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SPATIAL AND TEMPORAL VARIATION OF ZOOPLANKTON COMPOSITION NEAR WHALE SHARK SIGHTINGS IN PROBOLINGGO OF EAST JAVA, INDONESIA

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ABSTRACT

Whale shark occurrence in Probolinggo differs from other Indonesian locales, suggesting a link to zooplankton availability. Zooplankton composition and whale shark emergence are the focus of this study. From December 2017 to November 2018, six observation points were made each month. A plankton net filters and lugol preserves water. Olympus CX23 microscope observations were repeated twice. The spatial analysis revealed varying whale shark numbers at each station (Chi-square test, X2 = 1418.6, P <0.05), with six sharks observed at station PR_5. Zooplankton numbers were similar at each location. Temporal analysis revealed significant differences in whale shark appearance each month (Chi-square test, X2 = 81.04, P <0.05), with March and November having the highest appearance among the three individuals. The amount of zooplankton varied (Chi-square test, X2 = 148.61, P <0.05), with the highest abundance in April and March. Whale shark appearance and zooplankton composition were not correlated (r = 0.01, P< 0.05) both geographically and temporally. Whale sharks were linked to zooplankton kinds. Results indicate whale sharks are particularly interested in Acartia sp. (r = 0.3, P < 0.05). This suggests that whale sharks' appearance is determined by their demand for food, not zooplankton availability.
Keywords: aggregation, feeding habit, habitat use, *Rhincodon typus*

Variasi Spasial dan Temporal Komposisi Zooplankton di Wilayah Kemunculan Hiu Paus, Probolinggo, Jawa Timur, Indonesia

ABSTRAK

Pola kemunculan hiu paus di Probolinggo berbeda dengan lokasi lain di Indonesia, diduga kemunculannya memiliki hubungan dengan ketersediaan zooplankton. Tujuan penelitian ini adalah untuk mengkaji komposisi spasial dan temporal zooplankton serta mengaitkan dengan kemunculan hiu paus. Penelitian dimulai Bulan Desember 2017 - November 2018 dan terdapat enam titik pengamatan tiap bulannya. Air disaring menggunakan plankton net dan diawetkan menggunakan lugol. Pengamatan menggunakan mikroskop Olympus CX23 dengan dua kali ulangan. Hasil analisis spasial menunjukan adanya variasi terhadap keberadaan hiu paus di setiap stasiun (Chi-square test, X2= 1418.6, P <0.05) dengan kemunculan tertinggi diamati di stasiun PR_5 sebanyak enam individu. Namun, jumlah zooplankton tidak menunjukkan perbedaan yang signifikan di setiap stasiunnya. Berdasarkan analisis temporal, kemunculan hiu paus berbeda signifikan setiap bulannya (Chi-square *test, X2= 81.04, P <0.05), dengan bulan Maret dan November menunjukan kemunculan tertinggi, tiga individu. Terdapat variasi kelimpahan dalam jumlah zooplankton (Chi-square test, X2= 148.61, P <0.05), dengan kelimpahan terbesar terjadi pada bulan April dan Maret. Baik secara spasial maupun temporal, korelasi kemunculan hiu paus terhadap komposisi zooplankton tidak berhubungan (r= 0.01, P< 0.05). Selain itu, korelasi antara hiu paus terhadap jenis zooplankton yang ditemukan juga dilakukan, hasilnya menunjukan bahwa Acartia sp. terindikasi menjadi jenis target hiu paus tersebut (r= 0.3, P <0.05), dimana kemunculan hiu paus tidak dipengaruhi oleh jumlah zooplankton yang tersedia, namun berdasarkan kebutuhan kalorinya.*

***Kata kunci**: agregasi, kebiasaan makan, penggunaan habitat, Rhincodon typus*

INTRODUCTION

The whale shark (*Rhincodon typus* Smith, 1828) exhibits a significant migratory pattern that is strongly influenced by the availability of food sources (Yap-dejeto *et al.*, 2013). Whale sharks are zooplankton-feeding organisms, as confirmed by fecal observations and abdominal exams (Nelson and Eckert, 2007; Boldrocchi *et al.*, 2020). Whale sharks consume many kilograms of zooplankton on a daily basis, as recorded by Heyman *et al.* (2001), Hacohen-Domene *et al.* (2006), Nelson and Eckert (2007), Hernandez-Nava and Alvarez-Borrego (2013), and Boldrocchi *et al.* (2020). The zooplankton species that whale sharks prey on include Copepod, crab larvae, fish eggs, fish larvae, Chaetognath, and others (Heyman *et al.*, 2001; Rowat and Engelhardt, 2007; Meekan *et al.*, 2009; Boldrocchi *et al.*, 2018). These findings suggest that the patterns of whale shark aggregation varied throughout each observation region. The groups of copepod, Chaetognatha, and Ctenophore have an impact on whale sharks at the observation location in Djibouti, North Africa (Boldrocchi *et al.*, 2020). The tintinnid group is the predominant species in the waters of the Philippines, which are also home to a significant population of whale sharks (Yap-dejeto *et al.*, 2013). Unlike the waters of the Ningaloo Reef, the whale shark shares its habitat with herring fish, which feed on the copepod found in these regions (Wilson, 2002). Nevertheless, the fact that the whale shark is an ophthalmological animal does not imply a specific preference for its diet. Its primary objective is not only to devour huge amounts of food, but also to meet its energy requirements (Pierce and Norman, 2016).

Whale sharks inhabit several locations in Indonesia, including Cendrawasih Bay, Papua; Talisayan, Kalimantan; Botuborani, Gorontalo; and Bentar Beach, Probolinggo. In some locations, such as Cendrawasih Bay, Talisayan, and Botuborani, where local fishermen provide their catch, whale sharks no longer appear seasonally but permanently due to the presence of lifting nets (Himawan *et al.*, 2015). Even though there are lifting nets in Probolinggo, fishermen do not give their catch to whale sharks, so whale sharks appear seasonally in these waters (Kamal *et al.*, 2016). Researchers have conducted several studies on whale sharks in Probolinggo, including Kamal *et al.* (2016), Himawan (2017), Syah *et al.* (2018), and Kamal *et al.* (2020); however, only Kamal *et al.* (2016) and (2020) conducted research on zooplankton abundance at this location. Due to its opportunistic nature, the composition of zooplankton, or its preference for it, determines its appearance, causing it to change seasonally. Investigations into the spatial and temporal appearance of whale sharks in Probolinggo are necessary to complete and answer the question of their seasonal appearance. The aim of this study was to investigate a spatial and temporal of zooplankton composition in Bentar Beach, Probolinggo in six station for twelve months and then linking these findings to the presence of whale sharks. This research can provide additional data on whale sharks in Probolinggo and serve as the foundation for conservation efforts in this area.

RESEARCH METHODS

Study Site

The sampling stations were located in the tourist area of Bentar Beach, Probolinggo, which is part of the Madura Strait. We collected zooplankton samples at six stations (Figure 1) between December 2017 and November 2018. The station points selected were based on previous research (Kamal *et al.,* 2016; Himawan, 2017; Syah *et al.,* 2018; Kamal *et al.,* 2020) and reports from local people. We conducted the observation every month during daylight hours (06.00–14.00). The Laboratory of Marine Biodiversity and Biosystematics, Department of Marine Science and Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University conducted the observations.

Zooplankton Collection

We conducted a vertical sampling of zooplankton, commencing at a depth of one meter below the water's surface. The water was filtered using a plankton net with a mesh size of 150 µm, a 50 cm diameter mouth conical net, and put into 100 ml bottle sampling. Lugol's solution was used to preserve the sample for taxonomical investigation. We have successfully identified the organism, conducted a thorough count, and calculated the density by expressing the number of individuals per cubic meter. For observations, we utilized an Olympus CX23 microscope set at a magnification of 10x. To collect the sample water, we obtained 1 ml and placed it in a Sedgewick-Rafter (SRC). Morphological observations were performed with identification books authored by Catellani and Edward (2017). We performed zooplankton identification at the genus level. If the morphological analysis cannot identify the specimen, we will categorize it at the higher taxonomic level.

Statistical Analysis

Statistical analyzes were performed and visualized in R studio (v. 3.6.2, http://r-projekt.org). Spatial and temporal visualization of composition data used bar

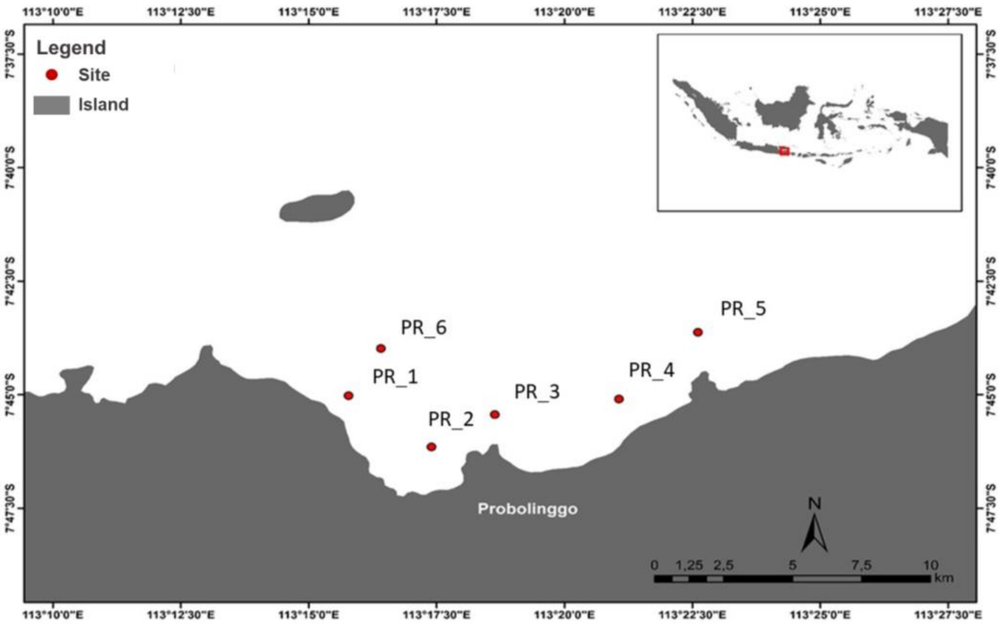

Figure 1. The sampling location was Bentar Beach, Probolinggo, East Java. It divided into six station, (PR_ 1, PR_2, PR_3, PR_4 and PR_5, PR_6).

plots from the ggplot2 package. Data were analyzed by non-parametric statistics and are recommended for this study (Nelson and Eckert, 2007). Kruskal-Wallis test was used to test statistical differences between zooplankton composition between station and month. Kruskal-Wallis test was used to test statistical differences between zooplankton composition, station and month. Besides, Kruskal-Wallis was used to the whale shark sighting between station and month. Pearson correlation tests were carried out to test a relationship between zooplankton composition and individual whale shark encountered (Nelson and Eckert, 2007; Hernández-nava and Álvarez-borrego, 2013; Boldrocchi *et al.,* 2018; De Veaux *et al.,* 2018).

RESULTS AND DISCUSSION

Spatial Variation

These observations' spatial variations refer to the horizontal surface distribution at each station (McManus and Woodson, 2012). We have divided these observations into six observation points, drawing on previous research (Kamal *et al.,* 2016; Himawan, 2017; Syah *et al.,* 2018; Kamal *et al.,* 2020) and information from local residents, particularly whale shark tourism activists in Probolinggo. Based on the Kruskal-Wallis analysis, the appearance of whale sharks at the six stations showed significant differences (chi-square test, X^2= 1418.6, P <0.05). Dunn's follow-up test revealed that almost all stations showed significant differences between one station and another. PR_5 found the most whale sharks at each station, with six individuals, whereas PR_4 and PR_6 did not find any individual whale sharks (Figure 2). We conducted the Pearson correlation test to examine the relationship between the appearance of whale sharks and the spatial abundance of zooplankton. The relationship between whale sharks and zooplankton abundance formed a non-significant negative correlation (r= -0.01, P >0.05).

The existence of whale sharks is influenced by various factors including temperature, the level of disturbance they experience, and food availability (Hacohen-Domene *et al.,* 2006; Rowat and Gore, 2007; Whitehead *et al.,* 2019; Marliana *et al.,* 2018). Tropical coastlines with high temperatures are often an attraction for whale sharks. Various investigations (Compagno, 1984; Yap-dejeto *et al.,* 2013; Ketchum *et al.,* 2013; Whitehead *et al.,* 2019; Maharani *et al.,* 2014; Wulandari *et al.,* 2018) have detected this occurrence, and some have recorded temperatures exceeding 35 ^{o}C (Robinson *et al.,* 2013). According to research conducted by Wulandari *et al.* (2018), the temperature range in Probolinggo waters fluctuates between 28.2 to 31.3 ^{o}C. The higher water temperature in Probolinggo can be caused by various factors, including the large influx of fresh water from rivers and relatively calm wave conditions (Nuriyanto *et al.,* 2019). The presence of fresh water molecules causes increased friction between salt water and fresh water from rivers, resulting in higher temperatures near the coastline than offshore (Tarigan and Edward, 2000). Apart from that, the remote location of PR_5 station and farthest from tourist activities at Bentar Beach is a contributing factor to the presence of sharks at this station.

The total number of zooplankton identified was twenty-three taxa, consisting of seventeen genera and six identified at the higher taxa level (family, class, or order). Different results from Utojo and Mustofa's (2016) research on zooplankton in Probolinggo waters revealed seven genera. The Kruskal-Wallis test on the composition of zooplankton in Probolinggo shows that there are no significant differences between station (chi-square test, X^2= 61.465, P >0.05). Several studies, such as Mirón *et al.* (2014), Cardenas-Palomo *et al.* (2015), and Witalis *et al.* (2024), support the findings of

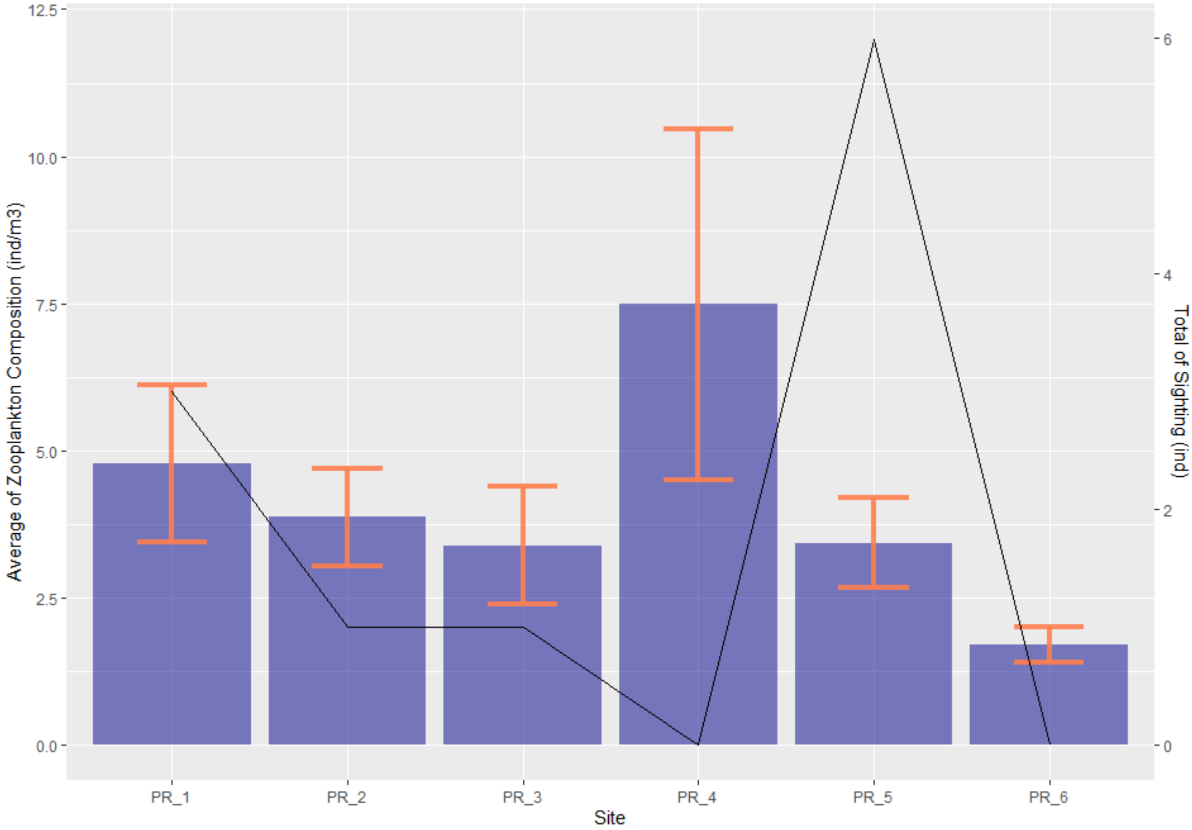

Figure 2. Average of zooplankton composition (Bar plot ± SD) and total of *Rhincodon typus* sighting (black line) recorded for each sampling station in one years.

this study. All these studies demonstrate that there were no significant differences in composition of zooplankton between station in same location. Physical oceanographic factors have no effect on the horizontal spatial distribution compared to the vertical spatial distribution, especially in trophic area (McManus and Woodson, 2012; Azevedo and Bonecker, 2003; Kâ *et al.*, 2012; Mirón *et al.*, 2014). Station PR_4 had the highest average abundance (7.49 ± 2.98 ind/m^3), while station PR_3 had the lowest average abundance (1.69 ± 0.31 ind/m^3) (Figure 2).

The highest number of taxa identified was at station PR_4, totaling 21 taxa and consisting of sixteen genera and six large groups. Several environmental conditions, such as high levels of nutrients, influence the abundance of zooplankton at station PR_4, which indicated can increase the abundance of phytoplankton as a food for zooplankton (Klais *et al.*, 2016).

The genus *Tintinnopsis sp.* accounted for the highest percentage of zooplankton at six stations, with 33%, followed by the Nauplius group at 31%, and fish eggs at 10%. We most often find the *Tintinnopsis sp.* species at point PR_1 (8.1% of the time), the Nauplius group at point PR_4 (14% of the time), and fish eggs at point PR_4 (Figure 2). Due to its cosmopolitan nature, *Tintinnopsis sp.* frequently inhabits neritic areas that are relatively shallower and undulating (Dolan and Pierce, 2013). This cosmopolitan nature is what causes *Tintinnopsis sp.* and *Stenoseniella sp.* to be the main contributors to spatial abundance formation (Feng *et al.*, 2015). However, this study did not find *Stenoseniella sp.* but found other genera, such as *Favella sp.* and *Leprotintinnus sp.*, supporting spatial abundance in Probolinggo waters (Dolan and Pierce, 2013). Environmental variables, particularly nutrition, exhibit a strong correlation with

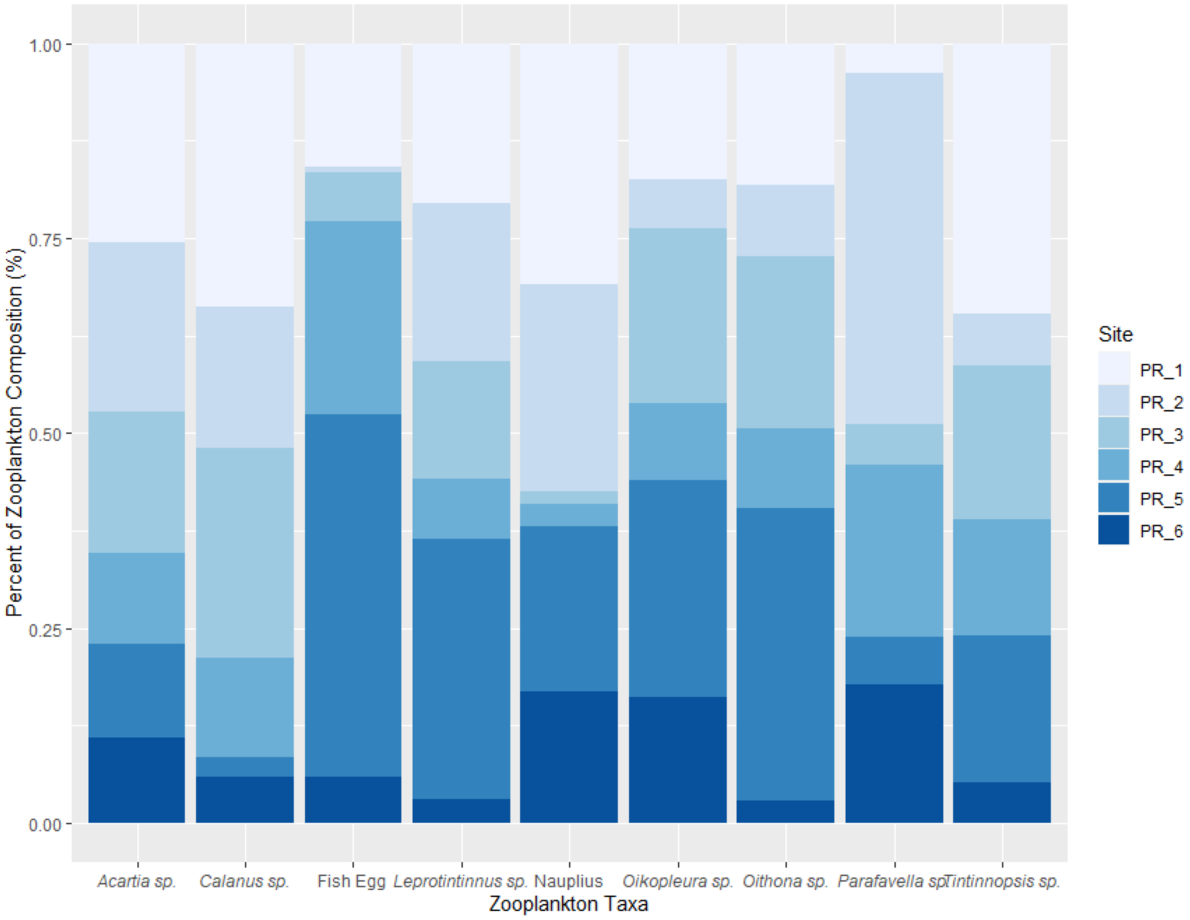

Figure 3. Percent of zooplankton composition (%) for ten taxon dominant in each station at Bentar Beach, Probolinggo.

tintinnid groups (Feng *et al.*, 2015; Jiang *et al.*, 2016; Xu *et al.*, 2016). Research by Feng *et al.* (2015) and Rakshit *et al.* (2017) suggests that the Tintinnids group, closely related to environmental variables, can serve as a bioindicator to distinguish water quality status. Apart from the tintinnids group, there is a Nauplius group that dominates these waters (Takashi and Uchiyama, 2008). Nauplius is a group that prefers relatively warm temperatures and is rich in nutrients. Nauplius itself consists of several forms, such as copepods, barnacles, crabs, and others. The Nauplius stage has a small and almost identical shape, so this shape will be difficult to identify at the species level (Jungbluth *et al.*, 2017).

Rivers heavily influence Probolinggo Beach, which features relatively calmer waves (Nuriyanto *et al.*, 2019). Mangrove forests, which encircle these waters, trap organic material input from rivers and the sea itself, thereby increasing the concentration of nutrients in the surrounding waters (Nugroho *et al.*, 2018; Boldrocchi *et al.*, 2020). Anthropogenic activity from rivers greatly influences the beach itself, leading to an increase in the concentration of nutrients entering it. If the numbers are appropriate, coastal areas will become areas with high levels of biodiversity and productive ecosystems (Kemp and Boynton, 2012). While the appearance of whale sharks does not correlate with the abundance of zooplankton in the area, we must consider other factors, like whale shark disturbances, to make the whale shark zone a crucial area for assessing the adequacy of the whale shark population. Establishing whale shark ecotourism areas necessitates the implementation of management strategies and regulations.

Temporal Variation

The eleven whale sharks were spotted throughout the course of one year. The Kruskal-Wallis analysis revealed that the presence of whale sharks in Probolinggo varied significantly each month (Chi-square test, X^2= 81.04, p <0.05). The Dunn's follow-up test indicates significant variations in the frequency of whale shark sightings throughout the year, with the greatest number observed between the months of March and November. In total, three individual whale sharks were recorded throughout this period (Figure 4). The seasonal occurrence of whale sharks in Probolinggo is consistent with previous research conducted by Boldrocchi *et al.* (2020), Whitehead *et al.* (2019), Reyes-Mendoza *et al.* (2021), and Cardenas-Palomo *et al.* (2015). The seasonal appearance of it is influenced by various factors, such as the availability of food (Ketchum *et al.*, 2013; Rohner *et al.*, 2015; Marcus *et al.*, 2016). Prior research (Whitehead *et al.*, 2020a; Hernandez-Nava and Alvarez-Borrego, 2013; Boldrocchi *et al.*, 2020; Robinson *et al.*, 2016; Ryan *et al.*, 2017) has investigated the correlation between these variables. Whale sharks in the waters of Probolinggo engage in active surface feeding, which involves swimming on the surface, constantly opening and closing their mouths, and filtering food items through their gill apparatus (Figure 5) (Motta *et al.*, 2010; Cade *et al.*, 2020). The correlation analysis conducted to examine the

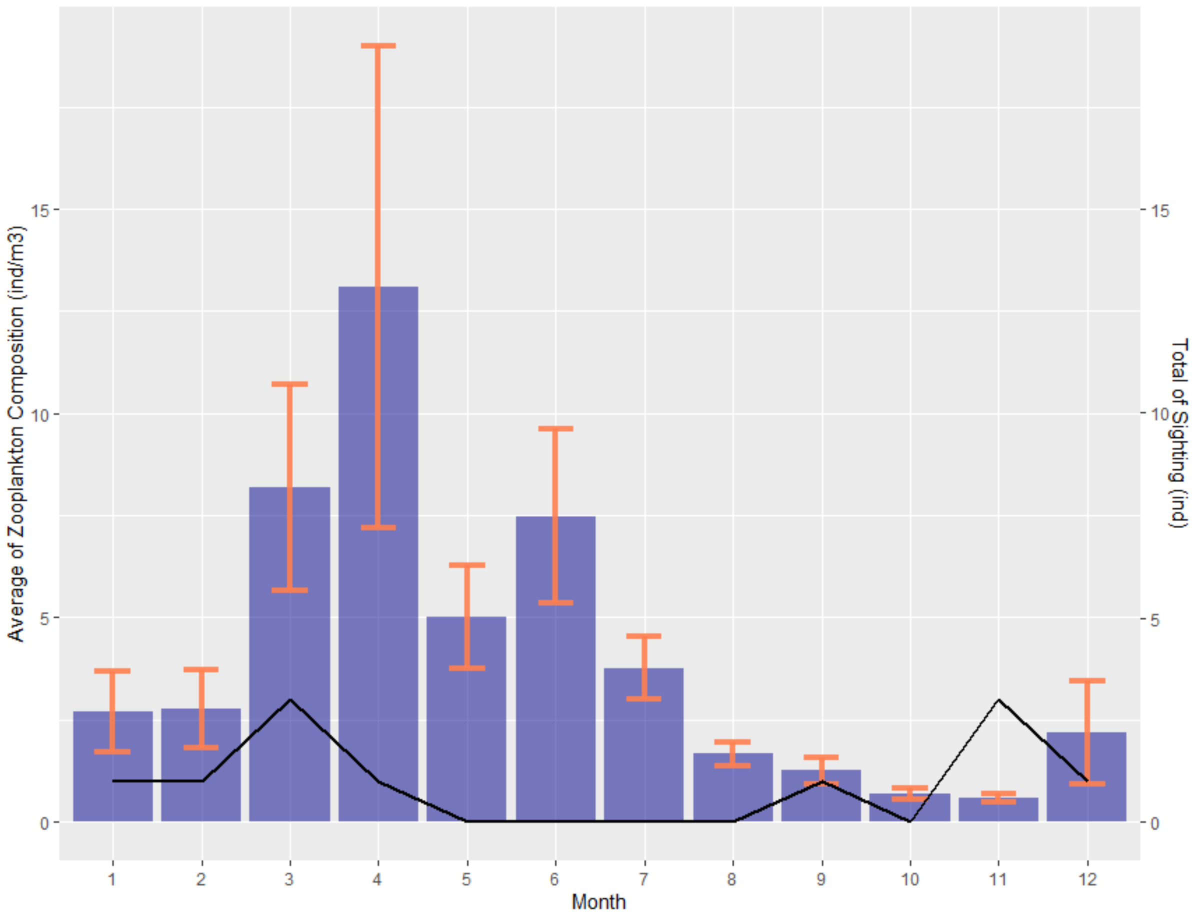

Figure 4. Average of zooplankton composition (Bar plot ± SD) and total of *Rhincodon typus* sighting (black line) recorded for each month in six station, Bentar Beach, Probolinggo.

Figure 5. A whale shark feeding actively at the surface. The whale shark will open the mount and move actively for filter the water.

relationship between the occurrence of whale sharks and the quantity of zooplankton per month reveals a weak positive connection that is not statistically significant ($r = 0.01$, $P > 0.05$).

The abundance of zooplankton in Probolinggo waters has a temporal pattern. The highest abundance of zooplankton was in March (8.19 ± 2.52 ind/m^3) and April (13.10 ± 5.89 ind/m^3) (Figure 4). Kruskal-Wallis analysis showed that reporting of zooplankton in each month was significantly different (Chi-square test, $X^2 = 148.61$, $P < 0.05$). DunnTest shows significant differences in December–August, December–June, and December–March. Research by Whitehead *et al.* (2020b) and Reyes-Mendoza *et al.* (2021), which found temporal differences in species composition and reported zooplankton, supports the results of this study. Temperature and water clarity have an influence on the composition of zooplankton each month (Witalis *et al.*, 2024). The same genus and group, namely *Tintinnopsis sp*. (35%), Nauplius (22%), and fish eggs (10%), dominate the percentage of spatial and temporal distribution of taxa. The most common times to find *Tintinnopsis sp*. are in January (65%), April (45%), and July (25%) (Figure 6).

The Tintinida group has the ability to dominate microzooplankton communities (Fonda Umani and Beran, 2003; Yap-dejeto *et al.*, 2013). Several factors, including temperature, salinity, and water clarity, influence the abundance of the tintinnid group (Wang *et al.*, 2014). Based on research by Rakshit *et al.* (2017), tintinnid community structure has a positive correlation with environmental variables, especially temperature and chlorophyll a. Low levels of water clarity are associated with Chlorophyll A. If water clarity increases, environmental variables such as temperature and concentration of chlorophyll a will be low (Rakshit *et al.*, 2017). The tintinnid group is not the main target of whale sharks in an ecosystem because this group has a small biomass value (Bojanić *et al.*, 2005). However, the tintinnid group is often associated with other microzooplankton groups and has an important role in

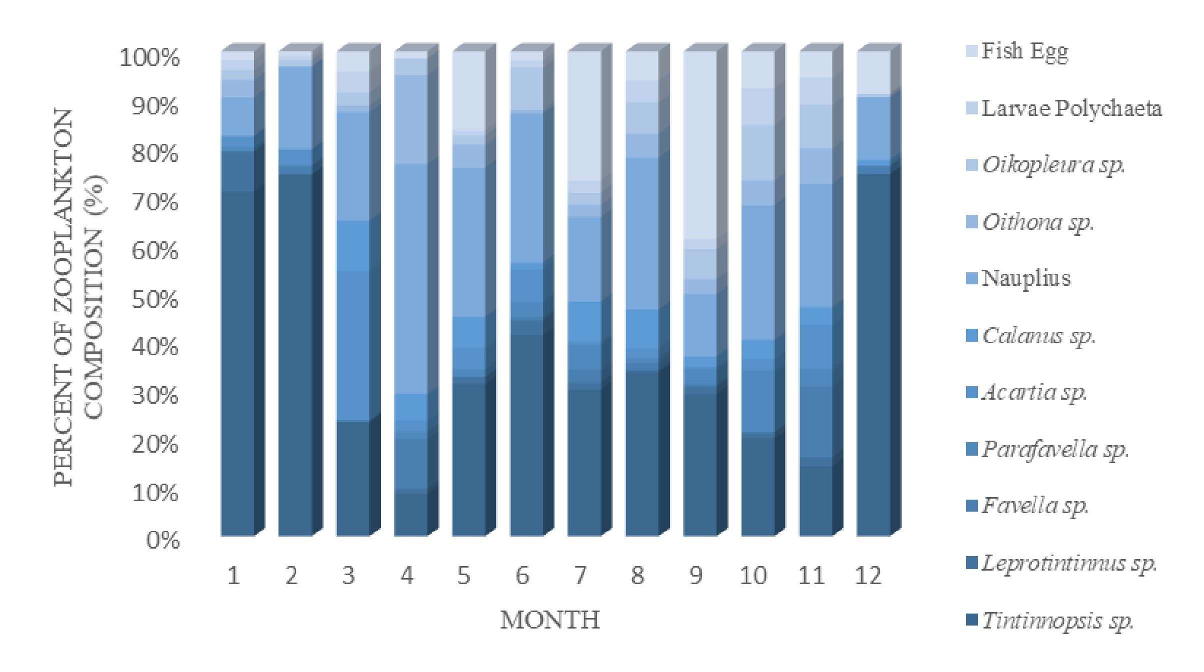

Figure 6. Percent of zooplankton composition (%) for each station in Pantai Bentar, Probolinggo.

channeling energy flows in the ecosystem to higher trophic levels (Conway, 2012; Stoecker and Pierson, 2019). Certain months, such as April and July, exhibit the highest reproduction or growth rates, contributing to the abundance of Nauplius and fish eggs in this study (Dar *et al.*, 1970; Turner, 2002). However, we were unable to identify either the Nauplius group or the fish eggs at the genus level, making it impossible to define a dominant level of reproduction or growth. Apart from that, Nauplius crustaceans are abundant because this group has effective movement to avoid predators and the ability to find prey is relatively effective, so the respiration rate in this group is relatively low (Marshall and Orr, 1966; Kiørboe, 2011).

Tintinnopsis sp., Nauplius, and fish eggs dominated the Probolinggo waters in this study both spatially and temporally, but there was no correlation with whale shark appearance. In this study, we conducted a correlation analysis to examine the impact of whale shark appearances on specific genera or groups. The findings showed that among the whole genus and group, *Acartia sp.* has a positive correlation with the sighting of whale sharks (r= 0.3, P <0.05). Other species, such as *Calanus sp.* (r= 0.1, P >0.05) and Polychaeta larvae (r= 0.2, P >0.05), also had a positive but not significant with the sighting of whale sharks in Probolinggo. Research by Whitehaed *et al.* (2020a) yielded different results, indicating that the copepod group in the waters of Bahia de La Paz, Mexico, did not exhibit a significant correlation, while the "other" group demonstrated a relationship with the appearance of whale sharks. Theoretically, the concentration or density of their food influences the appearance of whale sharks (Heyman *et al.*, 2001; Wilson *et al.*, 2001; de la Parra Venegas *et al.*, 2011; Rowat *et al.*, 2011; Reyes-Mendoza *et al.*, 2021). In areas where whale sharks are most prevalent, their aggregations react to a variety of food sources, including zooplankton blooms (Ketchum *et al.*, 2013; Cárdenas-Palomo *et al.*, 2015; Lavaniegos *et al.*, 2017) and fish spawning (Heyman *et al.*, 2012). Because whale sharks are opportunistic creatures (Pierce and Norman, 2016), they will congregate in a location that can meet their nutritional needs (Rohner *et al.,* 2015; Boldrocchi *et al.,* 2020). Apart from that, the whale shark's feeding strategy also determines and will be different for each region (Lawson *et al.,* 2019), where in Probolinggo waters the whale shark's feeding type is active surface feeding. Active surface feeding is the way whale sharks eat by opening their mouths while moving on the surface (Motta *et al.,* 2010; Cade *et al.,* 2020). This condition causes the whale shark to move more, potentially increasing the current entering its mouth and influencing the amount of zooplankton it filters. This condition is the same as for baleen whales (Potvin and Werth, 2017; der Hoop *et al.,* 2019).

The abundance of zooplankton and the appearance of whale sharks in Probolinggo are two forms of basic information. The Probolinggo Waters area, renowned for whale shark tourism activities, can utilize this basic information. We can use this data to understand the food availability of whale sharks and to influence their emergence. Therefore, we can create a platform that recommends policies for whale sharks and minimizes their disturbance during foraging events.

CONCLUSION

The temporal variations in the zooplankton composition and whale shark presence are significant. There is no apparent correlation between the zooplankton composition in Probolinggo and whale sharks. Nonetheless, a substantial correlation has been found between the emergence of whale sharks and some taxa, including Acartia sp. The zooplankton composition at each of the six observation stations did not significantly differ from one another in the spatial analysis; however, there were distinct variations in the appearance of whale sharks at each station. There is no discernible relationship between the distribution of zooplankton and the presence of whale sharks.

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