# The distribution of organic waste discharged from super-intensive vaname shrimp (*Litopenaeus vannamei*) ponds monitored using stable isotopes

[1]Mudian Paena, [2]Rajuddin Syamsuddin, [2]Chair Rani, [2]Haryati Tandipayuk

[1] Doctoral Program, Agricultural Science Graduate School, Hasanuddin University, Makassar, Indonesia; [2] Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia. Corresponding author: M. Paena, mudianpaena@yahoo.co.id

**Abstract**. The penaeid shrimp *Litopenaeus vannamei* (vaname) has become a mainstay of tropical shrimp aquaculture since 2003. Super-intensive pond technology for vaname shrimp production is known to discharge high levels of organic waste due to the high stocking density and large volume of feed used. This organic waste will become dispersed in the coastal waters around super-intensive ponds. The research aimed to detect the distribution of organic waste discharged from two super-intensive shrimp ponds, using stable isotopes of C and N. Sediment, seagrass, and *Sargassum* samples were collected around the outlets of two super-intensive ponds. Stable isotope concentrations of these samples were analysed, as well as those of the feed used by the shrimp farms. While there was no evidence of either C or N uptake in sediment type 1, sediment type 2 had elevated levels of both N ($\delta^{15}N$) and C ($\delta^{13}C$) fractions. Sargassum only showed evidence of C ($\delta^{13}C$) uptake, over around 5 ha. However seagrass samples showed uptake of shrimp farm organic waste over more than 9 ha, based on both N ($\delta^{15}N$) and C ($\delta^{13}C$) fractions. These results indicate that seagrass could provide bio-filtration for organic waste from super-intensive shrimp ponds.
**Key Words**: super-intensive aquaculture, organic waste, development issues, aquaculture fisheries.

**Introduction**. The penaeid shrimp *Litopenaeus vannamei* (vaname) has become a mainstay of tropical shrimp aquaculture since 2003 (FAO 2006). The development of super-intensive pond technology for vaname culture has proven both feasible and profitable; however there is considerable concern over the high levels of organic waste discharged into coastal waters by super-intensive shrimp farms due to the high stocking densities and high volume of feed used. As stated by Wu (1995), environmental impacts from aquaculture vary greatly depending on the species cultured, aquaculture technology used, stocking density, feed type, and the site, especially hydrographical factors. Furthermore, according to Gowen et al (1991), the impacts of organic waste discharge are not limited to the immediate vicinity of the source, are likely to promote eutrophication, and can cause widespread changes in surrounding ecosystems. Observed impacts may include declines in the biomass, density, and diversity of benthos, plankton and nekton, modification of natural food webs, and phase shifts.

Organic waste discharged by super-intensive ponds will inevitably be transported and distributed by currents in the coastal waters around the shrimp farm. The distance to which the waste is transported will be determined by current strength, direction, and tidal regimes. Strong currents and high tidal range will tend to carry the waste over greater distances and be more effective in diluting the pollution burden, thus reducing the environmental impacts. Conversely, when currents are sluggish and the tidal range is small, the organic waste will tend to remain close to the source, resulting in higher concentrations with greater impact on the underwater environment. As pointed out by Iwama (1991), the transport distance of organic waste from aquaculture facilities in a marine environment is a function of current speed, water depth, and the total organic waste discharged. The later factor is especially important in terms of significant environmental impacts close to the organic waste source. According to Olsen et al

(2008), the capacity of coastal ecosystems to assimilate water-borne nutrients is mediated by two principal mechanisms: (1) the absorption of nutrients by phytoplankton and transfer through the food chain to higher trophic levels, and (2) the dilution of nutrients and organic matter, mediated by the hydrodynamic characteristics of the site in question.

Potential negative impacts from aquaculture discharges can include changes in sediment chemistry as well as seagrass community dynamics, in particular benthic macrofauna, meiofauna and bacterial communities (Holmer 1992; Findlay & Watling 1997; Hargrave et al 1997; Pergent et al 1999; Pearson & Black 2000; La Rosa et al 2001; Mirto et al 2002). From a spatial perspective, the extent of organic waste impacts needs to be understood in order to seek appropriate organic waste management solutions, before and after discharge into the environment. In particular, Hu et al (2009) point out that the composition, distribution and source of organic matter in marine sediments is crucial to the understanding of mechanisms to regulate the release of organic matter into the marine environment.

The distribution of organic waste around super-intensive ponds could be tracked through studying stable isotope fractions, in particular those present in marine sediments and seaweeds (macroalgae). Aberson et al (2016) found that stable isotopes could be used in studies on the polychaete worm *Hediste diversicolor* and the macroalga *Ulva* spp., organisms considered to be indicators of organic waste pollution. *H. diversicolor* preys on organic waste consuming microphytobenthos, while *Ulva* spp. organic waste from the water column.

The primary biogeochemical cycles of biological importance are those of the elements carbon, nitrogen, oxygen, sulphur, and metals, all of which are connected to the presence, transport, and transformation of organic matter. Differences in the chemical composition and isotope ratios of organic matter can help determine its origin and age, how it was transported and assimilated in a given environment (Kruger et al 2016). In particular, stable isotopes in organic and inorganic particles have been used to study ecological processes and deposition history in marine environments (Naidu et al 1993; Schubert & Calvert 2001). Stable isotopes of carbon and nitrogen have also been used to determine the accumulation of heavy metals in fish bodies (Liu et al 2018). Laboratory simulations show that stable isotopes can be used to trace the source and distribution of Pb in the waste burning process (Li et al 2017).

The ratio of the ($\delta^{15}N$) stable isotope of nitrogen is considered as a suitable choice for analyses aiming to identify the source(s) of nitrogen present in marine systems (Costanzo et al 2001; Carballeira et al 2013). In particular, the analysis of stable isotope ratios has been used successfully to determine trophic relationships between organisms, to identify the source or origin of organic waste (terrestrial and marine), and in the analysis of environmental impacts (Risk & Erdmann 2000; Costanzo et al 2001). Measurements of stable isotopes of organic carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in sediment can enable the identification of organic waste sources (Mahmood et al 2016). Stable isotopes of organic carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have also been used to study the impacts of aquaculture waste (Jiang et al 2013). Furthermore, stable isotopes of C and N have been used to study the distribution or dispersion of organic waste discharges from aquaculture through tracing the signature of organic matter contained in feed. The detection of feed signature has been successful in organic matter within benthic sediment (Sarà et al 2004) as well as in seagrass (*Enhalus acoroides*) and the macroalgae *Sargassum bacciferum*. Thus, this research aimed to apply the sable isotope ratio method to detect the dispersal and distribution of organic waste discharged from super-intensive shrimp ponds in Labuange Bay, District Barru, South Sulawesi Province, Indonesia.

**Material and Method**. The underlying principle of stable isotope ratio analysis is to determine the similarity or difference in $\delta^{13}C$ and $\delta^{15}N$ content between samples collected from the environment and organic waste discharged from super-intensive vaname shrimp aquaculture. In this case, samples for the detection of organic waste were taken from the benthic sediment, seagrass, and macroalgae (*Sargassum*) in coastal waters around two super-intensive shrimp farms which both discharge the organic waste from their ponds into Labuange Bay.

The sampling stations were selected based on a current model for the waters around the outlets of the super-intensive ponds (Figure 1). Sediment, seagrass and *Sargassum* samples were collected from 6-7th September 2016 during the dry season. The timing of sample collection was based on the assumption that primary production (phytoplankton and macroalgae) is greatest during the dry season, and thus the isotope signal should also be at a maximum at this time of year (Margalef 1985). In general, background isotope composition of sediment and macroalgae should remain constant or become enhanced during the rainy season, however spatial variability tends to be more marked and meaningful than temporal variability (Smith 1996; Hargreaves 1998).

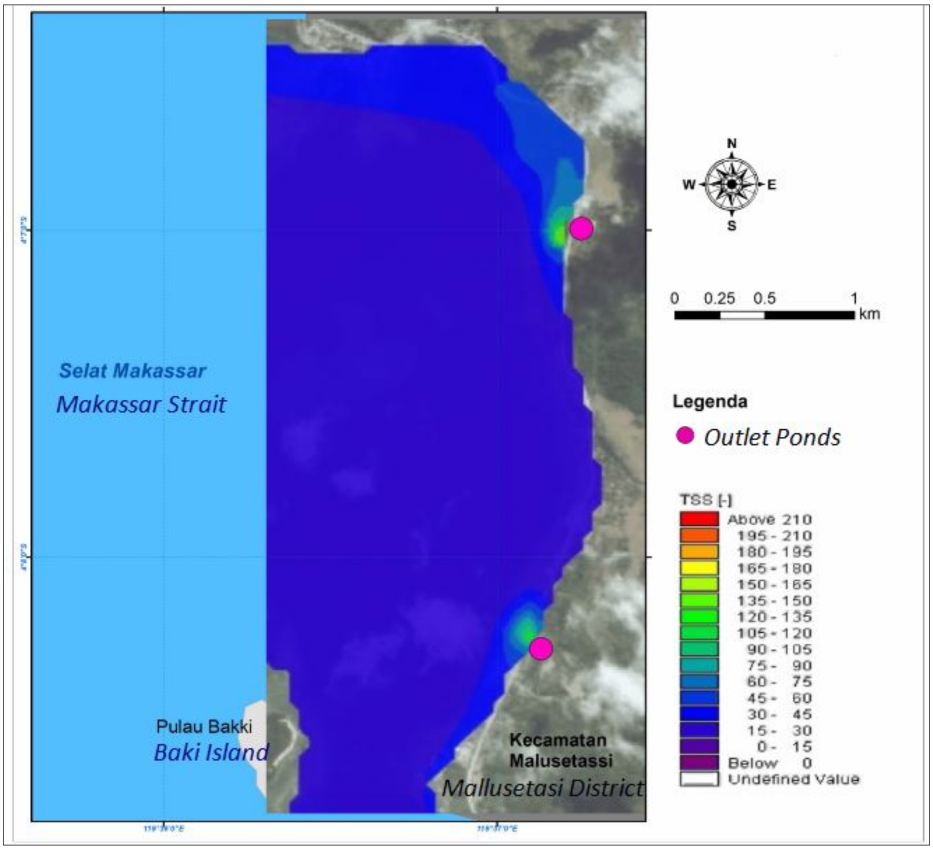


Figure 1. Current and organic waste transport model for Labuange Bay near the outlets of two super-intensive shrimp ponds.

Sediment was collected at a time when the ponds were in use, at four points along each of the transects which were aligned perpendicular to the predominant current direction. The transects were set at 25, 50, 100 and 200 metres from the pond outlets respectively (Mazzola & Sarà 2001). Sediment samples were collected using a van Veen grab. Samples from each transect were combined, and prepared for analysis, after which a subsample of 200 g was taken. Sampling of seagrass and *Sargassum* is done by making a transect parallel to the coast that cuts off the seagrass and *Sargassum* communities. Then the transect is divided into three sampling points, i.e one point at the center and 2 points on both ends of the transect. The three samples from the three points are then composite. Samples of the feed used in the shrimp farms were also collected. In total, the final analysis was performed on two samples each of feed, sediment and seagrass, and one *Sargassum* sample, making a total of 7 samples.

The carbon and nitrogen isotope ratios of the sediment, seagrass, and *Sargassum* samples were analysed at the Indonesian National Nuclear Agency Laboratory. Firstly, all samples were treated with acid (2 N HCl), then rinsed with decarbonised water and dried at 60$^{o}$C for 24 hours. The isotope composition of each sample was determined using a

Finnigan Delta-Plus spectrometer, to provide isotope concentration values expressed in parts per thousand (Sarà et al 2004).

The stable isotope ratios were calculated according to the formula from Sarà et al (2004):

$$\delta^{13}C \text{ or } \delta^{15}N = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] x\, 10^{3}$$

$$\text{Where: } R = \frac{^{13}C}{^{12}C} \text{ or } \frac{^{15}N}{^{14}N}$$

The fraction or percentage of contamination of each sample (with feed-containing organic waste) was calculated using the equation from Mongelli et al (2013):

$$(\delta^{13}C \text{ or } \delta^{15}N)sample = X(\delta^{13} or\ \delta^{15}N)contaminant + (1X)(\delta^{13}C \text{ or } \delta^{15}N)reference$$

where: $X$ : contaminant fraction;

$(\delta^{13}C \text{ or } \delta^{15}N)_{sample}$: sample isotope ($^{13}C$ or $^{15}N$) concentration (‰);

$(\delta^{13}C \text{ or } \delta^{15}N)_{contaminant}$: contaminant isotope ($^{13}C$ or $^{15}N$) concentration (‰);

$(\delta^{13}C \text{ or } \delta^{15}N)_{reference}$: reference isotope ($^{13}C$ or $^{15}N$) in the absence of contamination (‰).

**Results and Discussion**. The results of the stable isotope analyses (for $\delta^{15}N$ and $\delta^{13}C$) are shown in Table 1, using reference concentration of stable isotopes $\delta^{15}N$ and $\delta^{13}C$ from uncontaminated seagrass. Isotope concentrations are expressed in parts per thousand (‰). The stable isotope concentrations for the feed were 5.35 and 7.11‰ for $\delta^{15}N$, and -23.77 and -25.52‰ (two samples) for $\delta^{13}C$, and used as reference values for the contaminant. Stable isotope concentrations for the five other samples ranged from -7.47 to 8.20‰ for $\delta^{15}N$, and from -13.55 to -7.74‰ for $\delta^{13}C$. These different isotope concentrations can be seen as the finger print of each sample, indicating the likelihood of influence from the surrounding environment.

Table 1

Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) concentrations

| *No.* | *Sample* | $\delta^{15}$*(N‰)* | $\delta^{13}$*(C‰)* |
|---|---|---|---|
| 1 | Seagrass-1 | 8.2 | -11.81 |
| 2 | Seagrass-2 | 3.3 | -13.55 |
| 3 | *Sargassum* | -2.58 | -12.38 |
| 4 | Feed-1 | 7.11 | -23.77 |
| 5 | Feed-2 | 5.35 | -25.52 |
| 6 | Sediment-1 | -3.38 | -7.74 |
| 7 | Sediment-2 | -7.47 | -12.48 |
| 8 | Seagrass (Thornton & McManus 1994) | -2 | -10 |

The stable isotope data were plotted to show the correlation between $\delta^{15}N$ and $\delta^{13}C$ (Figure 2). This plot shows that the values for seagrass and *Sargassum* samples fall between the mixing line for the feed (contaminant) and the (uncontaminated) reference seagrass. The sediment-2 sample had the lowest $\delta^{15}N$ concentration, and appears depleted compared to the other samples; conversely, the seagrass-1 sample had the highest $\delta^{15}N$ concentration, and seems enriched. The low $\delta^{15}N$ concentration of sediment-2 is likely due to a low contribution of nitrogen from shrimp feed waste, indicating that the sediment is not much affected by organic waste from the ponds; on the other hand, the high (enriched) $\delta^{15}N$ concentration in seagrass-1 indicates a significant impact from the feed-containing waste.

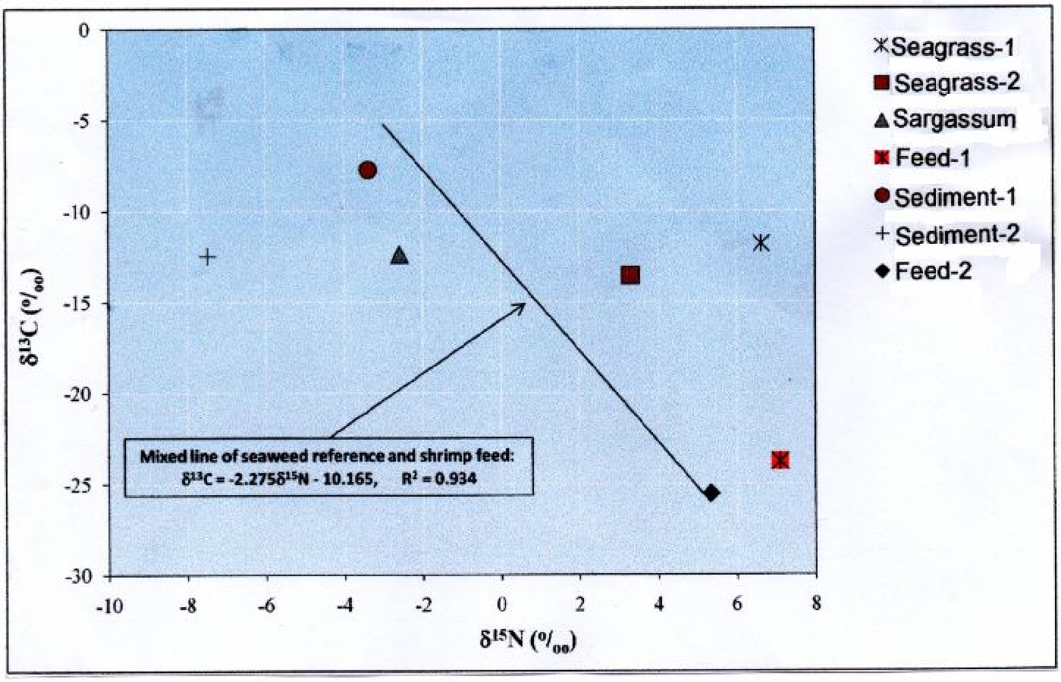


Figure 2. Correlation between $\delta^{15}$N and $\delta^{13}$C for all samples and the reference seagrass values.

Although seagrass-1 appears to have the highest level of contamination, both seagrass-1 and seagrass-2 samples indicate much higher levels of contamination than in the other samples (*Sargassum* and sediment). The seaweed sample *Sargassum* is indicative of an intermediate level of contamination, while the two sediment samples do not show evidence of contamination.

Based on the highest isotope concentrations of feed-1 ($\delta^{13}$C = -23.77 ‰ and $\delta^{15}$N = 7.24 ‰), the contribution of the contaminant to each sample is shown in Table 2. These data indicate that in the sample seagrass-1, the vast majority (around 95%) of the nitrogen-15 ($\delta^{15}$N) concentration and 13% of the carbon-13($\delta^{13}$C) concentration most likely originated from the feed-containing organic waste discharged from the shrimp ponds. It should be noted that N generally comes from the protein and C from the carbohydrate contained in shrimp feed. The seagrass-2 sample had a somewhat lower (58%) level of nitrogen-15 but a higher (26%) carbon-13 level, the latter double the value for seagrass-1. The seaweed (*Sargassum*) sample showed carbon-13 uptake (17%) but no nitrogen-15 was detected. The lack of N and relatively low C contaminant uptake by *Sargassum* could be due to the seasonal nature of *Sargassum* mats, unlike the seagrass which is rooted in place and thus exposed to organic waste from the super-intensive shrimp ponds throughout the year.

Table 2

Estimated contaminant fraction from shrimp feed by sample

| *No.* | *Sample* | *$\delta^{15}$N-based contaminant fraction (%)* | *$\delta^{13}$C-based contaminant fraction (%)* | *Inferred conclusion* |
|---|---|---|---|---|
| 1 | Seagrass-1 | 0.95 | 0.13 | uptake |
| 2 | Seagrass-2 | 0.58 | 0.26 | uptake |
| 3 | *Sargassum* | 0 | 0.17 | uptake of $^{13}$C |
| 4 | Feed-1 (contaminant) | 1 | 1 | - |
| 5 | Sediment-1 | 0 | 0 | no uptake |
| 6 | Sediment-2 | 0 | 0.17 | uptake of $^{13}$C |
| 7 | Seagrass (Thornton & McManus 1994) | 0 | 0 | - |

Nitrogen-15 was not detected in either of the sediment samples, but carbon-13 was detected (17%) in sample sediment-2, similar to the *Sargassum* sample. Burford et al (2002) found that sediment is generally only slightly contaminated or not contaminated by organic waste due to the swift remineralisation processes mediated by microbial communities which facilitate anorganic diffusion. In contrast to the sediment, which does not readily absorb contaminants present in the water column, seagrass and *Sargassum* are living organisms which readily absorb nutrients from their environment, and can thus more readily become contaminated. The extent of coastal waters contaminated by organic waste discharged from each of the super-intensive shrimp farm outlets is shown in Figure 3. For the more northerly farm, the respective extent of areas around the outlet where seagrass and *Sargassum* showed evidence of contamination were around 3.44 and 3.15 ha respectively. For the second (southern) farm, contaminated areas around the outlet based on seagrass and *Sargassum sp.* were 5.88 and 1.89 ha respectively.

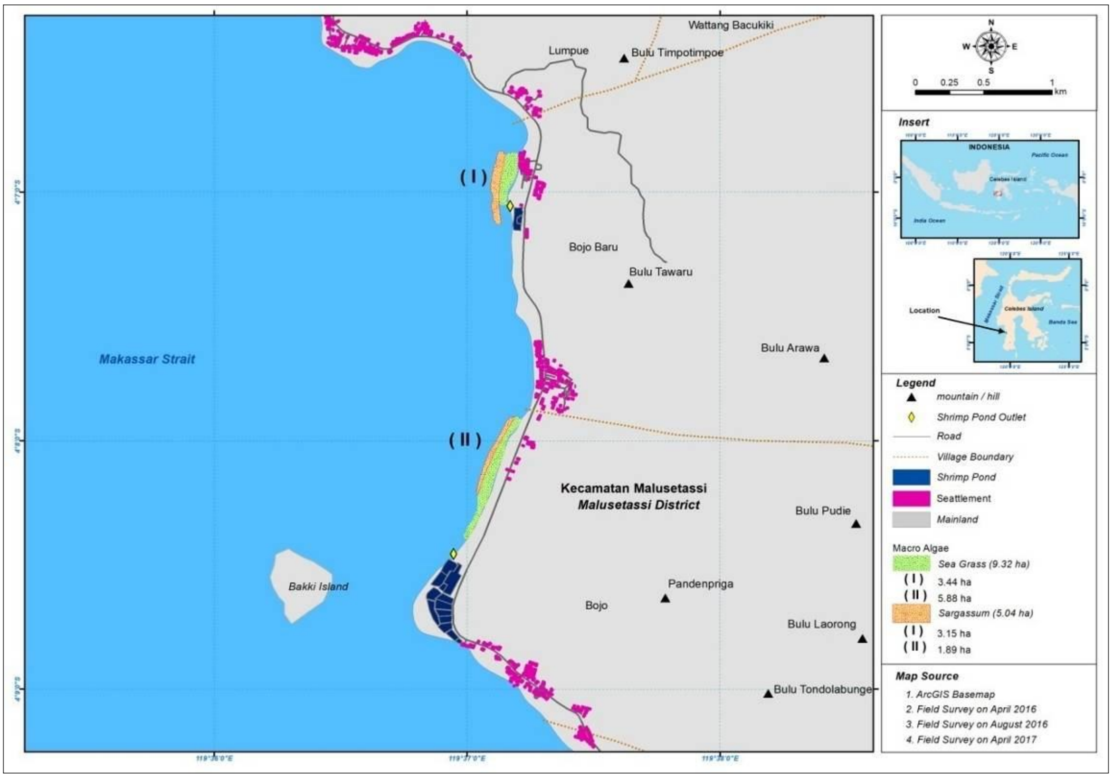


Figure 3. Extent of areas in Labuange Bay showing evidence of super-intensive shrimp pond organic waste contamination based on seagrass (green) and macroalgae (orange) stable isotope analyses.

**Conclusions**. The relation between N ($\delta^{15}$N) and C ($\delta^{13}$C) stable isotope fraction present in the feed used in the super-intensive shrimp farms with those present in seagrass around the farm organic waste discharge outlets indicate that organic waste is both dispersed and accumulated by organisms in the surrounding waters. The findings also indicate that seagrass may play a role in the biofiltration of organic waste discharges. Conversely, N ($\delta^{15}$N) and C ($\delta^{13}$C) fractions from shrimp feed were absent in the sediment, especially in seagrass meadows. This absence is likely due to uptake of dissolved N ($\delta^{15}$N) and C ($\delta^{13}$C) by seagrass, while the strong tidal currents likely limit the deposition of particles to very low levels which can be processed and diffused through abiotic and microbial mechanisms.

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Authors:
Mudian Paena, Doctoral Program, Agricultural Science Graduate School, Faculty of Marine Science and Fisheries, Hasanuddin University. Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Kota Makassar, Sulawesi Selatan 90245, Indonesia, e-mail: mudianpaena@yahoo.co.id
Rajuddin Syamsuddin, Faculty of Marine Science and Fisheries, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Kota Makassar, Sulawesi Selatan 90245, Indonesia, e-mail: rajuddin_syamsuddin@yahoo.com
Chair Rani, Faculty of Marine Science and Fisheries, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Kota Makassar, Sulawesi Selatan 90245, Indonesia, e-mail: erickch_rani@yahoo.com
Haryati Tandipayuk, Faculty of Marine Science and Fisheries, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Tamalanrea, Kota Makassar, Sulawesi Selatan 90245, Indonesia, e-mail: haryati_fikpunhas@yahoo.com

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