Effect of salinity on the growth and feed conversion of rabbitfish, *Siganus canaliculatus* (Park, 1797), fed with seaweed (*Gracilaria changii*)

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Abstract. The low production of the rabbitfish *Siganus canaliculatus* and the high price fluctuations of the seaweed *Gracilaria changii* cultured in brackishwater ponds are an opportunity to develop multitrophic aquaculture between *S. canaliculatus* and *G. changii*. The study aimed to analyze the effect of salinity on the growth and food conversion of *S. canaliculatus* fed with *G. changii*. The study used a completely randomized design with three treatments and three replicates. The treatments included three different salinities, namely 35, 30, and 25 ppt. During the study, *S. canaliculatus was* fed fresh *G. changii* ad libitum, starting with 500 g. The availability of the feed in the tanks was checked every day and a 500 to 1,000 g of seaweed *G. changii was added* if its availability decreased. The remaining feed was weighed at the end of the study. The culture period was 60 days. The length and weight of the stocked Rabbitfish *S. canaliculatus* were 12.3±2.1 cm and 36.47±18.29 g. The stocking density was 10 Rabbitfish *S. canaliculatus* for each tank. The length and weight at the end of the study were 14.1±1.8 cm and 55.73±21.74 g. The increase in length and weight was 1.71±0.87 cm and 19.26±10.52 g. The absolute and relative length growth rates were 12.04 to 21.45 mm and 0.17 to 0.31 mm. The absolute and relative weight growth rates were 16.21 to 23.11 g and 0.23 to 0.32 g. The feed conversion was 62.26 to 75.95. The survival rate was 100% for all salinity treatments. The absolute and relative length growth of Rabbitfish *S. canaliculatus* was not significantly different between all salinity treatments. The total and relative weight growth of the rabbitfish *S. canaliculatus* was also not significantly different. The conversion of *G. changii* seaweed to Rabbitfish *S. canaliculatus* was not significantly different between all salinity treatments. The water quality parameters during the study supported the life of the Rabbitfish *S. canaliculatus*.
Key Words: Feed conversion, multitrophic aquaculture, natural food, environmentally friendly, alternative solution, brackishwater ponds.

Introduction. The low production of rabbitfish causes unmet demand, especially from restaurants (Abdusysyahid 2006). Rabbitfish is classified as a luxurious fish and is generally consumed by people with medium to high incomes (Nurdiana 2016). Limited production and consumers' high willingness to pay for it are opportunities for the development of rabbitfish farming. So far, its production depends on catch in nature. One of the potential locations for the development of rabbitfish cultivation is the brackishwater pond.

A species of rabbitfish that can be cultivated in brackishwater pond is *Siganus canaliculatus*. Farmers who have not harvested shrimp due to decreased brackishwater pond productivity can take advantage of this opportunity. In the last 25 years, the most significant problems faced by farmers were low carrying capacity due to environmental degradation and high rates of shrimp mortality in brackishwater ponds. These two problems caused brackishwater ponds in Indonesia to produce only about 50 kg ha^{-1} of tiger prawns for each growing period. This production was lower than the production of brackishwater ponds with simple (extensive) technology that produced 400-500 kg ha^{-1} of tiger prawns for each growing season (Ghufron & Kordi 2010). This low production led farmers to look for more profitable aquaculture alternatives. The cultivation of seaweed *Gracilaria changii* was one of the alternatives.

The cultivation of seaweed *G. changii* has become the main activity for the cultivation of brackishwater ponds (Arbit et al. 2019). However, seaweed cultivation also faces problems. Namely, the price fluctuates significantly so that economically seaweed cultivation does not contribute significantly to the improving the welfare of seaweed farmers. This problem requires a solution so that seaweed farmers can be more prosperous. One solution is the development of multitrophic aquaculture between seaweed and herbivorous fish, which have a higher economic value than seaweed. The rabbitfish is a suitable alternative.

The use of seaweed *G. changii* as a natural food for rabbitfish has advantages regarding the nutritional content, because seaweed *G. changii* has a complete nutritional content. Chemically, seaweed consists of water (27.8%), protein (5.4%), carbohydrates (33.3%), fat (8.6%), fiber (3%), and ash (22.25%) (Wirjatmadi et al 2002). Seaweed also contains enzymes, nucleic acids, amino acids, vitamins (A, B, C, D, E, and K), macro minerals (calcium and selenium), and micro minerals (iron, magnesium, and sodium). The amino acid, mineral, and vitamin content of seaweed is 10 - 20 times higher compared to land plants. Its complete nutritional content makes seaweed ideal for herbivorous fish (Rukmi et al 2012).

The use of seaweed *G. changii* as a natural food also has other advantages when used in live conditions. It can supply oxygen through photosynthesis during the day and absorb excess nutrients in the water, thus reducing the eutrophication of the brackishwater pond. Consequently, it is a type of more environmentally friendly production. The absorption of excess nutrients by seaweed *G. changii* can inhibit the development of toxic plankton and increase the productivity and profits of aquaculture, which can increase farmers' income (Burhanuddin & Hendrajat 2015). The presence of seaweed *G. changii* in culture media has been proven to reduce eutrophication of waters. For example, previous studies showed that seaweed could absorb excessive nutrients in the waters and shrimp metabolism waste (Badraeni et al 2020).

Multitrophic aquaculture of seaweed *G. changii* and rabbitfish *S. canaliculatus* in a brackishwater pond can be an alternative solution for seaweed farmers so that they do not continue to be caught in the problem of erratic prices. In addition to price considerations, multitrophic aquaculture seaweed cultivation and rabbitfish *S. canaliculatus* in brackishwater ponds are environmentally friendly because it does not require artificial food input, so it does not increase the eutrophication burden in brackishwater ponds (Tuwo et al 2019b). This multitrophic aquaculture can also increase the productivity of the brackishwater pond. The productivity of the brackishwater pond has decreased due to eutrophication, especially in South Sulawesi. South Sulawesi has a huge brackishwater pond – of 109 561 ha, which represents around 16% of the total area of 674 135 ha of brackishwater pond cultivation in Indonesia (BPS 2019). The success of multitrophic aquaculture in this huge brackishwater pond can improve the welfare of seaweed farmers.

Before carrying out multitrophic aquaculture of seaweed *G. changii* and rabbitfish *S. canaliculatus* in a brackishwater pond on a large scale, it is necessary to study the environmental factors that limit the production of rabbitfish *S. canaliculatus.* Salinity must be considered because the rabbitfish *S. canaliculatus* is a marine fish, so its life is highly affected by water salinity. The salinity fluctuation in brackishwater pond–due to rain and evaporation is thought to significantly affect rabbitfish because, under certain conditions, rabbitfish can only tolerate changes in the range of 5 ppt (Ghufron & Kordi 2005). Salinity is closely related to the osmoregulation mechanism that requires a large amount of energy. The osmoregulation affects the quantity of seaweed consumed by rabbitfish (Framegari et al 2012).

Until now, the cultivation of seaweed and rabbitfish has only been carried out in the sea, namely between the *Kappaphycus alvarezii* seaweed and the rabbitfish *Siganus* sp

(Amalyah & Idris 2019). In nature, the rabbitfish *Siganus* sp is a consumer of *K. alvarezii* seaweed consumer (Friedlander 2001). So far, there have been no research results on the growth of rabbitfish fed seaweed and cultivated at different salinities. Therefore, it is necessary to analyze the growth of rabbitfish at different salinities.

This study aimed to analyse the effect of salinity on the growth and feed conversion of the rabbitfish *S. canaliculatus* fed with *G. changii* seaweed. This study is expected to be a reference in evaluating the feasibility of multitrophic culture between seaweed *G. changii* and *S. canaliculatus* rabbitfish in a brackishwater pond, which has relatively large salinity fluctuations.

Material and Method. The rabbitfish *S. canaliculatus* used in this study had an average initial length of 12.52 ± 2.19 cm and an average initial weight of 37.78 ± 18.69 g. The seaweed used as natural food was *G. changii*. The tanks used are nine fiber tanks with a volume of 2 cubics. Each tank was equipped with an aeration and water pump for oxygen supply and water circulation in the tank.

A completely randomized design was used. There were three treatments and three replicates. The treatments were three different salinities, namely 35 ppt, 30 ppt, and 25 ppt. The selection of the three salinity levels is based on the variation in salinity in which certain rabbitfish species can grow appropriately (Framegari et al 2012). The position of the treatment tank was randomized to obtain the treatment arrangement as described in Figure 1.

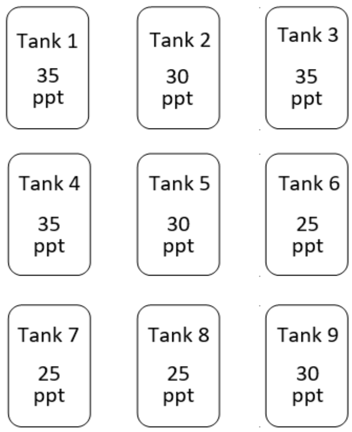

Figure 1. Position of the treatment tank after randomization.

Each tank was stocked with ten rabbitfish *S. canaliculatus*. The tank used had 2 sqm, so the density was five rabbitfish per sqm. Five rabbitfish per sqm is the highest density in rabbitfish culture in ponds, and this is the equivalent to 50 000 rabbitfish per hectare. Before stocking, rabbitfish *S. canaliculatus* were acclimatized for one week in an aerated tank. Acclimatization aims to adapt the rabbitfish *S. canaliculatus* to live in tanks and eating seaweed *G. changii*. Density refers to the density of rabbitfish cultivation in the brackishwater pond, which was 2 to 5 fish m^{-2} (Suharyanto 2008).

Before stocking in the tank, the initial length of the rabbitfish *S. canaliculatus* was measured using a ruler with a precision of 1 mm. In addition, their initial weight was measured with analytical balance with an accuracy of 0.01 g.

During treatment, rabbitfish *S. canaliculatus* were fed with seaweed *G. changii* ad libitum, starting with 500 g. The availability of seaweed *G. changii* in the tanks was checked every day. If there was little remaining seaweed *G. changii* left in the tank, 500 to 1,000 g of seaweed *G. changii* was added to the tanks. The weight of seaweed fed to the rabbitfish *S. canaliculatus* during the study was observed. The weight of uneaten seaweed *G. changii* was weighed at the end of the study.

Treatment lasted 60 days. The measured growth parameters of the rabbitfish *S. canaliculatus* were the length and weight growth rates. The absolute length growth of rabbitfish *S. canaliculatus* was calculated using the equation (Everhart et al 1975): $Lm =$

$Lt - Lo$, where Lm was absolute length growth (cm), Lo was the length at the beginning of the study or treatment (cm), Lt was the length at the end of the study or treatment (cm).

The growth per day or relative length of the rabbitfish *S. canaliculatus* was the absolute length divided by the study or treatment period (days) using the equation : $\mathrm{Lh} = \frac{Lt-Lo}{t}$, where Lh was the daily length growth (cm), Lo was the length of the fish at the beginning of the study or treatment (cm), Lt was the length at the end of the study or treatment (cm), t was the period of the study or treatment (days).

The absolute weight growth of rabbitfish *S. canaliculatus* was calculated using the equation (Everhart et al 1975): $Wm = Wt - Wo$, where Wm was absolute weight growth (g), Wo was fish weight at the beginning of the study or treatment (g), Wt was fish weight at the end of the study or treatment (g).

The daily or relative weight growth of rabbitfish *S. canaliculatus* was calculated using the absolute weight equation divided by the study period or treatment (days) using the equation: $\mathrm{Wh} = \frac{Wt-Wo}{t}$, where Wh was the daily weight growth (g), Wo was the weight at the beginning of the study or treatment (g), Wt was the weight at the end of the study or treatment (g), t was the period of study (days).

The fFeed Conversion Ratio (FCR) was calculated by using the equation (Djajasewaka 1990): $FC = \frac{F}{[(Wt+D)-Wo]}$, where FC was the feed conversion value, Wt was the total weight of fish at the end of the study or treatment (g), Wo was the total weight of fish at the beginning of the study or treatment (g), D was the total weight of fish that died during the study or treatment (g), F was the total feed consumed during the study or treatment (g). The water quality parameters measured were temperature, pH, dissolved oxygen, and nitrate.

The normality test was used to analyze whether the distribution of data is normally distributed or not. Data normally distributed were analyzed using a one-way anova to analyze whether treatment or salinity has a significant effect or not on the growth and feeding conversion of the rabbitfish *S. canaliculatus*. Normality and one-way anova were done using the SPSS program (Raharjo 2018).

Data that are not normally distributed were tested using the Kruskal Wallis non-parametric statistics test to analyze whether treatment or salinity has a significant effect or not on the growth and feed conversion of rabbitfish *S. canaliculatus* by using the SPSS program (Hidayat 2014).

Results

The survival rate in the study was 100% for all salinity treatments. The normality test indicated that the absolute length growth of rabbitfish *S. canaliculatus* was normally distributed ($p > 0.05$). The one-factor ANOVA test indicated that the absolute length growth of rabbitfish *S. canaliculatus* was not significantly different ($p > 0.05$) between all salinity treatments (25, 30, and 35 ppt) (Figure 2).

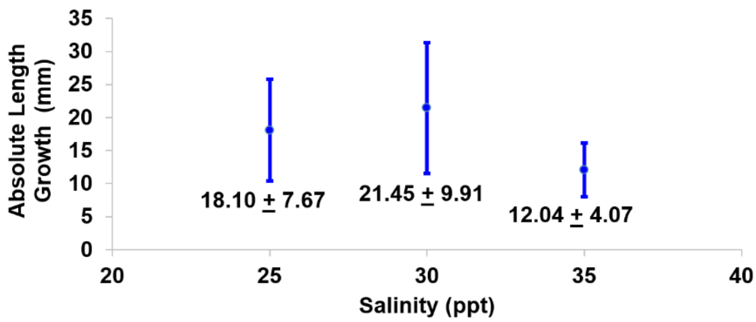

Figure 2. Absolute length growth of the rabbitfish *Siganus canaliculatus* at different salinities.

The normality test of the daily or relative length growth of rabbitfish *S. canaliculatus* indicated that it was normally distributed (p > 0.05). The one-factor ANOVA test indicated that the daily or relative length growth of rabbitfish *S. canaliculatus* was not significantly different (p > 0.05) between all salinity treatments (25, 30 and 35 ppt) (Figure 3).

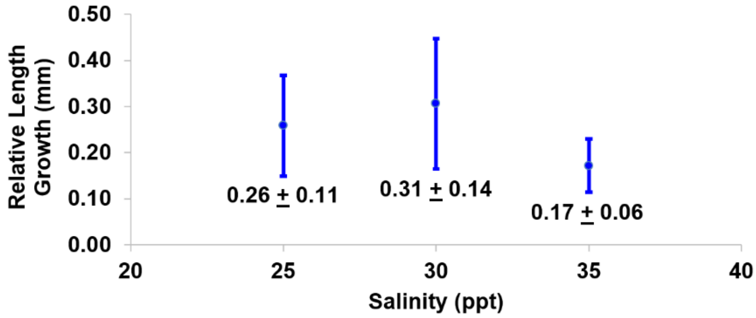

Figure 3. The daily or relative length growth of the rabbitfish *Siganus canaliculatus* at different salinities.

The normality test of the absolute weight growth of rabbitfish *S. canaliculatus* indicated that it was not normally distributed (p < 0.05). The Kruskal Wallis nonparametric statistical test indicated that the absolute weight growth of the rabbitfish *S. canaliculatus* was not significantly different (p > 0.05) between all salinity treatments (25, 30, and 35 ppt) (Figure 4).

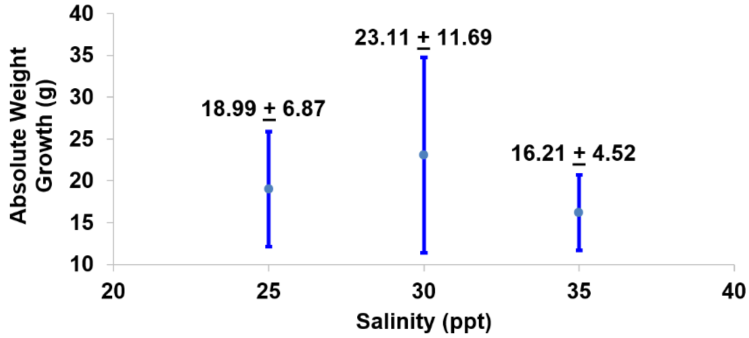

Figure 4. Absolute weight growth of the rabbitfish *Siganus canaliculatus* at different salinities.

The normality test of the daily or relative weight growth of rabbitfish *S. canaliculatus* indicated that it was not normally distributed (p < 0.05). The results of the Kruskal Wallis nonparametric statistics test showed that the daily or relative weight growth of Rabbitfish *S. canaliculatus* was not significantly different (p > 0.05) between all salinity treatments (25, 30, and 35 ppt) (Figure 5).

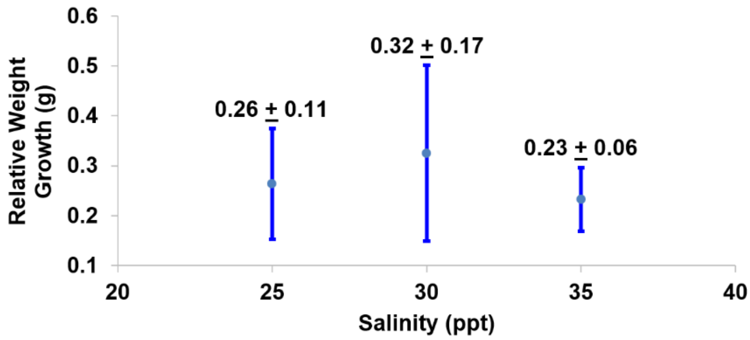

Figure 5. The daily or relative weight growth of the rabbitfish *Siganus canaliculatus* at different salinities.

The normality test indicated that feed conversion of rabbitfish *S. canaliculatus* fed *G. changii* seaweed was normally distributed ($p > 0.05$). The one-factor ANOVA test indicated that feed conversion of *S. canaliculatus* rabbitfish to fed seaweed *G. changii* was not significantly different ($p > 0.05$) between all salinity treatments (25, 30, and 35 ppt) (Figure 6).

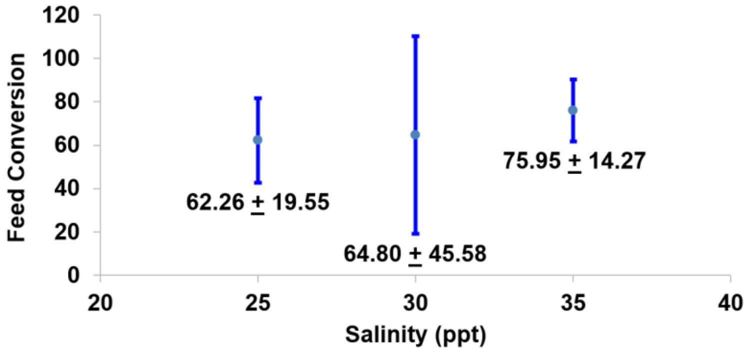

Figure 6. The feed conversion of rabbitfish *Siganus canaliculatus* fed to seaweed *Gracilaria changii* at different salinities.

In general, the water quality parameters were favorable for the life of Rabbitfish *S. canaliculatus* during the study or treatment (Table 1).

Table 1

The range of water quality parameter values measured during the study and the optimum range of water quality parameter values for fish and seaweed farming in ponds

Water quality parameter	**Measured range values**	**Optimum range values (Reference)**
Temperature (°C)	26.40 - 32.20 (28.93 ± 1.96)	25 - 34 (Ghufron & Kordi 2005; Affan 2012; Suci et al 2020)
pH	7.21 - 8.07 (7.71±0.26)	6.5 - 9.0 (Latuconsina & Wasahua 2011).
Dissolved Oxygen (ppm)	4.13 - 7.46 (5.15±1.21)	4.2 – 7 (Affan 2012; Suci et al 2020)
Nitrate (mg/l)	1.42 - 2.85 (1.87±0.43)	0.9 - 3.50 (Andarias 1991)

Discussion. The treatment salinity (25, 30, and 35 ppt) did not significantly affect growth. Almost similar results were found in previous studies, which indicated that at a salinity of 30-34 ppt, the rabbitfish *Siganus* sp fed *Kappaphycus alvarezii* seaweed could grow well in laboratory treatments (Framegari et al 2012).

Biologically, water salinity affects fish body fluids (Tuwo et al 2020; Tresnati et al 2021; Tuwo et al 2021a; Yasir et al 2021b). The salinity of the body fluids of freshwater fish is different from the salinity of the body fluids of seawater fish. The body fluid salinity of freshwater fish is about 14 ppt (Castro & Huber 2005), while saltwater fish is about 18 ppt (Sumich 1992). Fish regulate the salinity of their body fluids through the osmoregulation process. The osmoregulation process requires energy. The 25 ppt salinity treatment was closer to the salinity of the rabbitfish body fluids than the salinity of 30 and 35 ppt. The higher the salinity, the greater the energy required for the process of osmoregulation or regulation of fish body fluids. Although the difference in feed conversion was not statistically significant, the energy requirements for the osmoregulation process may explain why the feed conversion rate tends to be higher at 30 and 35 ppt salinity compared to 25 ppt salinity. At high salinity, fish will adapt to drink as much as possible and excrete salt through their gills to avoid dehydration and excess salt. The whole mechanism requires extra energy that could reduce the efficiency of the feed consumed (Framegari et al 2012). In addition to affecting the regulation of fish body fluids, salinity can also induce specific responses related to the immune system and other biological processes.

Although not statistically significant, feed conversion tends to be better at 25 ppt salinity. This feed conversion indicates that rabbitfish can tolerate a salinity range of up to 10 ppt. This range is greater than the salinity range that rabbitfish can tolerate in previous studies, which was only up to 5 ppt (Ghufron & Kordi 2005).

Feed conversion, which tends to be better at 25 ppt salinity, indicates that salinity is one of the environmental factors affecting feed consumption (Setiawati & Suprayudi 2003). Feed conversion, which tends to be better at 25 ppt salinity, indicates that salinity is one of the environmental factors affecting feed consumption.

This study indicated that the rabbitfish *S. canaliculatus* fed with seaweed *G. changii* had a slightly shorter mean absolute length growth (17.20 cm) than the rabbitfish *S. canaliculatus* (20.0 cm) reared in bottom net cages placed at three levels of seagrass density in seagrass ecosystems (Suci et al 2020). However, in terms of weight growth, the rabbitfish *S. canaliculatus* in this study fed with seaweed *G. changii* had an average absolute weight growth (19.44 g) that was much heavier than the rabbitfish *S. canaliculatus* (3.0 g) reared in bottom net cages at three levels of seagrass density in seagrass ecosystems (Suci et al 2020).

The rabbitfish *S. canaliculatus* in this study fed with seaweed *G. changii* had an average absolute weight growth (19.44 g) which was much heavier than the rabbitfish *S. canaliculatus* (7.67 g) that lived naturally at three levels of seagrass density in the seagrass ecosystem in the waters of Pulau Sembilan, Sinjai Regency (Ismail et al 2019).

Rabbitfish *S. canaliculatus* has a relatively similar growth rate in the salinity range of 25-35 ppt, so it is recommended that brackishwater pond farmers to keep this salinity range if they culture *S. canaliculatus* in brackishwater ponds.

Rabbitfish are often considered predators in seaweed cultivation. Other species in this genus have high grazing power. For example, at a density of 1,200 g m^{-2} of *Kappaphycus alvarezii* seaweed, a rabbitfish *S. guttatus* was able to graze 23.58% of it. However, at a thickness of 600 g m^{-2} seaweed *K. alvarezii*, one rabbitfish *S. guttatus* was able to graze of 12.4% (Amalyah & Idris 2019). Until now, no previous research has reported the grazing power of the rabbitfish *S. canaliculatus*.

Rabbitfish *S. canaliculatus* is an herbivorous fish with the highest preponderance index value in algae (58.3%), and followed by seagrass (20.5%), detritus (20.5%), and crustaceans (0.06%) (Selviani et al 2018). The preponderance index, or the most considerable portion index, is a formula used to determine the percentage of food in the fish stomach (Nikolsky 1963). One of the seagrass species found in the intestines of rabbitfish *S. canaliculatus* is *Gracilaria* sp (Selviani et al 2018).

This study indicated that 62 - 76 g of wet seaweed were necessary to produce 1.0 g of rabbitfish *S. canaliculatus*. This high feed conversion value, 1:62 to 1:76, was due to the very high water content of the seaweed *G. changii*. The results of previous studies indicated that to obtain 1.0 ton of dry weight of *Gracilaria* sp, it took 8.5 tons of wet *Gracilaria* sp (Suheri et al 2015). With the seaweed water content of 88.23%, producing 1.0 g of rabbitfish *S. canaliculatus* required 7.33 to 8.94 g of dried seaweed. This feed conversion value was very high compared to the artificial feed conversion value for the culture of rabbitfish *S. canaliculatus* in floating net cages, that is, 1:3 (Suci et al 2020).

So far, no previous researches reported feed conversion of herbivorous fish feed to aquatic plants or seaweed. On the other hand, many studies had reported feed conversion in herbivorous livestock, such as domestic goat *Capra aegagrus hircus*. Domestic goats given various species of tree leaves had feed conversions from 1:13.63 to 1:18.20 (for wet leaves) and from 1:3.65 to 1:4.89 (for dry leaves) (Kardiandi & Aku 2021). If it refers to the feed conversion of the rabbitfish *S. canaliculatus*, which is equivalent to the weight of dry seaweed, then the conversion value of rabbitfish *S. canaliculatus* feed is between the wet and dry leaf feed conversion values of domestic goat.

The results of previous studies have not reported the digestibility rate of herbivorous fish fed on aquatic plants or seaweed. The study of digestibility rate on herbivorous livestock, such as water buffalo *Bubalus bubalis,* showed that when it was fed with napier grass *Cenchrus purpureum* in the proportion of 70%, the rest of 30% being concentrate, the digestibility rate was 58.5% (Purnomoadi et al 2003). If 30% of the concentrate is considered completely digested, then the digestibility rate of napier grass was only about 30%. Water buffalo fed with oil palm industry by-products had feed conversion rates of 13.15-26.79 (Elisabeth & Ginting 2003). If it referred to the feed conversion of rabbitfish *S. canaliculatus* feed, which was equivalent to the weight of dry seaweed, then the feed conversion value of rabbitfish *S. canaliculatus* feed was better than the feed conversion of water buffalo fed with oil palm industry by-products (Elisabeth & Ginting 2003).

If using the average price of seaweed *G. changii* US$0.35 per kg dry (Rosmiati et al 2019), producing 1 kg of rabbitfish *S. canaliculatus* required a feed cost equivalent to US$2.55-3.25. Suppose that the price of rabbitfish of size 5 - 6 is US$5.46/ kg (Shopee 2021), it can be said that feeding seaweed to rabbitfish *S. canaliculatus* is much more profitable rather than selling dried seaweed as raw material, especially when the price of seaweed drops, which can reach only US$0.245 per kg of dry weight. The use of seaweed as food for rabbitfish does not require additional production costs. Seaweed sold dry requires post-harvest costs for drying of around US$0.105 per kg. Therefore, it is recommended that seaweed farmers apply multitrophic aquaculture between seaweed *G. changii* and rabbitfish *S. canaliculatus* in the brackishwater pond. It can provide greater profits, especially if the price of seaweed goes down. This study indicated that multitrophic aquaculture of herbivorous fish has a higher survival rate than carnivorous fish (Tuwo et al 2019a; Yasir et al 2021a). This higher survival rate of rabbitfish *S. canaliculatus* can generate higher profit compared to carnivorous fish. The price of carnivorous feed in the form of pellets or fish is more expensive than seaweed, so the production cost is more expensive. If carnivore mortality is high and, thus, production costs increase. Consequently, the production cost of rabbitfish is lower than the one of carnivorous fish. For these reasons, rabbitfish, which is classified as a luxury fish in seafood restaurants (and its price is the same or higher than carnivorous fish), is more profitable than carnivorous fish.

The physicochemical water parameters measured during the study were generally in the optimal range for the life and growth of rabbitfish *S. canaliculatus* (Table 1). The optimal salinity range for rabbitfish was 30 - 34 ppt (Framegari et al. 012), but 25 ppt salinity in the treatment in this study was not a problem because rabbitfish indicated the same feed conversion with 30 ppt and 35 ppt salinities. The water temperature range of 26.40 - 32.20 during the study was in the range of optimal values for fish farming (Latuconsina & Wasahua 2011). In general, the quantity of dissolved oxygen measured during the study was in the optimum range for cultivation (Affan 2012). The oxygen content was lower than the optimum range, but the difference was only 0.07 ppm. Oxygen is a crucial component in fish farming because the produced fish biomass quantity dependes on the availability of dissolved oxygen (Tuwo et al 2021b). Nitrate content was also in the optimum range for cultivation (Andarias 1991).

Conclusions. The salinity 25, 30, and 35 ppt did not have a significant effect on the growth and feed conversion of rabbitfish *S. canaliculatus* fed with seaweed *Gracilaria changii*. This study shows that rabbitfish *S. canaliculatus* can be multitrophically cultured with seaweed *Gracilaria changii* in brackishwater ponds with salinity of 25-35 ppt.

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