The biological evaluation of mud crabs (*Scylla olivacea)* in fattening activities using seaweed *Kappaphycus alvarezii* in ponds

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Abstract. Currently, the development of seaweed benefits leads to natural raw materials for feed. To support the success of fattening business of the mud crab *Scylla olivacea* feed is needed as a provider of energy for the activity of body cells. The purpose of this research was the biological evaluation of *S. olivacea* in ponds. The first step of this research was pond and feed preparation, using different doses of seaweed flour, namely 0% (Control), 10% (A), 20% (B), and 30% (C). Then feed was given as much as 10% of body weight with a feeding frequency of twice a day to *S. olivacea* with a weight of 130-180 g, and kept in a box for 35 days in the pond. The test parameters were related to the crab biology, namely absolute growth, survival rate and feed efficiency ratio. The results showed that the addition of 30% seaweed resulted in a significantly different response ($p<0.05$) in terms of absolute growth and feed efficiency, and in a similar response ($p>0.05$) in terms of survival rate. The conclusion of this research that is seaweed is effective for increasing the weight and feed efficiency in the business of *S. olivacea* fattening in ponds. Future developments of this research will be the application of seaweed to evaluate the body chemistry of *S. olivacea*.

Key Words: growth, feed efficiency, survival rate, biological evaluation.

Introduction. Seaweed *Kappaphycus alvarezii* is one of the leading aquaculture commodities in Indonesia. It is still a potential business and is the main preference in coastal areas both in Indonesia and in the world, due to the relatively easy and inexpensive cultivation techniques and shorter harvesting time (Parakkasi et al 2020). *K. alvarezii*, synonym *Eucheuma cottonii*, contains 9.76% protein, 1.10% fat, 26.49% carbohydrates, 46.19% ash, 5.91% crude fiber, 10.55% water (Matanjun et al 2009) and 42−44% carrageenan (Mochtar et al 2013). In addition, *K. alvarezii* also contains potassium minerals, calcium, magnesium, sodium, iron, zinc, iodine and vitamin C and E (Matanjun et al 2009).

K. alvarezii is the main producer of kappa-carrageenan which is widely used as gelling agent, stabilizer and feed thickener. Currently, the development of seaweed benefits leads to natural raw materials for feed. Seaweed is known to be used as a feed ingredient including fish feed, being a source of nutrients and energy, a binder, a thickening agent and a balance regulator (El-Deek & Brikaa 2009).

Seaweed generally contains essential minerals, nucleic acids, amino acids, protein and vitamin A, C, D, E, and K. Another important chemical class contained in seaweed is carbohydrates, in the form of polysaccharides such as agar. The polysaccharides and carrageenan present in K. alvarezii, are considered as an additional source of dietary fiber due to their unique physicochemical properties and structural diversity, which exert various effects on the physiological functions of the body. Carrageenan provides a wide variety of biological properties, including immune modulation, anti-cholesterol, anticancer, antiviral, antioxidant and lipid-lowering properties (Sokolova et al 2014). Potassium in sufficient quantities in *K. alvarezii* is capable of producing physiological changes (Wanyonyi et al 2017). Kappa carrageenan is a hydrocolloid used as a food additive, acting as a gelling agent, emulsifier (Chang et al 2017). The content of *K. alvarezii* plays a very important role in the growth process and supports the survival rate of crabs.

Mud crabs (*Scylla olivacea*) is one of the species commonly found in Indonesian waters among *S. serrata*, *S. tranquebarica* and *S. paramamosain* (Alimuddin et al 2019) and also one of the main fisheries commodities, with important economic value. The high market demand encourages aquaculture farmers to increase crab productions, both for enlargement, soft shell crabs, egg laying crabs and crab fattening. In principle, crab fattening maintains large crabs which, in terms of weight, are still below the standard of consumption size. Crab fattening has great potential to be developed because it only requires a small capital, short aquaculture time and simple technology (Karim et al 2017). In Indonesia, the maintenance time is of 14–21 days (Putranto 2007), 25–35 days in Vietnam (Dat 1999).

One of the determinants of the success of the crab fattening business is the feed needed to provide energy for the activity of body cells (Karim 2005). Apart from feed, the rearing container is also an important factor to consider in crab fattening activities. Crab box is a container for individual crab maintenance in order to prevent cannibalism and competition for space and food. Based on the above mentioned facts, a research was conducted on the effect of seaweed *K. alvarezii* on the biological evaluation of *S. olivacea* in the fatting activities using the crab box method in ponds.

Material and Method

Description of the study sites. This research was conducted in July-August 2020 in Tambak Desa Mabbiring, Sibulue District, Bone Regency, South Sulawesi Province (Figure 1). Water quality testing was carried out at FIKP Water Quality Lab, Unhas, Makassar.

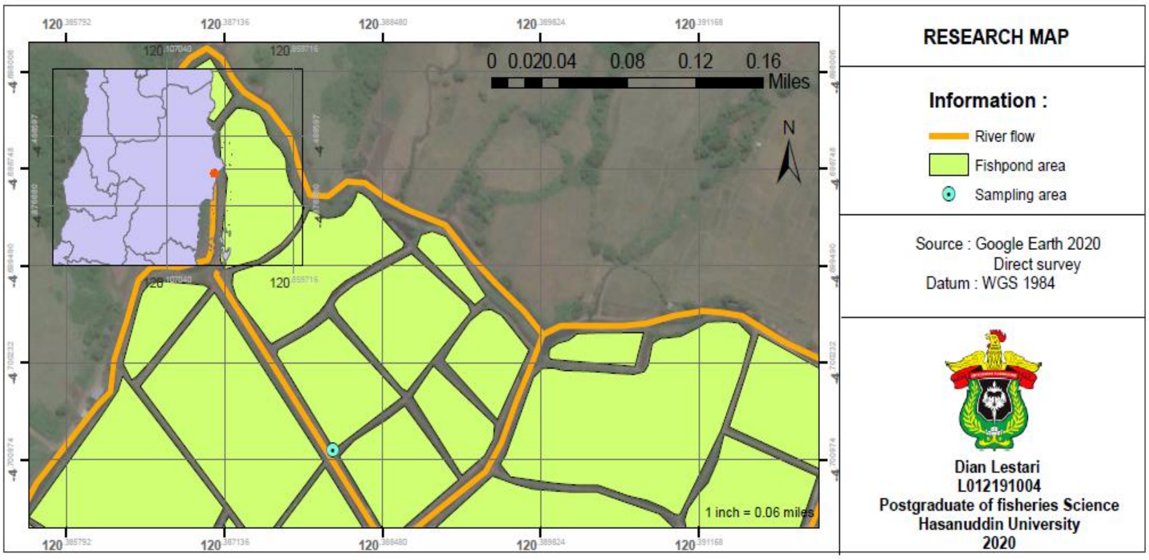

Figure 1. The location of research crab pond at Bone, South Sulawesi.

Making formulation feed. The test feed used was artificial feed in the form of meatball feed formulated with various concentrations of seaweed *K. alvarezii*. The feed ingredients used were *K. alvarezii meal*, fish meal, shrimp paste, corn meal, starch, fish oil, vitamin, mineral mix and carboxymethyl cellulose (CMC). The manufacture of feed started from weighing the raw materials according to their composition (Table 1), then continued with mixing all the raw materials until they were homogeneous. After that, the mixture was steamed for 20 minutes and then the feed was cut into pieces. The nutritional composition of *S. olivacea* feed used was about 47 to 48% protein content (Table 2).

Table 1

Test feed formulation

Feed raw materials	*Kappaphycus alvarezii doses*			
	0%	*10%*	*20%*	*30%*
Fish meal	51	48	45	42
Shrimp paste	10	10	10	10
Corn meal	2	6	9	13
Starch	30	20	10	0
Kappaphycus alvarezii meal	0	10	20	30
Fish oil	2	2	2	2
Vitamin min mix	2	2	2	2
CMC	3	2	2	1

Table 2

Test nutrition composition

Sample	*Composition (%)*					
	Moisture	*Crude protein*	*Crude fat*	*Crude fiber*	*NFE*	*ASH*
Feed A	12.65	46.93	5.68	0.81	32.24	14.34
Feed B	14.27	47.08	5.63	2.79	27.57	16.92
Feed C	15.75	47.51	5.63	4.76	22.49	19.61
Feed D	17.38	47.66	5.58	6.68	17.97	22.11

NFE-Nitrogen free extract.

Preparation of research containers. The preparation of the research container which is carried out starts from the preparation of the pond including drying and infusing water in the pond (Figure 1). The pond preparation is carried out to support the process of crab maintenance activities so that they can grow optimally (Karim 2013). After preparing the pond, the raft and crab box were installed (Figure 2). The raft in the study was made of 1.5 inch paralon pipe and the crab box was installed on the pipe raft. There are 120 crab compartments of a size of 20 x 14 x 15 cm.

Figure 1. Drying and infusing water in the pond at Bone, South Sulawesi (original).

Figure 2. Installing the raft and crab box at Bone, South Sulawesi (original).

Acclimatization of test animals. The test specimens used were 120 *S. olivacea* males, 30 at each treatment, with a body weight of 130-180 g. Before being spread, the crabs were weighed and then put in a tagging crab box. Furthermore, the crabs were fasted for 24 hours. During the acclimatization process, crabs were fed with trash fish alternating with artificial feed.

Maintenance of test animals. Maintenance of crabs was carried out in a crab box placed on a pipe raft. Maintenance was carried out for 35 days. Feed was given as much as 10% of body weight day^{-1}, twice a day, namely 30% in the morning (at 06.30 WITA) and 70% in the afternoon (at 05.30 WITA), related to the total feed given (Karim et al 2016). The uneaten feed was weighed.

Biological evaluation*.* Biological evaluations carried out in this research were: absolute growth, survival rate and feed efficiency ratio.
The absolute growth was calculated according to the following formula (Effendie 1979):

$$h = Wt - W0$$

Where:
h-individual average absolute growth (g);
Wt-individual average body weight at time t (g);
W0-individual average initial weight (g).
The survival rate was calculated according to the formula (Effendie 1979):

$$S (\%)=(Nt/N0)x100$$

Where:
S-survival rate (%);
Nt-numbers of living animals at the end of the research (crabs);
No-numbers of living animals at the beginning of the research (crabs);
The feed efficiency ratio was calculated according to the formula (Effendie 1979):

$$EP (\%) = (Wt-W0/F)x100$$

Where:
EP-feed efficiency (%);
Wo-weight of test animals at the beginning of the research;
Wt-weight of test animals at the end of the research;
F-quantity of feed consumed.

Statistical analysis*.* To determine the effect of treatment on the biological response of *S. olivacea*, analysis of variance (ANOVA) was used. When the treatment had a significant effect on these parameters it was proceeded to the W-Tukey test ($p<0.05$). Statistical analyzes were performed using IBM SPSS Statistics software version 24.

Results. Based on the analysis of variance ($p<0.05$), it was showed that the provision of seaweed *K. alvarezii* gave different responses in terms of absolute growth and feed efficiency of crabs. However, it gave a similar response to the survival rate.

Table 3
Absolute growth, survival rate, and feed efficiency (mean±standard deviation)

Treatment	*Absolute growth (g)±St dv*	*Survival rate (%)±St dv*	*Feed efficiency (%)±St dv*
0%	31.2333±1.2342[a]	76.6667±11.5470[a]	13.1770±0.2497[a]
10%	34.0667±4.0216[ab]	76.6667±15.2752[a]	13.7401±0.7136[a]
20%	42.1667±4.5742[b]	83.3333±11.5470[a]	16.1523±1.0758[b]
30%	54.8333±4.0550[c]	96.6667±5.7735[a]	19.0665±1.1663[c]

Different letters indicate significant differences between treatments at the level 5% (p<0.05).

Based on the results of the proximate analysis of the feed used (Table 2), it can be seen that with the increasing dose of seaweed *K. alvarezii*, the levels of protein, fat and carbohydrate feed also increased. Feed protein ranged from 47 to 48%, which plays a role in the growth process of crabs. A good range of protein content for *S. olivacea* feed is 34 to 54% (Anderson et al 2004). Crabs need feed to maintain life and growth, and will grow well if the available feed contains all the nutrient elements needed in optimal levels (Aslamyah & Fujaya 2010). The content of seaweed is generally essential minerals (iron, iodine, aluminum, manganese, calcium, soluble nitrogen, phosphorus, sulfur, chlorine, silicon, rubidium, strontium, barium, titanium, cobalt, boron, copper, potassium, and other elements), nucleic acids, amino acids, proteins, minerals, trace elements, flour, sugars and vitamin A, C, D, E, and K. Another important chemical content is carbohydrates in the form of polysaccharides such as agar. These contents are very important in the growth process and support the survival rate of *S. olivacea*.

Table 4 reports on the water quality measurements of the maintenance media including physical and chemical parameters: temperature, salinity, degree of acidity, dissolved oxygen and ammonia levels.

Table 4

The results of water quality measurement in the maintenance media

Parameters	*Value range*
Temperature (^{0}C)	22-25
Degree of acidity (pH)	6-8
Salinity (ppt)	3.5-14.73
Dissolved oxygen (DO) (ppm)	1.9-10.9
Ammonia (ppm)	0.004-0.007

Discussion. The absolute growth, survival rate and feed efficiency of crabs increased with the increasing dose of seaweed *K. alvarezii* (Table 3). Growth in crabs is characterized by changes in shape and size, weight or length due to differences in growth speed of different body parts. As an animal that has an exoskeleton or shell, growth in crabs is marked by molting (Warner 1997). Their growth depends on the increase in length and weight of each molting crab (Sulaiman & Hanafi 1992).

Growth is manifested by the addition of protein content, fat, carbohydrate, ash and water content in the body of organisms. In terms of energy, growth occurs when the energy consumed is greater than the energy spent on various body activities. If the environment is optimal, crab growth is greatly influenced by the feed given. Feed that contains complete and balanced nutrition will stimulate growth. Growth will occur when the feed consumed has a high protein content and a positive energy balance.. In this case, proteins are used as a building block of the body for growth, while fat and carbohydrates are used as an non-protein energy sources. Feed protein can be used efficiently for the formation of new tissue. When the availability of energy from non-proteins in the feed is higher, the proteins consumed can be used more efficiently to to be stored in the body. Providing the right amount of feed is very important in fattening: it represents the highest operational expense and it affect thequality of water, inhibiting the growth of *S. olivacea*, when added in excess (Maulana et al 2012).

The high survival rate of *S. olivacea* was due to the maintenance in individual crab boxes, thus preventing cannibalism, which is the main factor causing death in crab rearing. This is also supported by the results of the research studies conducted by Fadnan (2010) and Tugiyono (2011), showing that *S. olivacea* that are kept at a density of 1 crab m^2 can produce a survival rate 100%, while the survival rate of *S. olivacea* that are maintained with a density of 2-4 individuals m^2 only reach 50-77.77%. Thus, *S. olivacea* that are kept in a single room (crabs are raised individually in a rearing container) do not experience competition for space or feed, so they are safe from threats and disturbances from other crabs.

Feed efficiency shows the proportion of feed that can be used for growth. The more available the feed in the maintenance container, the higher the chances to be consumed, increasing the growth of *S. olivacea* (Maulana et al 2012). Moreover, a higher protein content improves the feed conversion ratio (Karim 2005). The use of feed is very dependent on the suitability of the quantity and quality of feed given. The efficiency of feed utilization is also influenced by environmental factors, age and feed ingredients used. Energy used can be reduced if crabs are kept in optimal environmental conditions.

The water quality of the crab rearing media is the temperature ranging from 22-25°C (Table 4). Water temperatures lower than 20°C can cause activity and appetite to drop dramatically. Very high temperatures are also not tolerable for *S. olivacea*. Temperatures above 42.1°C can cause death. The optimum temperature for *S. olivacea* is 20-42°C (Kordi 2007). A temperature that is less or more than the optimum temperature will affect the growth of crabs because of a decrease in metabolic reactions. Fluctuations will cause stress on the *S. olivacea* and can lead to death. High temperature waters tend to increase the growth and molting period of crabs (Karim 2013).

The salinity of the maintenance media during the research was 3.5-14.73 ppt. Crab is an organism that can tolerate a wide range of salinity. Crabs are able to tolerate a salinity of 1-42 ppt. The process of adaptation to salinity conditions is carried out through the osmoregulation process, which is the process of regulating the osmotic pressure in the body to match the osmotic pressure of the medium. This osmoregulation process requires a certain amount of energy obtained from the feed consumed (Karim 2013). Although the salinity during the research was classified as low, many adult *S. olivacea* were able to live successfully at low salinity because of their ability to regulate osmosis well (Soim 1995). Salinity is one of the most important abiotic factors affecting aquatic organisms (Misbah et al 2017).

The degree of acidity of the maintenance media during the research was 6-8. The slightly acidic conditions of mangrove crabs can still survive with a fairly good pH tolerance ability. At high or low pH, there will be an increase in energy use or a decrease in energy production as well as a suppression of the energy metabolism. The optimum pH for maintanance of *S. olivacea* ranges between 6.5-8 (Karim 2013).

Dissolved oxygen in the maintenance media is 1.9-10.9 ppm. Dissolved oxygen content needed by crabs is ≥3 mg L^{-1}. Low dissolved oxygen will cause a reduced appetite, and a low level of utilization, which affects physiological processes such as behavior, survival rate, metabolism, molting and crab growth. Low dissolved oxygen levels cause the inability of organisms to provide high energy requirements for organisms to eat well (Karim 2013).

The ammonia level of the maintenance media is 0.004-0.007 ppm. The optimum ammonia content in crab aquaculture is <0.1 ppm. Ammonia is the main product compound of nitrogen waste, which comes from the waste of organic materials containing nitrogen compounds such as protein and residual excretion of organisms. Ammonia also comes from undigested feed, feces and food waste. In *S. olivacea* aquaculture, ammonia is an indicator of amino acid catabolism and adenylate deamination in the purine nucleotide cycle. In higher concentrations, ammonia will poison organisms (Karim 2013).

Conclusions. Based on the results of the current research, it can be concluded that the addition of 30% of seaweed *K. alverezii* to the feed gives a significant response to the biological evaluation of *S. olivacea* maintaned in the fattening system in ponds.

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