Zooplankton Tintinnopsis dominance in the Estuary of Polong River, Pangkep Regency, South Sulawesi, Indonesia

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Abstract. Genus Tintinnopsis is a microzooplankton from the protozoa (ciliate) group that lives in various habitats in marine waters. Like other zooplankton, Tintinnopsis holds important roles in the food chain. An investigation on the zooplankton community focusing on species composition had been conducted in July 2020 in Polong River estuary, Pangkajene District, South Sulawesi. Sampling was carried out at four stations, each with three replications. Seventy-five liters of seawater were filtered using a plankton net 25 (0.06 mm) into a 100 mL sample bottle and added with 2mL of 1% Lugol. Zooplankton species were counted and identified by putting 1ml of water sample on a Sedgwick Rafter counter and observed on a microscope. Zooplankton identification followed Identification of Marine Plankton and Marine Plankton A Practical Guide. The number of zooplankton differences amongst stations was analyzed statistically by using a one-way ANOVA. Results showed that Tintinnopsis were found in all stations, and were most abundant in station 2, followed by stations 1, 3, and 4. There were two other zooplankton species found in the area, i.e. nauplii Copepoda (stations 1, 3, and 4) and Apocyclops sp (station 2). Each species composition was 80.60%, 18.62% and 0.78% for Tintinnopsis sp., nauplii copepods and Apocyclops sp., respectively. One-way ANOVA also showed no significant differences in the individual abundances amongst station (CI 95%; (p>0.05).

1. Introduction

Polong river is one of many river estuaries in Pangkajene Archipelago (Pangkep) regency, South Sulawesi, Indonesia. Around the mouth of the river, there are several shrimp and milkfish ponds which are a source of income for the people of Pangkep Regency. The types of shrimp pond cultivation in the area are intensive, traditional-plus, and traditional. In traditional-plus farming, artificial diets were applied when the shrimps are between 15-30 days old, hence before reaching that age range, shrimps consume natural food. In traditional farming, during the maintenance period, shrimps feed on naturally occurring organisms. Natural food in the pond environment is in the form of plankton. Porto Neto et al., (2009) found Copepod zooplankton to dominate (45%) the total plankton in Litopenaeus vannamei commercial shrimp farm in Northeastern Brazil [1]. In traditional shrimp farming in Ceará, Piaui and Rio Grande do Norte states - Brazil, copepods are dominant (83%), followed by polychaetes (5%), barnacles (5%), protozoans (3%), ciliates (2%), gastropods (1%) and others (1%) [2]. Copepods are metazoan zooplankton that is dominant in food webs that support fisheries [3]. To maintain an ecological balance in the aquatic environment, zooplankton prey on phytoplankton and microzooplankton.
The genus Tintinnopsis is a microzooplankton from the protozoa (ciliate) group that lives in various habitats in marine waters. In its environment, tintinnopsis plays an important role, among others, as a food source for higher levels of zooplankton such as copepods, small crustaceans, cnidarians, tunicates, rotifers, ostracods, cladocerans, mollusks and fish larvae [4,5]. Some early studies from Govoni et al. (1983) and Turner et al. (1983) identified Tintinnopsis on the intestinal and fecal contents of copepods and fish larvae [6,7]. Predators of tintinnopsis are generally copepods, including the families Acartidae, Aetideidae, Metridinidae, Paracalanidae, and Tortanidae. On the south-west coast of India, there were four species of tintinnopsis found in two Litopenaeus vannamei farms, namely T. minuta, T. entzil, T aperta, and T fusus. It was found that T. minuta had the highest abundance (500 cells l-1) [8]. Also, in an intensive pond of Litopenaeus vannamei in Long Vinh Vietnam, protozoan and copepod zooplankton dominated the digestive tract of the cultivated shrimps [9].

In addition to its role as a food source for high levels of zooplankton and fish larvae, tintinnopsis also plays a role in reducing bacteria and harmful algal blooms (HABs). They feed on bacteria and algae, including HABs chaetoceros, Gymnodium, and alexandrium [3,10]. Microzooplankton such as ciliates and dinoflagellates eat dissolved food waste or microparticulate and can reduce 70% of organic matter in waters [3]. Research on tintinnopsis (Choreotrich group, a small marine ciliate) has been carried out in the Chilika lagoon, India [11] and widely known for its important role, however, there is still a lack of studies on tintinnopsis in Indonesia. Hence this study aims to determine the abundance of zooplankton in the estuary of Polong river, Pangkajene Islands (Pangkep) Regency.

2. Methods

2.1. Study Site

Sampling was conducted in the estuary of Polong river, Pangkep regency, in June 2019 (Figure 1). There were four stations with three replications (sub-stations) each. The distance from each sub-stations was 500m, which started from the shoreline towards the open sea. Research stations were determined based on the traditional port location (station 1), mangrove area (station 2), adjacent to fish and shrimp ponds (station 3), and nearby the jetty owned by Tonasa cement manufacturer (station 4).

Figure 1. Map showing study site in the estuary of Polong river, Pangkep regency
2.2. Zooplankton Samplings
Seventy-five liters of seawater were filtered (using a plankton net 25; 0.06 mm) into a 100 mL sample bottle and added with 2mL of 1% Lugol. The water salinity was measured by using a hand-held refractometer, whereas temperature and dissolved organic matter (DOM) by using a DO meter. A pH meter was used to measure water pH. Water flow was measured by using the float method. The total organic matter was prepared based on the Winkler method then analyzed by using a spectrophotometer (HAC-USA type LPG 422.99.00012) with 660 nm wavelength [12].

2.3. Zooplankton Analysis
Zooplankton was analyzed by using the Sedgwick rafter cell (SRC) method. Sedgwick rafter chamber was filled with a 1mL of the filtered water sample and placed on a microscope for species and abundance observation. The number and species of zooplankton in 100 squares (from 1000 squares) was counted and identified following [13,14].

2.4. Data Analysis

2.4.1. Zooplankton Abundance. The number of zooplankton observed was calculated using the following formula [12]

\[
\text{No./ml} = \frac{C \times 1,000 \text{mm}^3}{L \times D \times W \times S} \text{……………….. (1)}
\]

Where:
C = number of organisms counted,
L = Length of S-R cell (each strip) (mm)
D = Depth of S-R cell (depth of a strip) (mm)
W = Width of a strip (mm)
S = number of strips counted

2.4.2. Species composition. Species composition was calculated by using the following formula:

\[
\text{Species composition(\%)} = \frac{A}{A+B+C+\ldots} \times 100\%\text{……… (2)}
\]

A=Number of individuals of a species; A, B, C = Total number of species

2.5. Statistical Analysis
To test any differences of zooplankton abundance among stations, a one-way ANOVA was applied and continued with a post hoc Tukey HSD.

3. Results

3.1. Physical and chemical water parameter
Physical and chemical water parameters (i.e. temperature, salinity, pH, and dissolved oxygen) of Polong river estuary, were relatively the same amongst stations (Table 1). The Dissolved Organic Matter (DOM) was relatively low in station 1 and relatively high in station 4. The Total Suspended Solid was relatively the same, except in station 4.
Table 1. Physical and chemical water parameter of the estuary of Polong river, Pangkep regency

| Physico-chemical Parameters                  | Stations |
|---------------------------------------------|----------|
|                                             | I       | II    | III   | IV     |
| Temperature (°C)                            | 29      | 29    | 29    | 29     |
| Salinity (ppt)                              | 32      | 32    | 32    | 32     |
| pH                                          | 6.42    | 5.59  | 5.66  | 5.65   |
| Dissolved Oxygen (mg/L)                     | 4.8     | 3.43  | 3.23  | 4.8    |
| Dissolved Organic Matter (mg/L)             | 27.6    | 30.1  | 30.8  | 45.8   |
| Total Suspended Solid (mg/L)                | 41.17   | 42.13 | 38.29 | 32.61  |
| Current direction (°)                       | 323     | 320   | 279   | 314    |
| Current speed (m/sec)                       | 0.2     | 0.2   | 0.1   | 0.1    |

3.2. Zooplankton abundance
There were 3 species of zooplankton found in the Estuary of Polong River, Pangkep regency. Two species (Tintinnopsis sp and nauplii copepods) were found in stations 1, 3, and 4. Whereas in station 2, another species (Apocylops sp) was also identified. This resulted in 3 species altogether in station 2. The most abundant zooplankton was Tintinnopsis sp followed by nauplii Copepoda and Apocylops sp. It also showed that station 2 had the most abundant of Tintinnopsis, followed by stations 3,1 and 4 (Figure 2).

Figure 2. Individual abundance for each zooplankton species found in all stations in the estuary of Polong river, Pangkep regency.
3.3. Species composition

*Tintinnopsis* sp. dominated all stations in Polong river estuary, Pangkep regency (Figure 3). The highest percentage of species composition was *Tintinnopsis* sp (80.60%) followed by nauplii copepods (18.62%), and *Apocyclus* sp (0.78%) (Figure 3).

![Figure 3. Zooplankton species composition in four sampling stations, at the estuary of Polong river, Pangkep regency](image)

### 3.4. Statistical analysis

One-way ANOVA showed no significant differences in zooplankton abundance between stations and between individuals (CI = 95%; p >0.05) (Table 2).

|                 | Sum of Squares | df | Mean Square | F     | Sig.  |
|-----------------|----------------|----|-------------|-------|-------|
| **Stasiun**     |                |    |             |       |       |
| Between Groups  | 9,750          | 6  | 1.625       | 1.548 | .324  |
| Within Groups   | 5,250          | 5  | 1.050       |       |       |
| Total           | 15,000         | 11 |             |       |       |
| **Individu**    |                |    |             |       |       |
| Between Groups  | 3,000          | 6  | .500        | .500  | .788  |
| Within Groups   | 5,000          | 5  | 1.000       |       |       |
| Total           | 8,000          | 11 |             |       |       |

### 4. Discussion

Water temperature and salinity of Polong river estuary were the same for all stations which can support the life of *Tintinnopsis*. Rakshit D et al., (2016) stated that in nature, tintinnopsis are considered as eurythermal and euryhaline organisms [15]. In a 2007 survey conducted in the Philippine Sea and eastern China Sea, Tintinnids were found at temperatures above 26°C and salinity...
above 34 PSU [16], then in 2008, it was found at temperatures of 26-29 °C and salinity 34-35 PSU. Tintinnopsis lives in the estuary and mangrove areas with temperature and salinity varying between 22.5 - 33.8°C and 2.9 - 34.5 PSU. In the cold and summer months of the Japanese sea, the tintinnid was dominated by Tintinnopsis beroidea [17], while in the Yellow Sea, South Korea, Tintinnopsis karajacensis and T. radix were dominant in winter and T. karajacensis in summer [10] with temperature 14.2 ± 0.8°C and reached a maximum in August i.e. 27.8 ± 0.7°C. Salinity variation in the Gulf of Naples was between 36.52-38.32 PSU [18]. In this study, water pH was tended to be acidic (Table 1). Increasing pH can cause a decrease in the growth rate of tintinnid [19] According to [3] some protists are very sensitive to pH. pH values > 8.8 will cause death. Other studies have found tintinnopsis at pH 7.9-8.22 [15]; 7.37-7.6 [20].

The dissolved oxygen concentration observed at each station was below 5.0 mg / L. Tintinnopsis can withstand low oxygen conditions in waters with a concentration of 3.24-4.89 mg / L [15.20]. Total suspended solids (TSS) was tended to have a high concentration. The Polong River estuary is influenced by river runoff so that suspended organic matter does not settle to the bottom of the water, as a result, the estuary was more turbid than the offshore areas [21]. Dissolved organic matter is generally nutrients including nitrates, phosphates, and silica which are needed for the growth of phytoplankton. Zooplankton abundance is triggered by an increase in the food supply, such as phytoplankton [21]. Nutrient is considered as one of the most important parameters in the estuarine environment affecting the growth, reproduction, and metabolic activity of organisms.

There were three species of zooplankton found in Polong river estuary, namely Tintinnopsis sp, copepod nauplii, and Apocyclops sp. Tintinnopsis sp was the dominant species (80.60%), followed by copepod nauplii (18.62%) and Apocyclops sp (0.78%). Tintinnopsis abundance was higher nearby the estuary. The estuary or the coastal area is where organic matter and phytoplankton accumulate which is carried by the flow of water when there is an exchange of the water pond. Microzooplankton is a mineralization grazer of organic nutrients [22]. According to Rakshit D et al., (2016) Tintinnid is more tolerant of high organic pollutants [15]. Porto Neto et al., (2009) found 18% tintinnopsis in commercial shrimp Litopenaeus vannamei farm in Northeastern Brazil [1]. Vinh H P (2017) also found that protozoan and copepod zooplankton dominate the digestive tract of Litopenaeus vannamei in intensive ponds in Long Vinh commune, Vietnam [9].

Tintinnopsis sp is classified as Phylum: Ciliophora; Class: Spirotrichea; Subclass: Choretrichia; Order: Tintinnida. Tintinnids are unique unicellular ciliates among planktons as they build vase-shaped shells called loricae [10]. The Tintinnida Order is a microzooplankton ciliate that is dominant and abundant in marine shallow waters and is a cosmopolitan [20,23–25]. The genus Tintinnopsis is dominant in summer and winter (76 and 61%) on the coast of Digha Bay of Bengal [26]. Seven tintinnid species including Tintinnopsis minuta and T. beroidea, are in the coastal Mediterranean [18]. In the estuary and mangroves of Parangpettai, India, genus Tintinnopsis was the most abundant [27]. Tintinnipsi includes cosmopolitan microorganisms and can be found in tropical, subtropical, temperate, and cold climates, as well as marine and neritic coastal areas [24]. However, it is more common in coastal areas than in neritic areas [10,11]. Genera Tintinnopsis are abundant in coastal waters and estuaries [28]. Their abundance in coastal areas is also related to lorica (shells). To produce lorica, tintinnopsis requires small mineral particles contained in organic matter that sinks in shallow waters, especially lorica agglutinin [15]. Agglutinin lorica producing tintinnids are commonly found in coastal areas, whereas hyalin lorica producers (or without lorica at all) are commonly found in neritic areas [3]. Tintinnopsis radix produce lorica agglutin from mineral particles, and are abundant on water surface until the maximum depth of 50m Tintinnopsis karajensis which has large branching hyaline was found in Makassar waters, Indonesia by Busch in 1925 as well as in river estuary of Mempawah, Pontianak, West Borneo [3,29–31].

Copepods are small crustaceans found in fresh, brackish, groundwater, sea water and live in association with aquatic organisms. Adult copepods are 200 µm-2 mm in size, whereas copepod nauplii are generally 20-200 µm [32]. Like tintinnopsis, copepods have an important role in the food web, and their first consumes are captive and marine fish larvae and large size crustaceans [33,34].
Edun et al., (2018) reported that copepods *Acratia tonsa* are the diets for African catfish (*Clarias gariepinus*) larvae [35]. Also, anchovies and sardines consume zooplankton and prey dominantly on copepod nauplii during their life cycle [36]. Hansen (2011) also reported that Cod (*Gadus morhua*) larvae experience rapid growth and high survival in the copepods nauplii treatment compared to the rotifers treatment [37].

In our sampling, low number of copepod nauplii was found at Polong River estuary, Pangkep regency. This may be due to the sampling depth was carried out at a depth of 2-3 meters from the surface and carried out during the day, while copepods are included in the zooplankton group, which are generally found on the seabed and freshwater bottoms, Dubischara et al., (2002) found nauplii were concentrated above 50 m depth whereas adult copepods tended to be down in of 300-100 m depth [32].

Apocylops is included in the copepod group, which also serves as food for aquaculture ponds. *Apocylops royi* is the only cyclopoid species that is commercially cultivated as live food in the aquaculture industry in Taiwan. Like the others, copepod species can live in a wide variety of salinity and temperature, hence they are easily cultivated in ponds, estuaries, brackish and marine ecosystems. In addition to cosmopolitan life, copepod has a short generation time (2-5 days), hence having a fast development [38].

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