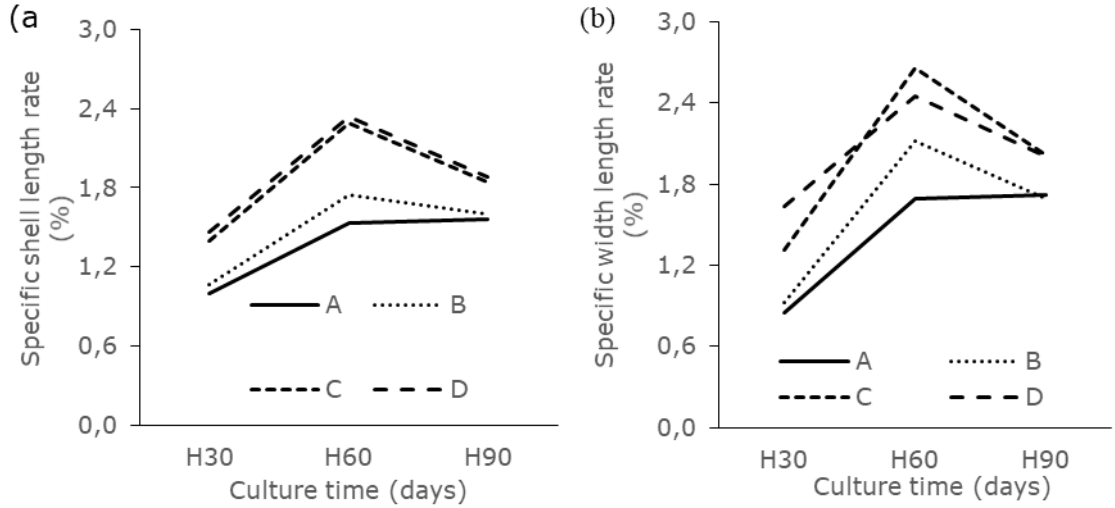


Figure 3. Specific growth rate: specific shell length rate (a) and specific shell width rate (b) of juvenile *T. chrysostomus* on 30 days, 60 days, and 90 days of culture at different salinity levels.

Different salinity levels have an influence on the specific shell width rate, as presented in Fig. 3. Overall, the specific shell length and width rate increased and reached its peak on the 60th day, then decreased on the 90th day. Specific shell length rate (SSLR) and specific shell width rate (SSWR) of juvenile *T. chrysostomus* tended to be faster on day 60. The PPS value on day 60 was 53 to 64% faster than day 30 for all treatments. According to (Setyono et al 2013), this presumably occurred due to the environmental conditions (temperature and pH) and feed absorption which reached the most optimum level at day 60. The growth rate of *Mytilus edulis* decreased after low salinity exposure for 283 days (Wing & Leichter 2011). Shell increment is caused by the formation of the organic matrix and shell calcification (Pourmozaffar et al 2020). However, physiologically stressful conditions such as salinity fluctuation can inhibit soft tissue and shell production (Hiebenthal et al 2012).

The specific growth rate at 26 ppt salinity was lower than the other treatments, presumably because the energy used for growth was depleted to balance the osmotic pressure. The metabolic process related to the salinity of the medium is osmoregulation activity. Osmoregulation is a homeostatic system, is the maintenance of balance through the regulation of fluid in the body with its environment (Maulana et al 2013). The D treatment obtained the optimal salinity in this study, causing the low osmoregulation activity in the *T. chrysostomus*, which required low energy consumption. On the other hand, the A treatment caused the osmoregulation activity to increase along with the increased amount of energy requirement. The energy used for growth will decrease if the energy for osmoregulation activity increases. The difference in salinity affects the development of larvae through the process of osmoregulation, and the value of the osmotic concentration of the media, which is not much different from the osmotic concentration of body fluids, will result in rapid body growth (Bulanin et al 2003). Moreover, Sabilu et al (2021) explained that salinity level influences the osmoregulation, nutrient utilization, and growth rate, as related to the capability of maintaining internal homeostasis in a hypersaline condition.

***Physiological response*.** Different salinity levels have a significant effect on physiological stress response parameters, namely in the fragility of hemolymph cells and body glucose levels. Stress can occur due to changes in new environmental conditions (Djai et al 2017). Changes in salinity can trigger stress in gastropods (Hiebenthal et al 2012). The value of hemolymph cell fragility and body glucose levels of gold-mouth snails in this experiment are presented in Table 2. According to Hastuti et al (2016), media with pH of 7 had the lowest stress response during maintenance which was supported by the optimum water quality. This study showed that the fragility of hemolymph cells (KSH)

was lower at higher salinity. The decrease and increase in salinity in mollusks can decrease and increase the osmoregulatory ability of mollusk hemolymph so that cells swell and shrink (Amin et al 2016). Higher salinity reduced stress on *Calyptraea*, so the survival rate of snails living at higher salinity will be greater (Diederich et al 2011).

The body glucose levels on the four salinity treatments in this study were significantly different, as the D treatment had the lowest body glucose level, and the A treatment had the highest body glucose. Higher value of body glucose level indicates that the *T. chrysostomus* has problems compared to other snails who have low body glucose levels. Osmoregulation activity requires hemolymph glucose as a source of metabolic energy. Energy requirement as glucose to improve the homeostasis during stress are produced through the processes of glycogenolysis and gluconeogenesis (Ockwell 2008). Thus, under stress conditions, there is a reallocation of metabolic energy into activities to improve the homeostasis (Adiyana et al 2014). According to Jiang et al (2013), increased glucose level is a secondary marker for stress response in freshwater crayfish. Therefore, stress occurred during the maintenance period in low salinity media could cause low growth rate in *T. chrysostomus*. This condition could also be found in the lobster which showed a low growth rate due to stress condition in the lobster (Supriyono et al 2017).

Based on the explanations above, we conclude that salinity has a significant effect on the physiological response and production performance of gold-mouth snails at the juvenile stage. The best conditions in the culture media for *T. chrysostomus* were at the 32 and 35 ppt salinity. The 35 ppt salinity was the optimum treatment in this study, whereas the survival rate, growth rate, hemolymph cell fragility value, and body glucose level obtained the best values compared to the other treatments.

Conclusions. Salinity has a significant effect on the physiological response and production performance of *T. chrysostomus* at the juvenile stage. The best condition in the culture media for *T. chrysostomus* were at the 32 and 35 ppt salinity. The test parameters at these two salinities were not significantly different. The 35 ppt salinity was the optimum treatment in this study, whereas the survival rate, growth rate, hemolymph cell fragility value, and body glucose level obtained the best values from the other treatments.

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Conflict of interests. The authors declare no conflict of interest.

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