Distribution of microalgae and omega-3 as food for *Sardinella lemuru* in Bali Strait

B Suprakto¹, T Harijono², I G P G R Yudana², A Kiswanto², A Fauziah¹,²,³,⁴

¹Marine and Fisheries Education Center, Jakarta, Indonesia
²Jembrana Marine and Fisheries Polytechnic, Bali, Indonesia
³Sidoarjo Marine and Fisheries Polytechnic, Sidoarjo, Indonesia
⁴Corresponding author: anna.apsidoarjo@gmail.com

Abstract. *Sardinella lemuru* is a small pelagic fish that contains high omega-3 which is distributed in the eastern Indian Ocean including the Bali Strait. Lemuru including high-fat fish with omega-3 content. This different fat content depends on the size of the fish, maturity, season, and type of food. The research objectives were to obtain abundance and diversity of microalgal composition as food and determine the level of omega-3 in the stomach of lemuru. The research was conducted at the 8 station with different waters characteristic. The data obtained were analyzed using t-test. The omega 3 content of lemuru in the second transitional season was 35.25% higher than the fish caught in the western season (21.4%). Lemuru feed on phytoplankton dominated by the Bacillariophyceae group with *Chaetoceros gracilis* in the second transitional season. These phytoplankton suspected important role as raw materials for forming omega 3 in the body of lemuru.

1. Introduction
*Sardinella lemuru* is a small pelagic fish that contains high omega-3 which is distributed in the eastern Indian Ocean including the southern coast of East Java, Bali and Lombok. Lemuru include high-fat fish with varying fat content. This different fat content depends on the size of the fish, maturity, season, diet and so on. This is because lemuru in nature eat a lot of plankton and microalgae which produce a lot of omega-3 fatty acid components. Thus, plankton is the key to forming omega-3 in lemuru because marine phytoplankton is the primary producer of omega-3 in the food chain.

One of the waters in Indonesia that has a high potential for capture fisheries is the Bali Strait, with its main fishery resource, namely lemuru fish (*Sardinella lemuru*). Lemuru is an especially important and specific fishery resource in the waters of the Bali Strait because it has a role in the business and economic activities of the local community [1]. Lemuru is a type of fish that has a high Omega-3 content. Research conducted [2] states that lemuru has high omega-3 unsaturated fatty acids, which is around 36.96%. The content of omega-3 fatty acids is relatively high compared to other types of pelagic fish, namely anchovies, sela, and mackerel.

Lemuru production in the waters of the Bali Strait fluctuates greatly from year to year, where at certain times the production is quite low but at other times the production is quite high. Based on fish landing data at the Muncar Fishing Port, in 2006 and 2007 there was an abundance of lemuru production which reached more than 50,000 tons per year. This high potential is inseparable from the fact that the Bali Strait has high water productivity due to the upwelling phenomenon that occurs seasonally in the southern waters of Java associated with the Indian Ocean. However, in the last few years, lemuru...
production has decreased significantly. This is presumably due to changes in environmental conditions and overfishing [3].

The presence of fish in the waters is greatly influenced by temperature. Water temperature is one of the important factors in the life of organisms in the sea, it can affect the metabolic activity and reproduction of these organisms, as well as an indicator of the phenomenon of climate change [4]. However, the presence of fish is also thought to be influenced by other environmental parameters such as the spread of phytoplankton. Phytoplanktonic act as primary producers in the food chain in waters, which in turn can affect the fertility of the waters and the presence of fish.

Water fertility indicators can be measured from the chlorophyll-a content. Chlorophyll-a is the most common pigment found in phytoplankton and plays a role in the photosynthesis process [5]. The presence of high chlorophyll-a concentrations, especially in coastal areas, indicates the presence of sufficient plankton to maintain the viability of economically important small pelagic fish [6]. Some conditions that have a direct effect on sea surface temperature and chlorophyll-a are the upwelling phenomenon and the direction of the Armondo current (Indonesian Monsoon current), so it is assumed that in the east monsoon there is more upwelling with a higher value of chlorophyll-a and the number of points in the eastern monsoon. Low temperature waters surrounded by warm temperatures around it, then the Armondo current brings lower temperatures to the waters of the Bali Strait compared to the western monsoon, because in the east monsoon Armondo brings low temperature water masses from the Indian Ocean [7].

Potential fishing areas can be identified by looking for information on oceanographic parameters preferred by lemuru, so that later it can save operational costs of fishing and prevent illegal fishing size. Therefore, research is needed to determine the oceanographic and biological parameters of the marine environment, especially microalgae as bioindicators and food for lemuru in the waters of the Bali Strait which are closely related in determining potential lemuru fishing areas.

The purpose of the study was to obtain data on hydro-oceanographic parameters (physics & chemistry) in the Bali Strait fishing ground during the transitional season II and west season; Obtain plankton data (abundance and diversity of species) in the waters in the two seasons; Obtaining the composition of phytoplankton and zooplankton in the hulls of lemuru caught in the fishing ground area during these seasons; Determining the levels of omega-3 fatty acids in lemuru meat; Determining the relationship between abundance and diversity of plankton species in the waters as a food source for lemuru fish with omega-3 fatty acid content of lemuru fish in different seasons.

2. Material and Methods

2.1. Composition of lemuru stomach content
The total 20 lemuru caught in the fishing ground were taken to determine the average body length of the fish and their hulls were taken to analyze the composition of the phytoplankton and zooplankton therein.

The number of plankton found in the stomach of lemuru is calculated using the volumetric method, while the composition of lemuru food is calculated using the index of preponderance [8].

To determine the type of plankton which is the main choice for lemuru food, an index of electivity is carried out by comparing the number of plankton in the stomach of lemuru with the abundance of plankton types in the waters using the formula.

2.2. Analysis of omega-3 fatty acid levels from fish meat
Fatty acid extraction was carried out by the method [9] and then methylated using the method [10]. 3 Lemuru organism were taken for each sampling and cleaned on their abdomen, then heated in the sun to dry. The dried fish meat is taken from the dorso-lateral part of the body (the three fish are mixed), then weighed as much as one gram, then crushed in a glass cup and extracted by adding 10 ml of petroleum ether (PE), centrifuged at 3000 rpm for 2 minutes later filtered. After filtering, let the mixture sit for about 15 minutes so that the petroleum ether evaporates and gets its fat. The fat that has been separated from the meat is then methylated by adding 10 ml of BF3 in 20% methanol (BF3CH3OH)
then shaken with a shaker for about 30 minutes. The top layer is the fatty acid methyl ester which is separated and analyzed using Gas Chromatography (GC) at Vicma Lab Laboratory, Cibinong.

2.3. Aquatic environment parameter sampling
The measurement of physical-chemical parameters is carried out in two ways, namely in situ and ex situ. Measurements of environmental physical-chemical parameters were carried out at each observation station. Water quality parameters measured in-situ include dissolved oxygen (DO) using a DO meter, YSI brand, type 550A, which is also a temperature measurement in degrees Celsius, and salinity using a refractometer. The physical-chemical parameters of the waters which were measured specifically, including phosphate, ammonia, and nitrate were tested at the Surabaya Industrial and Standardization Research Institute. Sampling at the research location and sample analysis in the laboratory refer to the APHA method [11]. The test is done in duplicate.

2.4. Microalgae (phytoplankton) sampling
Sampling of microalgae (phytoplankton) was carried out by filtering 50 L of seawater using Wildco 12 "100 μm Nitex® mesh plankton net, rope length = 2 M [12]. The filtered water in the plankton net is then collected into a 250 ml sample bottle, 10% lugol solution is added, until it is tea-colored and deposited for 4 days [13]; [14], then the sediment is taken and identified using an identification book. The samples were analyzed in the Laboratory of Environmental Biology, Sidoarjo Marine and Fisheries, Polytechnic

2.5. Identification and calculation of microalgae abundance
Identification of the types of microalgae was carried out using references [15]; [16] and [17]. The abundance of microalgae cells is calculated based on the following equation [14].

\[ N = \frac{n \times (L_s/L_p) \times (vol.1/vol.s)}{vol.2} \]

dimana :
N = microalgae abundance (cell/ml)
n = the number of cell (sel)
Ls = total of Sedgewick rafter cell area (mm²)
Lp = the area of Sedgewick rafter cell was observed (mm²)
vol. 1 = the volume of water sample from precipitation (ml)
vol. 2 = the volume of water precipitated (ml)
vol.s = the volume of sedgewick rafter cell (ml)

2.6. Data analysis
The research material is hydro-oceanographic parameters, plankton in the waters of the Bali Strait, lemuru fish and plankton in the stomach of lemuru fish. Sampling was carried out in the waters of the Bali Strait in November (representing the transitional season II) and February (representing the west season), then the analysis was carried out at the BBAP-Situbondo, and the Laboratory of Organic Chemistry UGM-Jogjakarta. This study uses descriptive methods and data analysis using regression and t-test.

3. Result and discussion

3.1. The dynamics of water quality parameters in the waters of Bali Strait
Hydro-oceanographic parameters in the waters of the Bali Strait in the second transitional season have lower temperature and salinity, while DO (Dissolved Oxygen) and chlorophyll-a concentrations in that
season are higher than in the west monsoon. Nutrient measurements (phosphate, nitrate, total organic matter) in the second transitional season were higher than those in the west monsoon and at a depth of 20 m the value was higher than in the surface layer, for both seasons as Table 1.

Table 1. The hydro-oceanographic parameters measuring of the Bali Trait in the transitional season

| No | Parameters           | depth  |<1m| 20m|
|----|----------------------|--------|----|-----|
| 1  | Tempt (°C)           |        | 29.00 | - |
| 2  | Salinity (ppt)       |        | 34.00 | - |
| 3  | DO (mg/l)            |        | 11.90 | - |
| 4  | Chlorophyl-a (mg/l)  |        | 1.42  | - |
| 5  | Phosphate (mg/l)     |        | 0.01  | 0.02|
| 6  | Nitrate (mg/l)       |        | 0.94  | 1.05|
| 7  | Silicate (mg/l)      |        | 1.85  | 0.91|
| 8  | Total organic mater (mg/l) | 41.71 | 54.35 |

Another uniqueness of the Bali Strait is the pattern of monsoon wind movement from the Asian continent to the Australian continent and vice versa, which is the dominant factor that influences the dynamics of oceanographic Indonesian waters [18]. In the west monsoon (December – February), the movement of monsoon winds from the Asian continent (high pressure) to the Australian continent (low pressure) passes through Indonesian waters, bringing moist air masses, causing an increase in precipitation when passing through Indonesian waters. The opposite process occurs when entering the east season (June-August).

According to [3], a decrease in sea surface temperature is always followed by an increase in chlorophyll-a which indicates that the increase in chlorophyll-a is influenced by the upwelling process. The phenomenon of water mass reversal brings nutrients needed by phytoplankton from the deep layers to the surface. So that this process becomes important in the hydro-oceanographic cycle and the life of plankton. Chlorophyll-a is an indicator of the presence of phytoplankton in the waters of the Bali Strait.

[19], stated that the oceanographic conditions of the Bali Strait are influenced by monsoon winds. In the east season (June - August) the temperature is relatively low and the concentration of chlorophyll-a increases, so that the highest productivity occurs in this season, this is due to oceanographic conditions including the upwelling phenomenon. While the west season (December - February) is the opposite. This pattern begins to emerge when entering the east monsoon which is characterized by low sea surface temperatures and high salinity [3]. When upwelling occurs, nutrients in the waters such as nitrate and phosphate which are very important for the development of phytoplankton increase sharply. This results in an increase in the abundance of phytoplankton [6].

The results of the study at the beginning of the second transitional season showed that both the waters and the stomach contents of the lemuru were dominated by phytoplankton of the type *Guinardia striata* [20], or *Chaetoceros gracilis* and *Rhizosolenia delicatula*. While the results of the measurement of water nutrients show numbers that support the life of phytoplankton, namely nitrate (NO3) 0.598 mg/l and phosphate (PO4) of 0.025 mg/l [21].

3.2. Plankton abindance and diversity in waters fishing ground

The abundance of phytoplankton in the transitional season II is high. The important components of the season (August 2020) are class Bacillariophyceae or commonly called diatoms of the genus *Rhizosolenia* and *Chaetoceros*. The genus *Rhizosolenia predominates*, especially *Chaetoceros gracilis* because it reaches 87% of all phytoplankton at both depths (<1 m and 20 m). While class Bacillariophyceae (diatoms) reached 95.9% of the total phytoplankton in both depths, the rest are class Dinophyceae (Table 2)
Table 2. Abundance (N) and diversity (H') of plankton in Bali Strait waters in transitional season II

| No | Species                  | Class/Subclass | D 1m N  | H'  | D 20m N | H'  |
|----|--------------------------|----------------|----------|-----|---------|-----|
| 1  | *Chaetoceros gracilis*   | Bacillariophyceae | 21000    | 0.53| 13040   | 0.48|
| 2  | *Chaetoceros coarctatus* | Bacillariophyceae | 404      |     | 448     |     |
| 3  | *Coscinodiscus sp*       | Bacillariophyceae | 28       |     | 212     |     |
| 4  | *Odontella sinensis*     | Bacillariophyceae | 12       |     |         |     |
| 5  | *Pyrocystis fusiformis*  | Bacillariophyceae | 8        |     | 0       |     |
| 6  | *Rhizosolenia alata*     | Bacillariophyceae | 204      |     | 40      |     |
| 7  | *Rhizosolenia delicatula*| Bacillariophyceae | 1320     |     |         |     |
| 8  | *Rhizosolenia hebetata*  | Bacillariophyceae | 396      |     | 12      |     |
| 9  | *Rhizosolenia stolterfothii* | Bacillariophyceae | 884     |     | 396     |     |
| 10 | *Ceratium extensum*      | Dinophyceae      | 0        |     | 4       |     |
| 11 | *Ceratium furca*         | Dinophyceae      | 20       |     | 104     |     |
| 12 | *Ceratium fusus*         | Dinophyceae      | 8        |     | 4       |     |
| 13 | *Ceratium karsteni*      | Dinophyceae      | 16       |     | 0       |     |
| 14 | *Ceratium longirostrum*  | Dinophyceae      | 4        |     | 8       |     |
| 15 | *Ceratium massilience*   | Dinophyceae      | 20       |     | 0       |     |
| 16 | *Ceratium tripus*        | Dinophyceae      | 28       |     | 36      |     |
| 17 | *Peridinium granii*      | Dinophyceae      | 260      |     | 104     |     |
| 18 | *Stentor rosseli*        | Dinophyceae      | 24       |     | 8       |     |

**Zooplankton**

| No | Species                  | Class/Subclass | D 1m N  | H'  | D 20m N | H'  |
|----|--------------------------|----------------|----------|-----|---------|-----|
| 1  | *Acartia clausi*         | Copepoda       | 12       | 1.28| 36      | 1.88|
| 2  | *Centropages typicus*    | Copepoda       | 0        |     | 16      |     |
| 3  | *Cyclopoid sp*           | Copepoda       | 0        |     | 60      |     |
| 4  | *Microcalanus pumilus*   | Copepoda       | 8        |     | 64      |     |
| 5  | *Microsetella norvegica* | Copepoda       | 12       |     | 28      |     |
| 6  | *Nauplius of Pareuchaeta norvegica* | Copepoda | 44       | 156 |     |     |
| 7  | *Pareuchaeta norvegica*  | Copepoda       | 4        |     | 36      |     |
| 8  | *Paracalanus parvus*     | Copepoda       | 0        |     | 20      |     |
| 9  | *Doliolum sp*            | Tunikata       | 0        |     | 4       |     |
| 10 | *Carestoderma edule*     | Bivalve         | 0        |     | 4       |     |

Bacillariophyceae in tropical waters generally found in high abundance because it has a high rate of relatively fast recruitment compared to other types of other plankton. The type of zooplankton nauplius from *Parechaeta norvegica* is a species that has a high abundance compared to zooplankton another is 40% of the total zooplankton. Of all the zooplankton that identified, 98% are copepod class, the rest are Tunikata class and Bivalve (Table 2). Zooplankton are more commonly found at a depth of 20 m due to the availability of food, namely phytoplankton found at this depth, so that zooplankton do not need to reach surface to get the food (phytoplankton).

According to the diversity index (H') Shannon-Wiener, Bali Strait waters in the transitional season II (August 2020) for groups of phytoplankton each depth (< 1 and 20 m) is 0.53 on surface layer and 0.48 in depth. While the diversity (H') zooplankton at the surface were 1.28 and 1.88 for a depth of 20 m. According to [22], this table indicates that community stability aquatic biota in moderate condition or at the level of pollution currently.

In the second transitional season of phytoplankton, Bacillariophyceae (diatoms) were abundant up to 95.9% of all phytoplankton. One of them is *Chaetoceros gracilis* which is abundant for 87% of all phytoplankton at both depths. Meanwhile, in the west season, the abundant zooplankton was the...
copepod class, which accounted for 88.3% of all zooplankton and was dominated by the species *Pareuchaeta norvegica* and *Acartia clausi*. The t-test showed that the abundance of phytoplankton in the transitional season II and the west season was significantly different (p < 0.05). Likewise, the t-test of zooplankton abundance in the two seasons showed a significant difference (p < 0.05).

According to [23], *Sardinella longiceps* is a phytoplankton eater, it is suspected that there is a change in food habits after the fish grow up. Reinforced by [24], in his research at Karwar that juvenile *Sardinella longiceps* (50-130/145 mm) is a carnivore that eats zooplankton as the main food. While adult fish (more than 130/145 mm) eat phytoplankton. In the same place, Noble in his research in 1961-1964 concluded that in July-September the stomach contents of *Sardinella longiceps* were filled with diatoms, while in December-February it was dominated by zooplankton, with a ratio of 3:2 [25].

The diversity of phytoplankton and zooplankton in both seasons indicates that the stability of the Bali Strait marine biota community is in moderate condition or is at a moderate level of pollution. The composition of plankton in the hulls of lemuru caught in the transitional season II and west season in the fishing ground area is directly proportional to the abundance of phytoplankton in the waters. In the second transitional season, the composition of phytoplankton stands out up to 98%. While in the west season, the composition of zooplankton reaches 83%. The results of the t-test both the abundance of phytoplankton and zooplankton in the two seasons showed a significant difference (p < 0.05).

### 3.3 Omega-3 Fatty acid content of lemuru

The omega-3 fatty acid content of fish caught in the second transitional season was 35.35% higher than fish caught in the western season (21.4%) of the total fatty acids in the fish meat. Lemuru eat phytoplankton dominated by *Chaetoceros gracilis* in transitional season II, their meat contains high omega-3 fatty acids. These phytoplankton play an important role as raw materials for the formation of omega-3 in the body of lemuru, and it is suspected that these fish have genes that contribute to the formation of enzymes that trigger the doubling of omega-3 amounts.

**Table 3.** omega-3-GCMS analysis results Lemuru meat in transitional season II (August 2020)

| No | Sistemati | Trivial | Formula | % relatif |
|----|----------|---------|---------|-----------|
| 1  | 9,12,15-oktadecatrienoic Acid | Asam Linolenat | C18:3 (n-3) | 7,18 % |
| 2  | 5,8,11,14,17-Eikosapentenoic Acid | Eikosapentaenoat (EPA) | C20:5 (n-3) | 12,57 % |
| 3  | 4,7,10,13,16,19-Docosahexaenoic Acid | Dokosaheksaenoat (DHA) | C22:6 (n-3) | 7,78 % |
|    | Total omega-3 | | | 27,53 % |

GC-MS analysis showed that lemuru in this season contained 32 types of fatty acids in its meat. Of the total fatty acids, the percentage of omega-3 fatty acids is relatively high. This is presumably because lemuru fish eat a lot of phytoplankton. According to [1], this microalga (phytoplankton) contains the main source of omega-3 which is indispensable in the food chain, because this omega-3 cannot be synthesized by aquatic biota.

Lemuru eat zooplankton and phytoplankton. Zooplankton is the main food, with a percentage of 90.52% - 95.54%. While the phytoplankton group is 4.46% - 9.48%. In the composition of zooplankton, copepods occupy the highest percentage in the stomach contents of lemuru [4]. The content of omega-3 in the body of lemuru or other marine fish is not always the same. In addition to food ingredients, differences in omega-3 production are also caused by several factors such as genetics and environmental characteristics [26]. As in previous studies [27], in his research stated that lemuru fish caught in the Lombok Strait contained omega-3 ranging from 23.5 to 30.6%. According to another source, lemuru caught in the Bali Strait contain 18 – 23.3% omega-3 [28]. The content of essential fatty acids, especially omega-3 from this lemuru (*Sardinella lemuru*) and the relatively cheap price can be used as highly nutritious food, especially in overcoming nutritional problems.
4. Conclusion

In the second transitional season of phytoplankton, Bacillariophyceae (diatoms) are abundant up to 95.9% of all phytoplankton. One of them is Chaetoceros gracilis was abundant for 87% of all phytoplankton in both depths. While in the west season the abundant zooplankton is copepod class is 88.3% of all zooplankton and is dominated by species of Pareuchaeta norvegica and Acartia clausi. Phytoplankton diversity and zooplankton in both seasons showed that community stability Bali Strait waters biota is in moderate condition or at the level of moderate pollution.

The content of omega-3 fatty acids in lemuru meat caught in the second transitional season is 35.35% higher than the western season (21.4%) of the total fatty acids present in the fish meat. Lemuru eats phytoplankton, especially Chaetoceros gracilis in the transitional season II, the meat contains omega-3 fatty acids tall. These phytoplankton play an important role as raw materials for the formation of omega-3 in the body of lemuru.

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