BIODIVERSITAS
Volume 22, Number 1, January 2021
Pages: 424-431

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220152

Effects of *Caulerpa lentillifera* added into culture media on the growth and nutritional values of *Phronima pacifica*, a natural fish-feed crustacean

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Manuscript received: 14 November 2020. Revision accepted: 28 December 2020.

Abstract. *Herawati VE, Pinandoyo, Ariyati RW, Rismaningsih N, Windarto S, Prayitno SB, Darmanto YS, Radjasa OK. 2021. Effects of* Caulerpa lentillifera *added into culture media on the growth and nutritional values of* Phronima pacifica, *a natural fish-feed crustacean Biodiversitas 22: 424-431. Phronima pacifica* is a microcrustacean commonly used as natural feed in fish farming as it has high nutrient contents and is suitable based on larval fish mouth gap size and mass cultivation capacity. However, *P. pacifica* is not produced optimally and its supply is inadequate, so that *P. pacifica* culturing techniques need to be improved. The culture of *P. pacifica* is mostly done using sea grape seaweed (*Caulerpa lentillifera*). The aim of this study was to investigate the optimal stocking density of *C. lentillifera* added into media culture on the growth and nutritional values of *P. pacifica*. We applied a complete random design experiment with four treatments of stocking density of *C. lentillifera* (i.e., 0 g/m^2 (Control), 20 g/m^2, 40 g/m^2, and 60 g/m^2) with 3 replicates for each treatment. The following parameters were analyzed, namely specific growth rate (SGR) of *C. lentillifera*, and the growth, biomass, growth rate, proximate analysis, and amino and fatty acid concentrations of *P. pacifica*. This study found that the addition of *C. lentillifera* into culture media significantly enhanced the growth of *P. pacifica*. The highest effect on the growth was observed in the treatment of 60 g/m^2 with *C. lentillifera* SGR value of 4.76 ± 0.12%/day, and *P. pacifica* growth rate, population density and biomass of 4.41 ± 0.12 ind/day, 53.81 ± 8.79 ind/L, and 1.14 ± 0.14 g, respectively. Similarly, the highest protein and fat contained in *P. pacifica* was obtained in the treatment of 60 g/m^2 with 60.23% protein and 10.24% fat. Furthermore, the highest fatty acid profile was C20: 5n-3 of 13.23% ± 0.08% and amino acid profile of 45.23% ± 0.01%. The application of the optimal stocking density of *C. lentillifera* in culture media could increase the growth and nutritional quality of *P. pacifica* which in turn could enhance aquaculture productivity.

Keywords: *Caulerpa lentillifera*, density, growth, nutritional quality, *Phronima pacifica*

INTRODUCTION

Amphipods serve as a natural source of feed for aquaculture activities (Rojano et al. 2014). Amphipods also have the potential to be used in integrated cultivation (IMTA) in ponds (Baeza-Rojano et al. 2013). Amphipod's shape is quite varied, there is no difference between the body size of marine and fresh amphipods. In some Amphipods, it has a thin, transparent cuticle and the muscles are weakly developed. Amphipods generally have slightly rounded bodies and are weak swimmers, being benthic-pelagic organisms. Some Amphipods have rather compact bodies with strong muscles and can swim very fast. Amphipods have a unique characteristic that is they can experience conglobation (rolling into a ball), which is turning their bodies round. The function of this conglobation is to protect itself from predators and to drown (Aoki et al. 2013). They are known as natural feed source applied in aquaculture activities (Baeza-Rojano et al. 2013) and has the potentials to be used in integrated aquaculture in ponds/fish cages (Jiménez-Prada et al. 2018). Amphipoda has six sub-orders, namely Pseudingolfiellidea, Hyperiidea, Colomastigidea, Hyperiopsidea, Senticaudata and Amphilochidea (Lowry and Myers 2017). One of the identified genera is *Phronima* spp. which belongs to Hyperiidea sub-order (Bishop and Geiger 2006).

Phronima pacifica is a species of Amphipoda microcrustacean that inhabits sea waters at a depth of 0-25 m below sea surface (Elder and Siebel 2015). *P. pacifica* has high nutrient contents, and it is commonly used to meet the nutrient requirements of brackish water fish larvae during the hatchery stage. In addition, the feed can fit through the mouth gaps of fish larvae, and it can be mass-cultivated (Herawati et al. 2014). *P. pacifica* is a non-selective filter feeder, therefore nutrients can be added through aquaculture media (Aoki et al. 2013).

In a food chain of aquaculture system, *P. pacifica* uses phytoplankton as one of the feeds in addition to bacteria and detritus in a culture media. As such, the type of culture media used greatly affects the nutritional quality of phytoplankton, bacteria and detritus which eventually affects the nutrient contents of *P. pacifica* (Damle and

Chari 2011; Herawati et al. 2020). One of such culture media is sea grape (*Caulerpa lentillifera)*.

C. lentillifera is a green seaweed of high economic value with the potential to drive development in the aquaculture industry. The entire *C. lentillifera* structure is a stem organ called a thallus. Thalli that die become detritus, which serve as feed for other organisms, so that seaweed provide habitat, shelter, spawning grounds, in addition, to feed that supports *P. pacifica* growth (Parker and Maria 2015). Nutrient concentrations in detritus affect the organic matter content in the culture media, and in turn the population density of *P. pacifica* as they can get the associated feed. The more sea grapes planted in a culture pond, the more *P. pacifica* individuals that will stick to the thalli.

However, to date, the optimal stocking density of *C. lentillifera* used as a culture medium in rearing *P. pacifica* is unknown. Previous study indicated that *Phronima* treated with fermented organic waste gave results with a peak population of 98 individuals / L that occurred on the 16th day of aquaculture, and the highest biomass of 0.51 g (Herawati et al. 2020). The highest nutrient content was obtained at the highest protein proximate analysis value of 58.90%, the proportion of the fatty acids comprised of eicosapentaenoic acid of 7.53%, and lysine amino acids of 44.16 ppm.

Only few studies have explored sea grape cultivation to support *P. pacifica* growth. The purpose of the present study was to investigate the optimal stocking density of *C. lentillifera* for *P. pacifica* growth and nutritional quality. To achieve this aim, varying stocking densities of *C. lentillifera* were applied in culture media, then the following parameters were to be analyzed, namely specific growth rate (SGR) of *C. lentillifera*, and the growth, biomass, growth rate, proximate analysis, and amino and fatty acid concentrations of *P. pacifica*. The results of the present study could facilitate the development of the *P. pacifica* aquaculture industry.

MATERIALS AND METHODS

Materials

The materials for experiment in the present study were 2 kg of *Caulerpa lentillifera* seeds, and *Phronima pacifica* with stocking density of 3 ind/L. The materials were obtained from the Brackish Water Cultivation Aquaculture Fisheries Center (*Balai Besar Perikanan Budidaya Air Payau*), Jepara, Central Java, Indonesia.

Experimental procedure

Before culturing *P. pacifica*, *C. lentillifera* stocking was first carried out for 7 days so that the nutrients in the organic fertilizer would be absorbed by *C. lentillifera*. Subsequently, *P. pacifica* stocking was performed at a density of 3 ind/L. During the *P. pacifica* rearing, up to 60 mL of organic fertilizer (cow manure about 50 g/container that has been dried) was added every 3 days. The container used was made of fiber with a tank size of 60 L, moreover, the water exchange was done every day.

The experimental design applied was a completely randomized design (CRD) with four treatments and three replicates for each treatment. The treatments were as follow: Treatment A (Control): 0 g/m^2 *C. lentillifera*; Treatment B: 20 g/m^2 *C. lentillifera*; Treatment C: 40 g/m^2 *C. lentillifera*; and Treatment D: 60 g/m^2 *C. lentillifera*.

Water quality parameters

Water quality parameters, including temperature, dissolved oxygen (DO), salinity and pH, were measured daily using a thermometer (Checktemp Digital Thermometer model HI98501, Hanna Instrument, Japan), a DO meter (AMTAST DO-820) and a pH meter (Jellas model PH-061), respectively. Water quality measurements were used to maintain *P. pacifica* individuals in stable condition. Water quality measurement data obtained are presented in ranges and compared with values in literature. The water quality data are presented in Table 1.

Data collection

Sampling for growth analyses was carried out every 2 days for *P. pacifica* and every 7 days for *C. lentillifera* up to 42 days of rearing. The analyses were performed at the exponential phase on the 10th day during *P. pacifica* rearing. The analysis was performed at the exponential phase because *P. pacifica* has the highest nutritional quality during its growth, therefore, it is also the recommended time for harvesting. Moreover, Fogg (1965) stated that the highest nutritional quality of *P. pacifica* is in the exponential phase so it is suitable as natural feed given in aquaculture.

Data analysis

Growth rate of Phronima pacifica

Growth rate was calculated according to Krebs (1972) using the following formula:

$$r = \frac{\ln N_t - \ln N_0}{t}$$

Where: r: growth rate (individuals/day); t: time required to achieve maximum growth (days); N_t: *P. pacifica* density on t day (individuals/L); N_0: *P. pacifica* initial density (individuals/L).

Table 1. Water quality parameters

Variables	Value ranges	Reference values
DO (mg/L)	3.5-4.5	2.6-4.9*
pH	8	8-9*
Temperature (°C)	28-30	30-38*
Salinity	28	25-28**
Nitrite (ppm)	0.031-0.075	0.056-1.329**
Nitrate (ppm)	0.008-0.440	0.063-0.03***
Phosphate	0.135-1.605	0.098-1.705***

Note: *: Fattah et al. (2014); **: Fattah et al. (2019); ***: Azizah (2006)

Population density of Phronima pacifica

Phronima pacifica was evaluated every 2 days. Since *P. pacifica* are attached to surfaces in clusters, sampling was carried out by stirring the culture media and then obtaining samples at the densest sampling points: front, middle, and back of the container as much as 1 L of water. The counting was done three times. After the evaluation was performed, the *P. pacifica* was put back in the container.

Increase of biomass of Phronima pacifica

Phronima pacifica biomass was determined by weighing at the beginning of stocking and weighing *P. pacifica* at the end of the rearing. The biomass weight was calculated by harvesting all *P. pacifica* using planktonets, then separating the water from the *P. pacifica*. Furthermore, the sample was weighed with an accuracy of 0.01 gram. The initial weight of *P. pacifica* was approximately 0.01 grams. The biomass was calculated using the formula presented by Krebs (1972):

$$W = W_t - W_0$$

Where: W: Increase in biomass (g); $W_{0:}$ Initial weight (g); W_t: Final weight (g)

Proximate analysis

The parameters used for proximate analysis were protein, fat, ash, crude fiber, and water. The analysis was performed once the sample is dried. The samples used for the analysis of amino acids and fatty acids were 10 grams of the dry weight each, and 20 grams of dry weight for proximate analysis. Lipid samples were analyzed using the gravimetric method using the weight of the sample to find the lipid levels. The proximate chemical composition of the samples was determined using a standard procedure (AOAC 2005; Herawati et al. 2017). Protein analysis was performed using the Kjeldahl method, while carbohydrate analysis was carried out manually based on the results of the proximate analysis.

Essential amino acid profile

The essential amino acid profile of *P. pacifica* was determined by examining its essential amino acid contents. Essential amino acid analysis was conducted using high-performance liquid chromatography (HPLC) type 1100 system with a Eurospher 100-5 C18, 250 × 4.6 mm column with a P/N: 1115Y535 pre-column. The effluents were: A) 0.01 M acetate buffer at pH 5.9; and B) 0.01 M MeOH acetate buffer at pH 5.9; THF> 80:15:5 Λ Fluorescence: Ext: 340 mm Em: 450 nm. About 2.5 g of sample was put into a sealed glass and 15 mL 6M HCl was added. Subsequently, the mixture was *vortexed* for homogeneity and subjected to hydrolysis in an autoclave at 110ºC for 12 h, before being cooled to room temperature and neutralized with 6M NaOH. After the addition of 2.5 mL 40% lead acetate and 1 mL 15% oxalate acid, approximately 3 mL of the mixture was filtered with a 0.45 µm Millex-HV filter (Merck KGaA, Darmstadt, Germany). Twenty-five microliters of the filtered mixture plus 475 µL of OPA anhydrolase solution were *vortexed* and incubated for 3 min for injection into the HPLC system. Finally, 30 µL of the final mixture was placed into the HPLC system. The amino acid composition of the sample was determined using a HPLC system (Shimadzu LC-6A, Shimadzu, Kyoto, Japan) (AOAC 2005; Herawati et al. 2017).

Fatty acid profile

The fatty acid profile of *P. pacifica* can be determined by analyzing its total fatty acid contents. The equipment used for the analysis was a QP-2010 Gas Chromatograph-Mass Spectrophotometer (GCMS) (Shimadzu) and the Mass Spectrophotometer had a 50 m long, 0.22 mm diameter Wall Coat Open Tubular CP-SIL-88 column (Agilent, Santa Clara, CA, USA), with analysis performed at 120-200°C column temperature range. The method employed was in-situ transcertification. One-hundred milligram samples of *P. pacifica* were homogenized using 4 mL of water. The obtained 100 µL homogenates were subsequently transferred into reaction tubes. One-hundred microliters of methylene chloride were then added, along with 1 mL of 0.5M NaOH in methanol. Once nitrogen was added and the tubes sealed, they were heated to 90ºC for 10 min. The reaction tubes were then cooled and 1 mL 14% BF_3 in methanol added. After nitrogen addition, heating ensued at the same temperature for 10 minutes. Afterward, the reaction tubes were cooled to ambient temperature, and 1 mL of water and 200-500 µL of hexane added. The mixtures were then *vortexed* for 1 min to extract the methyl ester of the fatty acid. After centrifuging, the upper layer of sample was ready for GC analysis. The fatty acid composition of the sample was determined using a gas chromatograph (Shimadzu) (AOAC 2005; Herawati et al. 2017).

Statistical analysis

Data obtained for *P. pacifica* included specific growth rate, relative growth rate, population density, and nutritional quality. The data were first tested for normality, homogeneity of variance and additivity. If the above assumptions were satisfied, one-way Analysis of Variance (ANOVA) was conducted to determine the effects of different sea grape stocking densities after 49-d rearing on *P. pacifica* growth. If there were significant ($P < 0.05$) or highly significant ($P < 0.01$) effects, the Duncan's Multiple Range Test (DMRT) was performed to determine differences between treatments, and to identify the optimal treatment.

RESULTS AND DISCUSSION

This study revealed that the density of *C. lentillifera* used in culturing activities affected the growth of *P. pacifica* based on the parameters of Specific Growth Rate (SGR) of *C. lentillifera*, and the growth rate, population density, biomass and nutritional quality of *P. pacifica*.

Specific growth rate of *Caulerpa lentillifera*

The result of Specific Growth Rates of *C. lentillifera* under various stocking densities is presented in Figure 1.

The highest SGR of *C. lentillifera* was observed under the treatment of 60 g/m^2 at 4.76 ind/day. According to the ANOVA results, the density *C. lentillifera* significantly influenced *P. pacifica* SGR ($P < 0.05$). In addition, according to Duncan's Multiple Range test, SGR under the treatment of 0 g/m^2 was lower than those of 20, 40, and 60 g/m^2. The SGR under the 20 g/m^2 is significantly lower than the SGRs under the 40 g/m^2 and 60 g/m^2 treatments, while there was no significant difference in SGR between the 40 g/m^2 and 60 g/m^2 treatments.

Growth rate of *Phronima pacifica*

The results of growth rate of *P. pacifica* under the four *C. lentillifera* stock densities are presented in Figure 2. The highest growth rate of *P. pacifica* was observed under the 60 g/m^2 treatment at 4.41 ind/day. Based on the ANOVA analysis of growth rates of *P. pacifica*, it showed that stocking density of *C. lentillifera* significantly influenced *P. pacifica* growth density ($P < 0.05$).

Population density of *Phronima pacifica*

Phronima pacifica population density exhibited a sigmoid growth trend, and the results obtained after culturing under different *C. lentillifera* stocking densities for 40 days and recorded every 2 days are presented in Figure 3. The exponential phase represents the multiplication of individual as the result of reproduction. The exponential phase was on the 10th day of observation, with the highest population being 61 ind/L, followed by the stationary phase, which occurred on the 18th day of observation with the highest population being 98 ind/L. The death phase fell under the 30th day of observation with the highest population density being 28 ind/L.

This data relates to nutritional analysis because *P. pacifica* has the highest nutritional quality observed during exponential phase. Therefore, the nutritional analysis was conducted during exponential phase.

The biomass of *Phronima pacifica*

Phronima pacifica biomass results on a wet weight basis are presented in Figure 4. The highest weight of *P. pacifica* biomass during the study was in the treatment D (1.14 g) and the lowest was in treatment A (0.83 g).

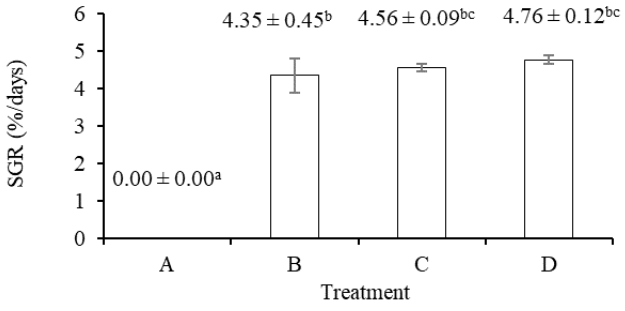

Figure 1. Specific growth rates of *Caulerpa lentillifera* under various stocking densities. A (Control): 0 g/m^2; B: 20 g/m^2; C: 40 g/m^2; D: 60 g/m^2. Different superscript letters indicate significant differences between treatments ($p < 0.05$)

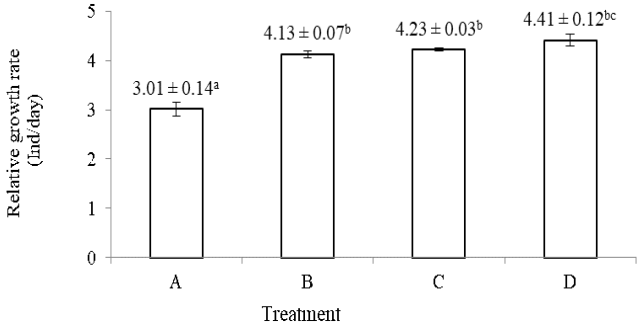

Figure 2. Relative growth rates of *Phronima pacifica* cultured under different *Caulerpa lentillifera* stocking densities. A (Control): 0 g/m^2; B: 20 g/m^2; C: 40 g/m^2; D: 60 g/m^2. Different superscript letters indicate significant differences between treatments ($p < 0.05$)

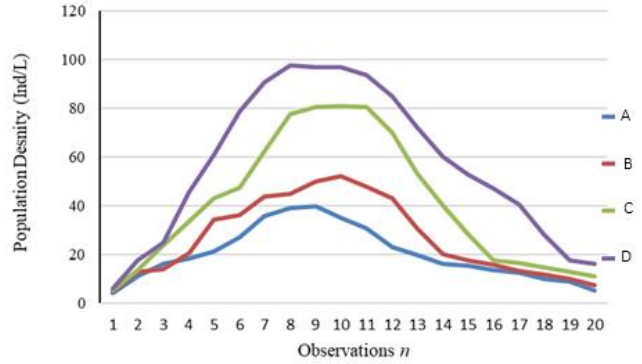

Figure 3. Trend of *Phronima pacifica* population density under varying *Caulerpa lentillifera* stocking densities for 40 days recorded every 2 days. A: 0 g/m^2; B: 20 g/m^2; C: 40 g/m^2; D: 60 g/m^2. Different superscript letters indicate significant differences between treatments ($p < 0.05$)

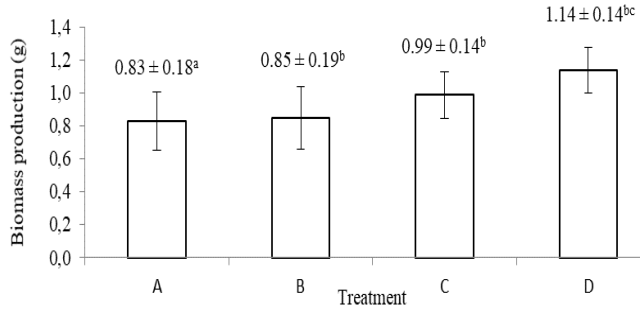

Figure 4. *Phronima pacifica* biomass under varying *Caulerpa lentillifera* stocking densities. A (Control): 0 g/m^2 B: 20 g/m^2 C: 40 g/m^2 D: 60 g/m^2. Different superscript letters indicate significant differences between treatments ($p < 0.05$)

Nutrient content in Phronima pacifica

The highest protein and fat contained in *P. pacifica* under different *C. lentillifera* stocking densities were produced by the 60 g/m^2 treatment with 60.23% and 10.24%, respectively, while the lowest levels were 45.26% and 6.04%, respectively, which were observed under the 0 g/m^2 treatment. The proximate analysis results are presented in Table 3.

Phronima pacifica cultured under different *C. lentillifera* stocking densities had varying fatty acid types. Among the fatty acids contained in *P. pacifica*, EPA was the type of fatty acid that is most commonly found. EPA (C20: 5n-3) was the most commonly found fatty acid type. *P. pacifica* cultured under the treatment of 60-g/m^2 stock density had the highest C20: 5n-3 (13.23% ± 0.08%), while the lowest EPA (C20: 5 n-3) was observed in *P. pacifica* cultured under the 0 g/m^2 treatment (6.19% ± 0.08%). DHA (C22: 6n-3) levels contained under the treatment of 60-g/m^2 stock density were the highest among other treatments (6.07 ± 0.03%), as well as the ARA content (C20: 4n-6) contained under the treatment of 60-g/m^2 stock density was the highest (8.23 ± 0.07%). The results total fatty acid in *P. pacifica* under varying *C. lentillifera* stocking densities are presented in Table 4.

Lysine was the most common type of amino acid found under the treatment of 60-g/m^2 *C. lentillifera* stocking density (45.23% ± 0.01%), followed by aspartic acid (37.94 ± 0.01%) and methionine (32.40 ± 0.07%). *P. pacifica* amino acid profiles under varying *C. lentillifera* stocking densities are presented in Table 5.

Discussion

This study found that the addition of sea grape (*C. lentillifera*) into cultured media significantly enhanced the growth of *P. pacifica*. *Caulerpa* spp. is native to warm tropical waters and is relatively easy to propagate. Several factors influence *Caulerpa* spp. growth, including salinity, temperature, nutrient availability and light (Mosquera and Salamanca 2016). According to Darmawati (2015), the population density of *P. pacifica* increased with an increase in stocking density. Similarly, in the present study, the highest *P. pacifica* growth rate was observed under the highest *C. lentillifera* stocking density (60 g/m^2). This is because *P. pacifica* not only feeds on algae but also detritus. Consequently, since *C. lentillifera* is an alga in which the entire plant structure is a thallus, the dead thallus would also become detritus, which also provides nutrients for *P. pacifica*.

Table 2. Population density of *Phronima pacifica* in each stage under varying *Caulerpa lentillifera* stocking densities

Growth phase	Population density (ind/L)			
	Treatment A	Treatment B	Treatment C	Treatment D
Lag	21 ± 0.25^a	24 ± 0.36^a	30 ± 0.18^b	40 ± 0.08^b
Exponential	28 ± 0.09^a	35 ± 0.78^b	60 ± 0.68^b	75 ± 0.04^b
Stationary	39 ± 0.25^a	55 ± 0.09^b	80 ± 0.48^b	98 ± 0.96^b
Death	19 ± 0.19^a	20 ± 0.08^a	18 ± 0.45^a	19 ± 0.98^a

Notes: Treatment A (*C. lentillifera* 0 g/m^2); B (*C. lentillifera* 20 g/m^2); C (*C. lentillifera* 40 g/m^2); D (*C. lentillifera* 60 g/m^2). Different superscript letters indicate significant differences between treatments ($p < 0.05$)

Table 3. Proximate analysis results for *Phronima pacifica* under varying *Caulerpa lentillifera* stocking densities

Treatments	Dry weight content percentage				
	Protein (%)	Carbohydrate (%)	Crude fat (%)	Ash (%)	Crude fiber (%)
A	45.26 ± 0.03^a	18.80 ± 0.02	6.04 ± 0.04^a	24.27 ± 0.07	5.63 ± 0.06
B	52.90 ± 0.04^b	17.72 ± 0.03	6.24 ± 0.03^a	17.69 ± 0.03	5.45 ± 0.08
C	55.45 ± 0.02^b	17.5 7 ± 0.05	8.57 ± 0.02^b	15.63 ± 0.03	3.78 ± 0.03
D	60.23 ± 0.01^c	15.22 ± 0.03	10.24 ± 0.03^c	11.86 ± 0.03	2.45 ± 0.08

Notes: Treatment A (*C. lentillifera* 0 g/m^2); B (*C. lentillifera* 20 g/m^2); C (*C. lentillifera* 40 g/m^2); D (*C. lentillifera* 60 g/m^2). Different superscript letters indicate significant differences between treatments ($p < 0.05$)

Table 4. Total fatty acid in *Phronima pacifica* under varying *Caulerpa lentillifera* stocking densities

Fatty acid profile (%)	Treatment of *C. lentillifera* stocking density			
	A (0 g/m^2)	B (20 g/m^2)	C (40 g/m^2)	D (60 g/m^2)
C14:0	9.52 ± 0.09^b	10.49 ± 0.06^c	5.41 ± 0.02^a	5.48 ± 0.09^a
C15:0	6.09 ± 0.08^a	7.18 ± 0.03^c	6.17 ± 0.04^b	6.15 ± 0.08^b
C16:0	5.14 ± 0.07^a	6.29 ± 0.09^b	7.97 ± 0.08^c	8.59 ± 0.04^d
C18:0	2.71 ± 0.05^a	6.65 ± 0.01^d	5.52 ± 0.03^c	4.91 ± 0.09^b
C18:1 n-9	3.07 ± 0.03^a	4.95 ± 0.03^b	4.89 ± 0.08^b	6.61 ± 0.01^c
C18:2 n-6	4.83 ± 0.09^a	5.46 ±0.07^b	6.49 ± 0.07^c	8.07 ± 0.02^d
C18:3 n-3	3.54 ± 0.02^a	4.76 ± 0.08^b	4.89 ± 0.03^c	7.32 ± 0.01^d
C20:2	2.30 ± 0.04^a	2.83 ± 0.02^b	6.02 ± 0.04^c	3.05 ± 0.03^d
C20:4 n-6	2.71 ± 0.03^a	4.15 ± 0.03^b	6.15 ± 0.09^c	8.23 ± 0.07^d
C20:5 n-3	6.19 ± 0.08^a	9.64 ± 0.07^b	10.88 ± 0.02^c	13.23 ± 0.08 ^{d}
C22:6 n-3	2.23 ± 0.05^a	3.08 ± 0.04^b	5.07 ± 0.01^c	6.07 ± 0.03^d

Notes: Different superscript letter indicates significant difference between treatments ($p < 0.05$)

Table 5. Total amino acid profiles of *Phronima pacifica* under varying *Caulerpa lentillifera* stocking densities

Amino acid (%)	Treatment of *C. lentillifera* stocking density			
	A (0 g/m^2)	B (20 g/m^2)	C (40 g/m^2)	D (60 g/m^2)
L-aspartic acid	18.92 ± 0.05^a	27.85 ± 0.07^b	29.52 ± 0.09^c	37.94 ± 0.01^d
L-serine	15.61 ± 0.02^b	14.76 ± 0.03^a	17.63 ± 0.08^c	17.62 ± 0.03^c
L-glutamic acid	16.61 ± 0.02^a	27.36 ± 0.02^c	24.35 ± 0.02^b	30.37 ± 0.07^d
Glycine	19.36 ± 0.06^d	17.33 ± 0.01^a	18.78 ± 0.07^b	19.19 ± 0.01^c
L-histidine	6.78 ± 0.02^a	8.25 ± 0.01^b	13.50 ± 0.05^c	20.70 ± 0.09^d
L-arginine	10.51 ± 0.01^a	15.85 ± 0.05^b	21.89 ± 0.08^c	27.28 ± 0.08^d
L-threonine	15.02 ± 0.09^a	18.47 ± 0.06^b	20.16 ± 0.03^c	20.37 ± 0.06^d
L-alanine	10.65 ± 0.03^a	12.51 ± 0.08^b	13.98 ± 0.01^c	15.51 ± 0.04^d
L-proline	15.25±0.09^a	18.08 ± 0.07^c	17.44 ± 0.04^b	19.00 ± 0.09^d
L-valine	20.24 ± 0.09^a	25.72 ± 0.05^b	28.13 ± 0.07^c	28.87 ± 0.03^d
L-methionine	11.10 ± 0.02^a	15.89 ± 0.09^b	24.40 ± 0.05^c	32.40 ± 0.07^d
L-Lysine HCl	21.57 ± 0.07^a	27.59 ± 0.03^b	35.99 ± 0.02^c	45.23 ± 0.01^d
L-isoleucine	19.97 ± 0.01^b	18.87 ± 0.02^a	21.50 ± 0.07^c	22.99 ± 0.04^d
L-leucine	22.44 ± 0.05^a	22.97 ± 0.02^b	25.46 ± 0.09^c	30.88 ± 0.05^d
L-phenylalanine	15.49 ± 0.07^b	14.41 ± 0.07^a	15.97 ± 0.05^c	16.98 ± 0.10^d

Note: Different superscript letters indicate significant differences between treatments ($p < 0.05$)

Phronima pacifica growth rate is influenced by individual density from the initial growth phase until the peak phase. According to Punnarak et al. (2017), differences in growth rate in *Phronima sp.* are due to several factors. In addition, Preciado et al. (2017) report that *Phronima* spp. are detritus eaters and some species are algae gazers, so that the higher the seaweed stocking density, the greater the growth rate of *Phronima* sp. Growth begins with a lag phase or an adaptation phase followed by the exponential phase, declining phase, stationary phase, and eventually the death phase.

The sigmoid curve observed in the present study was formed by the growth trends in the adaptation phase, exponential phase, stationary phase and until death phase. Lag phase is the initial growth phase where the growth rate of *P. pacifica* is still low. *P. pacifica* abundance is influenced by detritus availability in a culture container and conducive environmental conditions. The growth of *P. pacifica* individuals cultured using *C. lentillifera* under different stocking densities exhibited significantly different trends in lag phase ($P > 0.01$). The growth trends of *P. pacifica* during maintenance exhibits a sigmoid curve. The adaptation phase begins from 4-6 days of observation in each treatment, with the highest density being 25 ind/L, which was observed due to the preparation of the environment and adjustment to the organic matter concentrations in the *C. lentillifera* culture media, so that there was no considerable population growth. *P. pacifica* adapts much more rapidly to the new environment if the density of the culture media is similar to the density under natural conditions, so that growth would take longer under conditions where there is a difference between the concentrations of the culture media and in nature. Herawati et al. (2017) reported that differences in concentrations between culture media and cells in plankton will have an effect on the restitution of enzymes and concentrates to further levels of growth and the presence of nutrients in cells through the diffusion process as a result of differences concentration between culture media and body fluids. In addition, differences in *P. pacifica* population density could be due to differences in seaweed distribution, which would lead to differences in available nutrients in the culture media and detritus abundance under varying seaweed distribution. Furthermore, the amount of detritus, which is an organic food source for *P. pacifica*, a filter feeder, would vary under varying seaweed distribution.

In the exponential phase, the nutrient concentrations in *P. pacifica* are the highest, growth has not been maximized, and the number of *P. pacifica* individuals begins to increase. Based on research conducted by Fogg (1965) and Herawati et al. (2014), the exponential phase is the phase where the nutrient concentrations and plankton density have not reached peak periods. However, in the present study, feeding was carried out in the exponential plankton phase. The exponential phase is the phase with the nutrient concentrations in *Phronima* spp. was the highest, although growth had not been maximized. The exponential phase in the present study was observed on day 10th and the results are consistent with the findings of Darmawan (2014) in which *P. pacifica* population was in the exponential growth phase at 9 and 10 days. In all treatments in the present study, the exponential phase was observed at 4 days. The rate of *P. pacifica* population growth depends on the capacity of *P. pacifica* to produce seeds. According to Moosa and Aswandy (1984) and Fattah et al. (2014), the number of eggs produced by Amphipoda species varies greatly, and is influenced by several factors, including the type, age, weight, and size of the species, so that there is a correlation between the size and number of *P. pacifica* produced. The greater the size of the *P. pacifica*, the more eggs it will produce. Eggs produced by *P. pacifica* hatch range from 50%-75% (Aoki et al. 2013).

The stationary phase is a phase that occurs after the exponential phase, and is characterized by the lack of growth, or a decrease in the amount of relatively the same. The stationary phase generally represents a peak in population growth until a drastic decrease in the population due to mass death (Darmawan 2014; Herawati et al. 2015). The stationary phase was observed at 18 days with the highest population being 98 ind/L in the 60 g/m^2 treatment,

which was thought to be caused by abundant nutrient detritus in the culture media. Moore and Eastman (2015) stated that seaweed with good nutrition will have a lot of thalli, and the thallus of dead seaweed will form detritus which serves as a food source for other organisms such as zooplankton, especially *P. pacifica*.

The death phase could be caused by several factors, including high temperature, poor nutrient availability in water, poor pH conditions, contamination and decreased photosynthesis. Herawati et al. (2017) reported that contamination could decrease population density and decrease photosynthesis. A decrease in nutrient availability would lead to the death of bacteria. In addition, toxic conditions created by the death of algae would also adversely affect growth. In the final phase of *P. pacifica* culture, the population increase, which could have been caused by a reduction in the number of nutrients in the culture media (Jiménez et al. 2014).

The greatest increase in *P. pacifica* biomass was achieved in the 60 g/m^2 treatment with 1.14 g. The biomass reported in the present study is higher than that reported by Herawati et al. (2020), where the biomass of *Phronima* sp. was 0.51 g after 30 days of culture. The high biomass of *P. pacifica* cultured using *C. lentillifera* was because of the high growth rate and high nutrient content of *C. lentillifera*. According to Bishop and Geiger (2006), differences in population density at harvesting is closely associated with the nutritional content of the feed provided. In addition to the nutrient concentrations in culture media, environmental conditions influence the growth and biomass of *P. pacifica*. In Darmawan (2014), plankton growth trends were influenced by factors such as physical water conditions, type of feed and feed concentrations. Under optimal water quality and maximum feed availability consistent with the growth requirements of *P. pacifica*, the maximum growth rate would be achieved quite rapidly and with more population peaks. Water quality, which is one of the key environmental factors influencing *P. pacifica* growth and development, could be maintained during the rearing period through daily water quality measurements in-situ.

In the present study, the highest protein and fat nutritional quality were observed under the 60 g/m^2 treatment with 60.23% and 10.24%, respectively, while the lowest was observed in the 0 g/m^2 treatment with 45.26% and 6.04%, respectively. The nutritional quality results reported in the present study are higher than those reported in Herawati et al. (2020), where the highest protein and fat contents under *P. pacifica* mass culture using organic waste fermented using probiotic bacteria were 58.90% and 8.24%, respectively, while the lowest was 41.26% and 5.04%, respectively. The high protein and low-fat content in the present study were caused by high nutrient and detritus amounts present in the culture media as feed. Widianingsih et al. (2008) reported that the higher the nitrogen and phosphorus contents, the higher the protein in the culture media, while fat content is inversely proportional to protein content. The results of the study are consistent with the findings of Lim et al. (2011), where protein content was always inversely proportional to fat content.

The results of the present study are reinforced by of the findings of Zengin et al. (2013), Monroig et al. (2013), and Tocher (2015), when PUFA is a key nutrient required for the formation of long-chain PUFA, which, in turn, form EPA and 351 docosahexaenoic acids (DHA) depending on the species.

Phronima pacifica is one of the natural feeds given to shrimp to substitute *Artemia* spp. especially in the post-larval stage due to the high nutritional contents. During this stage, *Artemia* sp. is deficient or low in natural feed for EPA and DHA contents. Therefore, *P. pacifica* can be used as a substitute. Akbary et al. (2011) and Ali et al. (2017) reported that *Artemia* sp. used as natural feed for shrimp contain relatively low EPA and DHA contents, specifically its stadia post larvae, so that enrichment is required.

According to the results of the present study, the highest amino acid contents were observed in the 60 g/m^2 treatment with the highest lysine contents of 45.23%. Conversely, the lowest amino acid contents were observed in the 0 g/m^2 treatment with 21.57%. The amino acid contents observed in the present study are slightly higher than those observed by Herawati et al. (2020) (i.e. 44.16%) that used organic waste fermented with probiotic bacteria. According to Ovie and Ovie (2006), Valverde et al. (2013) and Herawati et al. (2015), lysine forms the structural framework of vitamin B1, has antiviral effects, facilitates the absorption of calcium, enhances appetite, and facilitates the production of carnitine that enhances the conversion of fatty acids into energy. The high lysine contents in *P. pacifica* used as natural feed could increase growth. In addition, it is a key ingredient in blood antibodies, strengthens the circulatory system and improves cells, while lysine deficiency can cause fin erosion and fish death (Nafisi et al. 2018).

In conclusion, the present study investigated the influence of varying stocking densities of *C. lentillifera* added into culture media on the growth, biomass and nutrition profiles of *P. pacifica*. Based on the results of the study, both 40 g/m^2 and 60 g/m^2 treatment resulted in high *P. pacifica* growth and biomass production. However, the highest nutritional quality based on proximate analysis, and fatty acid and amino acids profile analyses, were observed in *P. pacifica* mass-cultured under 60 g/m^2 treatment. The application of the optimal stocking density of *C. lentillifera* into *P. pacifica* culture media determined in the present study could increase the nutritional quality of *P. pacifica*, a natural fish feed, which in turn could enhance aquaculture productivity.

ACKNOWLEDGEMENTS

This research was funded by the Directorate of Research and Community Service, Directorate General of Strengthening for Development Research, The Ministry of Research, Technology and Higher Education of The Republic of Indonesia, fiscal year of 2020.

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