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Dr Petrus Hary Tjahja Soedibja:

Thank you for submitting the manuscript, "The natural food to support the growth rate of Nile Tilapia, Oreochromis niloticus (Linnaeus, 1758) for the poly mangrove ecosystem, Segara Anakan" to ILMU KELAUTAN: Indonesian Journal of Marine Sciences. With the online journal management system that v to track its progress through the editorial process by logging in to the journal web site:

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Reviewer A:

- Please discuss all data and compare to previous studies conducted in similar ecosystem

- Use the latest version of references (maximum 2013)

- Sent the article to certified English proofreader after revising the article

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1758) for the polyculture system in the mangrove ecosystem, Segara Anakan".

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The natural food to support the growth rate of Saline Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) for the polyculture system in the mangrove ecosystem, Segara Anakan

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Abstract

The polyculture of saline Nile Tilapia and Milkfish requires natural feeding to support brackish water fish production. Currently, no study has been done about the potential of natural food to support the productivity of the saline Nile Tilapia and Milkfish brackish water polyculture ~~system~~ in Tritih Kulon, Cilacap, Central Java. The potential natural food can be assessed through the abundance and diversity of plankton (phyto and zooplankton) analysis in the water body. This research analyzed the correlation between plankton diversity and abundance as natural feeding with saline Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) productivity in the brackish water polyculture ~~system~~ in the Tritih Kulon Village, Cilacap Regency, Central Java. The results showed that 21 plankton was identified from the research location, comprising 12 phytoplankton species and nine zooplankton groups. The plankton supports Nile tilapia and Milkfish productivity in the polyculture system, as indicated by low mortality (R^2= 0.825-0.908), high weight gain (R2=0.881-0.874) and high specific growth (0.87-0.91). In addition, the gut content in saline Nile Tilapia was 4.2-18.5 % (phytoplankton) and 7.7 – 15.6% (zooplankton). It could be concluded that natural food availability significantly supports saline Nile Tilapia productivity in brackish water culture.

Keywords: abundance, brackish water pond, fishery, monoculture, phytoplankton

---

Introduction

Aquaculture technology is divided into two different systems, i.e., monoculture and polyculture systems (Carbone and Accordi 2000; Cochard 2017; Hu et al. 2020; Zhu et al. 2022; Jansen et al. 2023). Aquaculture can be conducted in the mangrove ecosystem because mangrove has a good supply of natural feeding (Hilmi et al. 2021c, 2022a; Murniasih et al. 2022) to support brackish water aquaculture activity. A polyculture system was developed to increase fish productivity per unit area and to maintain water quality (Ekasari et al. 2015; Rose et al. 2015). Brackish water ~~P~~polyculture is ~~designed to support the~~ aquaculture system between two or more fish species or ~~organism~~ aquatic organism, for example, polyculture between milkfish and prawn or shrimp~~.~~ (Soedibya 2013; Rachmawati and Samidjan 2014; ~~Nuryanto et al. 2017~~; Soedibya et al. 2017). Other studies also reported polyculture between Milkfish and Nile Tilapia (Soedibya 2013; Mutia et al. 2018; Muyot et al. 2018). Some other studies also reported brackish water polyculture between prawn or shrimp and Nile Tilapia (Yang and Fitzsimmons 2004; Mutia et al. 2018; Prabu et al. 2019).

Natural food is essential to support brackish water fish polyculture. Fish natural food consists of plankton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacteria associated with detritus (Sukardjo 2004; Rougier et al. 2005; Nagelkerken et al. 2008; Mendoza and Henkel 2017; Andriyani et al. 2018). Generally, plankton is divided into phytoplankton and zooplankton (Henmi et al. 2017; Mendoza and Henkel 2017; Andriyani et al. 2020; Alam et al. 2021). ~~Besides natural food, phytoplankton is the primary producer in the aquatic ecosystem (Ishii and Kamikawa 2017; Karl and Church 2017; Alam et al. 2021)~~. Previous studies proved plankton availability and abundance are essential for fish growth and fisheries productivity (Henmi et al. 2017; Andriyani et al. 2020; Alam et al. 2021).

Plankton diversity and abundance have a high potential to support Nile Tilapia and other species in polyculture ~~production~~productivity, which has been reported from several regions (Yang

Commented [LL1]: Replaced by the latest one. Maximum 2013 and Fitzsimmons 2004; Nagelkerken et al. 2008; Soedibya 2013; Mutia et al. 2018; Kusuma et al. 2019). Some researchers reported plankton diversity and abundance in the polyculture pond of Nile Tilapia, *Oreochromis niloticus (*Sihombing et al. 2017; Muyot et al. 2018). Some other studies also found similar data in Milkfish *Chanos chanos* (Soedibya 2013; Ekasari et al. 2015; Kusuma et al. 2019). Currently, no report about plankton diversity and abundance in the polyculture of saline Nile Tilapia ~~milkfish~~ and ~~Nile Tilapia~~ milkfish in the brackish water pond in Tritih Kulon Village, District of North Cilacap, Cilacap Regency. These data are vital for developing the brackish water polyculture of both species in the Tritih Kulon Village.

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Saline Nile Tilapia, *Oreochromis niloticus (Linnaeus, 1758)* (Yang and Fitzsimmons 2004; Soedibya 2013; Ekasari et al. 2015; Kusuma et al. 2019) is a tropical fish that lives in shallow waters. This species lives in an aquatic ecosystem with various salinities (Suresh and Lin 1992; Basuki and Rejeki 2015; Kusuma et al. 2019; Prabu et al. 2019). This species can adapt and live in brackish water with a salinity of up to 25‰ (Japse and Caipang 2011). Nile tilapia consume a broad spectrum of feed and are classified as omnivorous fish. These species consume phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacteria associated with detritus (Mendoza and Henkel 2017; Neal et al. 2018; Takenaka et al. 2018; Teoh et al. 2018; Setyawati et al. 2019; Rahman et al. 2021). The ecological ~~characteristics~~ advantages of Saline Nile Tilapia have been ~~developed among~~utilized to develop brackish water aquaculture and had been polycultured with many fisheries commodities (Yang and Fitzsimmons 2004; Soedibya 2013; Ekasari et al. 2015; Muyot et al. 2018). Polyculture between Saline Nile Tilapia and Milkfish, Chanos chanos (Forsskal, 1775) ~~polycultures with the Nile~~ have long been ~~cultivated~~ carried out intensively in ponds. The feeding habits of milkfish, including herbivores, are the advantages of this species. This species is used as a superior product for cultured animals kept in polyculture with omnivores or carnivores. This condition causes the production of milkfish to be stable. Besides that, the specific taste causes milkfish to be in great demand. This species has been cultivated in a polyculture system with Tiger Prawn, milkfish, shrimp, and others (Soedibya 2013; Muyot et al. 2018)

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The Tritih Kulon Village resides in North Cilacap District, Cilacap Regency Central Java, Indonesia. The village has an area of 503.43 hectares consisting of land and swampy brackish water areas, including the mangrove and lagoon ecosystems (Hilmi et al. 2019, 2021c, d, e). The previous study utilized most saline water areas for shrimp aquacultures. At that time, shrimp farming has become a profitable business for the Tritih Kulon villagers due to high prices. Currently, shrimp production tends to decline and lead to business failure. This condition has persisted in South Java for the last four years, from Bantul to Cilacap Regencies. The defeat led to the shrimp pond in the Tritih Kulon Village becoming abandoned and unproductive. This study tried to provide a solution by providing information about the opportunities for using abandoned shrimp farming ~~land~~ brackish water pond in the Tritih Kulon Village as a polyculture area between saline Nile ~~t~~Tilapia and milkfish. A preliminary need is to study plankton diversity in a polyculture pilot pond of Saline Nile Tilapia, *O.* ~~*italics*~~*niloticus*, and Milkfish, *C. chanos*. This study aimed to analyze plankton diversity and abundance as natural ~~feeding~~ food in the brackish water polyculture pond of Saline Nile Tilapia, *Oreochromis niloticus (Linnaeus, 1758)* with Milkfish, *Chanos chanos* in the Tritih Kulon Village, District of North Cilacap, Cilacap Regency, Central Java.

Materials and Methods

Study area and time

The brackish water polyculture pilot pond ~~was~~ is located in the Tritih Kulon Village North Cilacap District, Cilacap Regency, Central Java, Indonesia (**Figure 1**) (Hilmi et al. 2021c, a). The pilot pond was a traditional earthen pond with an area of 2000 m^{-2} width. The research was conducted from August to October 2018.

Figure 1. Location of Tritih Kulon Village, North Cilacap District, Cilacap Regency Central Java, indicating the polyculture pond of *Oreochromis niloticus (Linnaeus, 1758)* and *Chanos chanos*

Procedures and data analysis

Pilot pond preparation for polyculture

Inflow and outflow of water occur naturally following tidal currents (Yang and Fitzsimmons 2004; Soedibya 2013; Muyot et al. 2018; Prabu et al. 2019). This study used a pilot earthen brackish water pond with an area of 2000 square meters (m^2). Pond preparation was initially by reversing the bottom of the pond for soil oxidation and killing fish disease-causing organisms. Neutralize soil acidity by adding ten sacks of lime to the pond surfaces and~~,~~ leaving the pool for two weeks without water. Afterward, the pond was fertilized using chicken manure and filled with water as deep as approximately 10 cm to allow plankton to grow. Then, the water depth was increased to about 1.5 m. We kept the depth at 1.5 m by adjusting the inlet and outlet holes as high as 1.5 meters.

Preparation, stocking, and cultivation of fish seeds

The seeds of *Oreochromis niloticus (Linnaeus, 1758)* and *Chanos chanos* were obtained from a governmental agency, namely the Center for Brackish Water Aquaculture Research (BBPBAP) Jepara, Central Java, Indonesia. The average length of Saline Nile Tilapia seeds was 3.1 ± 0.241 cm, with average body weight was 5.36 ± 0.21 g ~~(Yang and Fitzsimmons 2004)~~.

Both fish seed species were acclimatized for 2 hours before stocking in a pilot earthen pond. The stocking density for Saline Nile Tilapia was five individuals per m^2, with the total stocked fish being 10,000 seeds. In contrast, the stocking density of milkfish was set up for three seeds per square meter, meaning the total number of milkfish seeds was 6,000 individuals. During the first two weeks of rearing, the fish were fed with fine pellets and continued with ~~the number of~~the pellets size of 1000 after reaching a body length of 5 cm. After six weeks, we provided the fish with pellet number 2000 (Soedibya 2013).

~~Phytoplankton~~ Plankton collection and identification

~~Phytoplankton~~ Plankton samples were collected weekly for ten weeks of polyculture periods. Water samples were taken using a 10 liters bucket and filtered using plankton net number 25. We filtered the water samples ten times to have a total volume of filtered water of approximately 100 liters. The plankton was collected and remained in the collection bottle. Afterward, we removed the collection bottle from the plankton net. The filtered plankton was transferred into sample bottles and preserved using formalin until the final concentration became 4% and two drops of pure Lugol (Zafar 2004; Tsuji and Montani 2017; Andriyani et al. 2020).

~~Phytoplankton~~ Plankton identification was conducted under a binocular microscope with a magnification of 10 x 40. The ~~phytoplankton~~ plankton identification processes were as follows. One drop of sample water is placed on an object glass and covered. We carried out microscopic observations in 20 fields of view with up-down and left-right macrometer directions. ~~Phytoplankton~~ Plankton identification according to the figure in identification keys (Henmi et al. 2017; Sihombing et al. 2017). ~~Phytoplankton~~ Plankton abundance was calculated using the following formula (Arumugam et al. 2016; Andriyani et al. 2020; Asmarany et al. 2022).

Abundance (F) = [(A/B) x (C/D) x (E/F)] cell/litre

Note:
- A= Water volume in a bottle sample (30 mL)
- B= Water volume observed (0.06 mL)
- C= Cover glass width (484 mm^2)
- D= view field number (20)
- E= number of ~~phytoplankton~~ pankton individual
- F= total volume of filtered water (100 L)

Nile Tilapia growth

Saline Nile Tilapia growth was measured as the absolute growth of body weight (W_G), measured weekly for ten weeks. Body weight was measured using a balance with an accuracy of 0.01 g. Absolute body weight growth was calculated as follows (Palada-de Vera and Eknath 1993; Soedibya 2013; Muyot et al. 2018).

$W_G = Wt - Wi$

Note:
- W_G= absolute weight growth
- Wt= body weight after t-time
- Wi= initial body weight

Specific growth rate

The specific growth rate (μ) was calculated from the initial and final densities during the cultivation period, according to the method of Njouondo.

$$\mu = \frac{\ln(x2) - \ln(X1)}{t2 - t1}$$

Where X2 and X1 are the final and initial densities, respectively, t2 is the final cultivation time, and t1 is the initial cultivation time. The specific growth rate was noted as cells/day. This study calculated phytoplankton doubling time-based on the specific growth rate according to the equation

$$g = \frac{\ln 2}{\mu}$$

The relation between ~~phyto~~plankton to Nile Tilapia growth

This study estimated the potential of plankton to support saline Nile Tilapia growth based on the weekly plankton abundance and weekly saline Nile Tilapia growth. This study analyzed data about ~~phyto~~plankton diversity and abundance descriptively. Then we compared the result to the information available in previous publications. The potential of plankton to support Nile Tilapia growth was analyzed using Pearson correlation and regression (Karl and Church 2017; Hilmi et al. 2020)

Result and Discussion

Natural food ~~in polyculture system using the indicator of plankton~~ diversity and abundance

Natural food, especially plankton, in the polyculture system is required to support fish productivity, including in the saline Nile Tilapia cultivation. The ~~observation~~ diversity and abundance of natural ~~f~~food~~eeding~~ in the polyculture of saline Nile Tilapia and milkfish is summarized in Figure 1 ~~showed plankton in aquatic ecosystems~~. ~~Based on the microscopic identification of phytoplankton samples from the polyculture pond of Nile Tilapia and Milkfish can be shown in **Figure 2**.~~ The total number of natural foods was 21 plankton species, consisting of 12 phytoplankton species and nine zooplankton groups (**Table 1** ~~and **Figure 2**~~). ~~Whereas **Table 1** also describes the phytoplankton diversity and absolute abundance.~~

Table 1 showed that phytoplankton consisted of Cyanophyceae, Dinophyceae, and Bacillariophyceae with abundance between 455 cells/liter – 908 cells/liter and diversity between 1.44 – 2.23. Zooplankton consisted of Codonillidae, Arthropoda, Protozoa, Mollusca, and Crustacea with abundance between 253 cells/liter – 463 cells/liter and diversity between 1.88 – 2.6. The data indicated low plankton abundance were observed during study. The potential phytoplankton species were *Oscillatoria, Peridinium, Prorocentrum, Navicula, Nitzia, Rhizosolenia, Diatoma, Striatella, Pleurosigma, Chaetoceros, Coscinodiscus,* and *Fragillaria.* The total zooplankton abundance in Lampung and South Sulawesi ranges between 32,745 individuals.m^{-3} and 652,925 individuals.m-3, dominated by *Calanoida* and *Cyclopoida nauplii (Duggan et al. 2011).* This study found that *Tintinnopsis*, *Lapadella*, *Brachionus*, *Cephalodella*, and *Colurella* dominated the zooplankton. Some researchers also report that Indonesia has five new records of plankton species that are *Oithona decipiens, Oithona hebes, Oncaea atlantica group, Oncaea zernovi group, and Spinoncaea* spp (references?).

Different with Tigris River Iraq, which has 106 taxonomy units of zooplankton, including 65 taxa belonging to rotifers, 25 taxa to copepod, and 16 taxa to Cladocera with The Shannon–Weiner index of Rotifera varied from 0.67 to 3, 0.50–1.72 for Cladocera and from 0.91 to 2.51 for Copepoda. (Abdulwahab and Rabee 2015). The other condition shows that Delta Mahakam has 48 taxa phytoplankton belonging to Bacillariophyceae (35), Dinophyceae (6), Chlorophyceae (4), and Cyanophyceae (3) (Effendi et al. 2016). Lake Burdur Turkey has six zooplankton taxa: Hexarthra fennica, Brachionus plicatilis from Rotifera, and Arctodiaptomus burduricus from Copepoda. The average zooplankton density in Lake Burdur shows that 399,074 ind.m^{-3} is distributed by 51% H. fennica, 9% B. plicatilis, and 40% A. burduricus..(Gülle et al. 2010). Pearl River estuary, China, has 132 species of zooplankton (Honggang et al. 2012). In River Yeúilırmak, Amasya, Turkey has plenty of divisions, which are *Navicula cincta, N. cryptocephala,* and *N. rhyncocephala* (Soylu and Gönülol 2003). (Fang et al. 2012) also reports that the Xiaoqing River estuary has seven plankton species: *Skeletonema costatum, Tribonema affine,* and *Chlorella* sp.

The data showed that the abundance of Bacillariophyceae > Dinophyceae > Cyanophyceae with *Nitzia, Diatoma,* and *Chaetoceros* had high potency in the polyculture system. Whereas Rotifera, Chodonillidae, and Arthropoda had high potency as natural feeding. The distribution of natural feed of both phytoplankton and zooplankton (diversity and abundance) can be shown in **Figure 2.** Based on **Figure 2** showed that the diversity and abundance of phytoplankton > zooplankton. The variety of phytoplankton on stations 6, 7, 8, and 10 was greater than on other stations, whereas the diversity of zooplankton station 1 was the highest.

Table 1. The diversity and abundance of plankton in the polyculture system

Classis/Species	abundance (cell/liter)										confidence interval
	1	2	3	4	5	6	7	8	9	10	
Phytoplankton											
Cyanophyceae											

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Oscillatoria	822	121	251	311	184	286	384	611	167	282	341.9±217.29
Dinophyceae											
Peridinium	1.302	212	114	221	377	211	306	288	601	719	435.1±357.50
Prorocentrum		412	218	174	89	281	241	418	291	339	246.3±133.42
Bacillariophyceae											
Navicula	481	504	417	385	1.298	738	781	552	828	829	681.3±275.43
Nitzia	781	1.027	818	1.219	3.036	1.833	2.077	1.865	2.199	3.071	1,792.6±838.09
Rhizosolenia	241	438	221	371	218	638	721	811	719	693	507.1±234.34
Diatoma	1.799	2.385	1.755	1.544	311	1.539	2.190	3.082	2.710	2.804	2,011.9±808.14
Striatella	422	390	271	315	278	529	481	365	391	380	382.2±82.38
Pleurosigma	251	622	315	128	276	383	485	391	588	429	386.8±152.79
Chaetoceros	204	281	316	285	3.138	1.892	926	819	692	539	909.2±928.53
Coscinodiscus	151	189	326	618	261	522	419	791	839	549	466.5±239.83
Fragillaria		765	433	221	141	523	409	591	285	263	363.1±226.94
total	6454	7346	5455	5792	9607	9375	9420	10584	10310	10897	
abundance (cell/liter)	645	612	455	483	801	781	785	882	859	908	
Diversity	1,44	2,14	2,18	2,18	1,81	2,23	2,22	2,20	2,17	2,09	
Zooplankton											
Codonillidae											
Tintinnopsis	219	316	467	361	363	412	504	629	523	528	432.2±120.99
Arthropoda		263	329	442	388	327	628	721	572	422	409.2±204.20
Protozoa	241	421	329	289	207	284	286	275	319	318	296.9±57.15
Mollusca	352	327	287	391	319	321	319	428	329	409	348.2±45.85
Crustacea	252	316	337	209	281	255	777	489	417	379	371.2±165.69
Rotifera											
Lapadella	174	215	255	186	228	347	429	519	438	379	317±121.34
Brachionus	306	218	203	408	391	436	389	328	372	351	340.2±78.13
Cephalodella	231	242	429	291	207	230	281	361	369	328	296.9±72.97
Colurella	250	362	274	422	501	427	389	421	408	367	382.1±74.35
Total	2025	2680	2910	2999	2885	3039	4002	4171	3747	3481	
abundance (cell/liter)	253	298	323	333	321	338	445	463	416	387	
Diversity	2,06	1,95	1,92	1,88	1,88	1,94	1,85	1,85	1,89	1,93	

The species domination of zooplankton and phytoplankton is highly correlated with eutrophication (Xiong et al. 2003). Climate change also has an impact on potential zooplankton. During spring and summer, zooplankton was irregularly distributed through the water profile, where the highest average density was recorded at 10–5 m depth (66,007 and 66,734 Ind. m^{-3} (Khalifa et al. 2015). The seasonal potential of zooplankton dynamics in the southern Chukchi Sea is less influenced by the local growth of zooplankton and spring phytoplankton bloom and more influenced by the advection of zooplankton abundance (Kitamura et al. 2017).

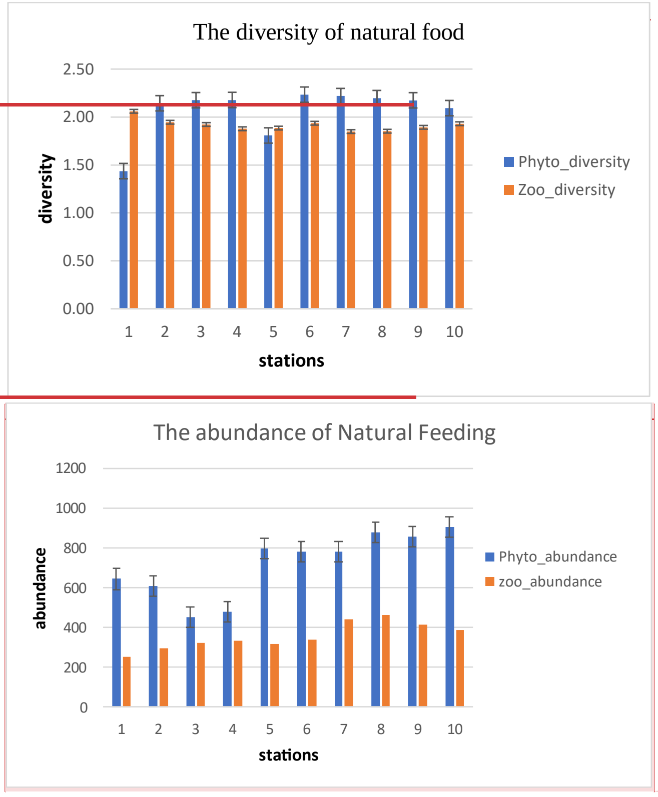

Figure 2. The potential (diversity and abundance) of ~~Natural feeding~~natural food (phytoplankton and zooplankton)

The other condition, the species composition of zooplankton, determined using hierarchical cluster analysis and indicator species analysis, showed the influencing of season condition. The winter community, characterized by warm water indicator species including Mesocalanus tenuicornis, Calanus pacificus, and Corycaeus anglicus, diverged into four communities throughout the spring and summer (McKinstry and Campbell 2018). The other location also shows the total potential 48 taxa phytoplankton from 26 genera and three classes, namely Bacillariophyceae (37 taxa), Cyanophyceae (10 taxa), and Schizomycetes (1 taxon) (Onyema 2007) which is influenced by variations in the physical environments (Ormańczyk et al. 2017). A previous study in Tanggerang coastal water also reported that Crustacean dominated 12 groups of zooplankton. The Morisita Index shows that the zooplankton in Tangerang coastal waters have been grouped in a patchy pattern distribution (Pratiwi et al. 2016). The distribution of zooplankton and phytoplankton is influenced by phosphate, nitrate, nitrite, dissolved oxygen, salinity, and depth water. (Putri et al. 2019)

The impact ~~of the abundance of zooplankton and phytoplankton as natural feeding~~of natural food diversity and abundance ~~toward~~on saline -Nile Tilapia growth in the brackish water Polyculture system

This study showed the effect of natural ~~feeding~~ food diversity and abundance ~~support~~ on Nile Tilapia ~~Growth~~ poductivity in the ~~Polycalutrure~~polyculture system **(Table 2**). The potential natural

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feedingfood to support saline Nile Tilapia productivity was indicated by , growth, and gut content of the Nile of Tilapia showed the abundance and diversitythe diversity and abundance of zooplankton and phytoplankton, as proved byin the saline Nile Tilapia gut content and fish growth. The potential natural feedingfood of phytoplankton 721 ± 163,2 ind/l (abundance) and 2,07 ± 0,25 (diversity) and zooplankton with value 358 ± 67,6 ind/l (abundance) and 1,91± 0,06 (diversity). The impact of natural feeding to support Nile Tilapia growth productivity was shown by low mortality (17,5 ±8,59 %), high weight growth rate (the fish mortality), (208.2 ± 22,5 gr) (weight rate), and hight specific growth rate (2,28 ± 0.77 %/day) (specific growth). The indicator potential of natural feeding food to support saline Nile Tilapia productivity was also showed by the present of natural food in Nile Tilapia gut. Natural food abundance in Saline Nile Tilapian gut wascan be shown by gut content, that is, 43-168 individuals/ml (phytoplankton) and 23-65 individuals/ml (zooplankton).

Table 2. The potential natural feedingfood, growth, and gut content of Nile of Tilapia

Station	Phytoplankton		Zooplankton		Nile Tilapia						
					Fish Growth			Potential of Gute content		Percent of Gute content	
	Abundance (ind/l)	Diversity	Abundance (ind/l)	Diversity	Mortality (%)	Weight absolute (gr)	Specific growth	Phytoplankton in gute content	Zooplankton in gut content	Percent of n gut content of phytoplankton	Percent of n gut content of zooplankton
							(%/day)	Ind/ml	Indv/ml	(%)	(%)
1.	645	1,44	253	2,06	28,67	194,03	1,48	46	34	7,1 (46/645)	13,4
2.	612	2,14	298	1,95	25,33	187,04	1,37	44	23	7,2	7,7
3.	455	2,18	323	1,92	27,33	171,3	1,3	43	31	9,5	9,6
4.	483	2,18	333	1,88	25,33	187,04	1,51	63	22	13,0	6,6
5.	801	1,81	321	1,88	10,67	223,1	2,71	64	35	8,0	10,9
6.	781	2,23	338	1,94	16,67	201,3	2,51	52	33	6,7	9,8
7.	785	2,22	445	1,85	15,33	229,4	2,94	33	36	4,2	8,1
8.	882	2,2	463	1,85	10,67	230,2	2,95	126	56	14,3	12,1
9.	859	2,17	416	1,89	7,33	224,6	2,82	99	65	11,5	15,6
10.	908	2,09	387	1,93	6,67	233,53	3,23	168	24	18,5	6,2
average	721	2,07	358	1,91	17,4	208,15	2,28			100,0	100,0
Stdev	163,1	0,25	67,59	0,06	8,59	22,54	0,77				

Fish growth and gut content can showproves the impact of natural feedingfood. Base on the data in **Table 2**, shows there was the positive correlation between natural food with and Nile Tilapia growth and gut content because the potential natural feeding (phytoplankton and zooplankton) has a positive impact on supporting Nile Tilapia growth. The gut content consisted of two parameters; potential and percentage of gut content. Both natural supply feeding from zooplankton and phytoplankton showed potential gut content in Nile tilapia 33-168 individuals/ml (phytoplankton) and 23 – 65 individuals/ml (zooplankton). The indicator of gut content also showed that the percent of gut content in Nile Tilapia was 4.2-18.5 % (phytoplankton) and 7.7 – 15.6 % (zooplankton).

Soedibya (2013), Soedibya et al. (2021), and Soedibya et al. (2017) stated that the increase in natural feeding has a positive impact on specific growth, survival rate, and gut content. The gut content indicates positive growth and survival rate of Nila Tilapia. The increasing gut content demonstrates the high ability of Nila tilapia to utilize natural feeding to support Nile tilapia growth in the fishpond. The Nile Tilapia, an adaptive species from a tropical region, can grow in salinities less than 18-20 pp (Soedibya 2013; Basuki and Rejeki 2015; Ekasari et al. 2015; Kusuma et al. 2019). The specific growth, weight absolute, and mortality of Nile tilapia are influenced by natural feeding, saline media, and diseases (Soedibya 2013; Basuki and Rejeki 2015)

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The Relation Between Natural ~~Feeding~~ Food with Nile Tilapia Growth in Polyculture system

The Relation Between Phytoplankton with Nile Tilapia Growth

Commented [LL11]: Discuss the results

The impact of ~~natural feeding (phytoplankton)~~phytoplankton on Nile tilapia growth is shown in **Figure 3**. The data showed that the relation between phytoplankton abundance with specific growth rate Y = 0.0044x – 0,8869 (linear) and Y = 0.000008X^2 – 0.0058 x +2.39 (best equation), with weight absolute, was Y = 0.1292 x + 115,02 (linear) and y = 0.0001X^2 – 0,010 x + 159,72 (best equation) and Nila Mortality Y = 51.88 -0,0478 x (linear) and Y = -0.0001X^2 + 0.1249 x -3,55 (best equation)

Commented [LL12]: Please used R value to show positive or negative correlation between phytoplankton abundance and growth or mortality

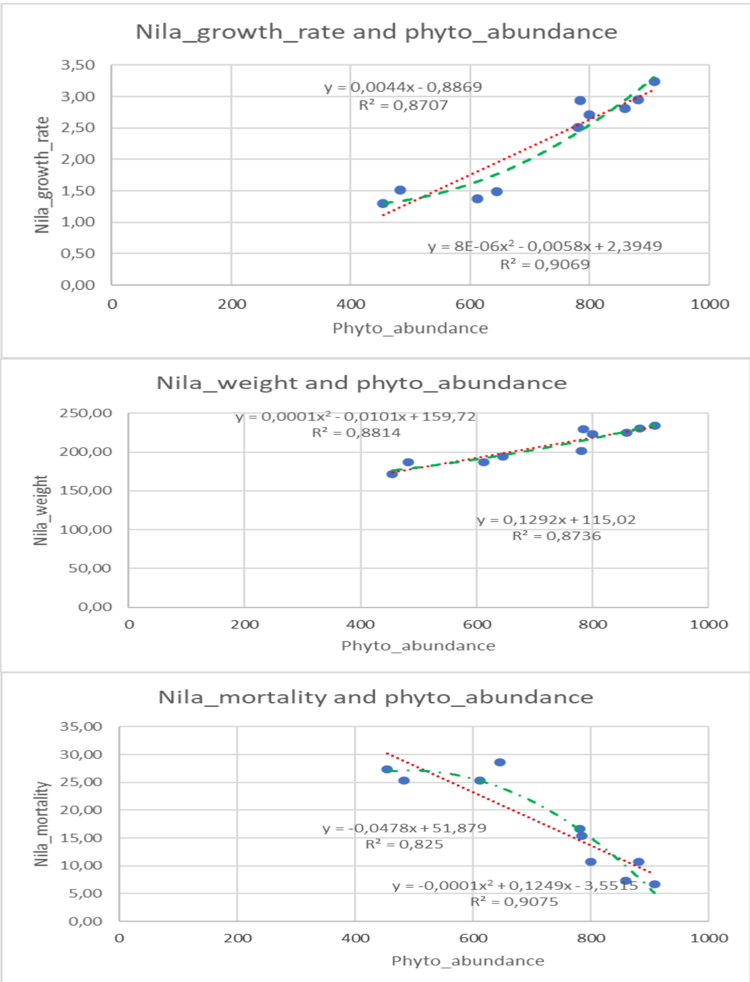

Figure 3. The relation between the abundance of phytoplankton with Nile Tilapia growth (mortality, growth rate, and weight

The Relation Between Zooplankton with Nile Tilapia Growth

The impact of natural feeding (zooplankton) on Nilapia growth is illustrated in **Figure 4**. The data showed that the relation between zooplankton abundance with specific growth rate Y = 0,0087x – 0,8167 (linear) and Y = 0.00000001X^4 – 0.010 x^2 - 2.433 x + 215,24 (best equation), with weight absolute Y = 0.2429 x + 121,26 (linear) and y = 0.0000001 X^4 + 0,00002X^3 +0,133 x -245,73 (best equation) and Nila Mortality Y = 49,75 -0,0904 x (linear) and Y = -0.000000006 X^3 + 0,0087X^2 +2,73 x^2 -34,23x +3385,8 (best equation)

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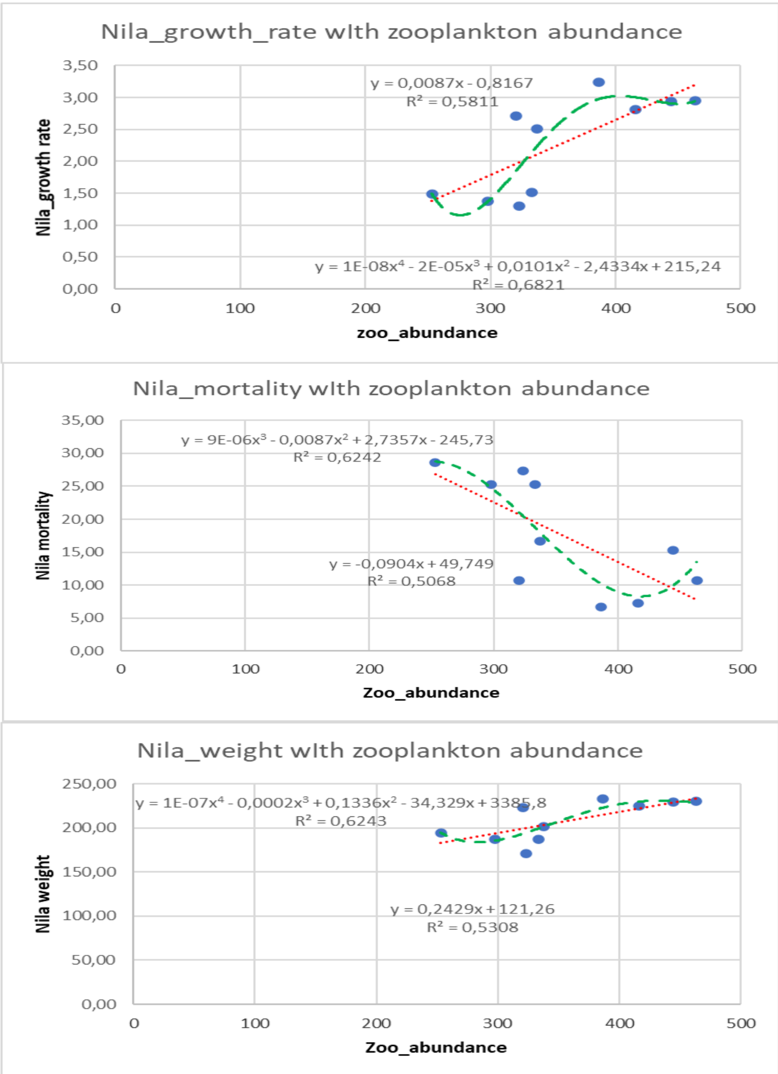

Figure 4. The relation between the abundance of zooplankton with Nile Tilapia growth (mortality, growth rate, and weight

The data in **Figure 4** showed that the abundance of phytoplankton had a positive correlation with specific growth and weight growth absolute and had a negative correlation with the mortality of Nile Tilapia. Similar to the impact of phytoplankton to support Nila growth, zooplankton also had a positive correlation with specific growth and weight growth absolute and had a negative correlation with the mortality of Nile Tilapia (Figure 5). The relation between zooplankton and phytoplankton with Nile tilapia growth shows that Nile tilapia is Herbivore / Omnivore, a Low trophic level feeder). It can be grown by adding Algae, bacteria, and detritus (bio flocs). Moreover, Nile tilapia has a fast growth rate, adaptable to different conditions, including high stocking densities, is highly disease resistant, and is tolerant of poor water quality (Prabu et al. 2019). Besides the natural feeding, Nila tilapia also was influenced by water salinity, water fertility, pH, DO, and pollution condition (McKinstry and Campbell 2018; Hilmi et al. 2021b, 2022b)

Conclusion

Phytoplankton and zooplankton are the potential natural food supporting Nile tilapia growth in polyculture in the Tritih Kulon Cilacap, as indicated by a positive impact on Nile Tilapia growth. A total number of 21 plankton species consisted of 12 phytoplankton species and nine zooplankton groups. Increasing weight and rate of growth of Nile tilapia and Milkfish in Tritih Kulon Aquaculture proves the potential of plankton. Nile Tilapia growth was 17,5 ±8,59 % (the fish mortality), 208.2 ± 22,5 gr (weight rate), and 2,28 ± 0.77 %/day (specific growth). The indicator of percent of gut content also showed that the percent of gut content in Nile Tilapia was 4,2-18,5 % (phytoplankton) and 7,7 – 15,6 % (zooplankton). Natural feeding (phytoplankton and zooplankton) positively supports the growth of Nila Tilapia, both specific growth, absolute weight, and mortality.

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The natural food to support the growth rate of Saline Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) for the polyculture system in the mangrove ecosystem, Segara Anakan

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Abstract

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The polyculture of saline Nile Tilapia and Milkfish requires natural feeding to support brackish water fish production. Currently, no study has been done about the potential of natural food to support the productivity of the saline Nile Tilapia and Milkfish brackish water polyculture ~~system~~ in Tritih Kulon, Cilacap, Central Java. The potential natural food can be assessed through the abundance and diversity of plankton (phyto and zooplankton) analysis in the water body. This research analyzed the correlation between plankton diversity and abundance as natural feeding with saline Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) productivity in the brackish water polyculture ~~system~~ in the Tritih Kulon Village, Cilacap Regency, Central Java. The results showed that 21 plankton was identified from the research location, comprising 12 phytoplankton species and nine zooplankton groups. The plankton supports Nile tilapia and Milkfish productivity in the polyculture system, as indicated by low mortality (R^2= 0.825-0.908), high weight gain (R2=0.881-0.874) and high specific growth (0.87-0.91). In addition, the gut content in saline Nile Tilapia was 4.2-18.5 % (phytoplankton) and 7.7 – 15.6% (zooplankton). It could be concluded that natural food availability significantly supports saline Nile Tilapia productivity in brackish water culture.

Keywords: abundance, brackish water pond, fishery, monoculture, phytoplankton

Introduction

Aquaculture technology is divided into two different systems, i.e., monoculture and polyculture systems (Carbone and Accordi 2000; Cochard 2017; Hu et al. 2020; Zhu et al. 2022; Jansen et al. 2023). Aquaculture can be conducted in the mangrove ecosystem because mangrove has a good supply of natural feeding (Hilmi et al. 2021c, 2022a; Murniasih et al. 2022) to support brackish water aquaculture activity. A polyculture system was developed to increase fish productivity per unit area and to maintain water quality (Ekasari et al. 2015; Rose et al. 2015). Brackish water ~~P~~polyculture is ~~designed to support the~~ aquaculture system between two or more fish species or ~~organism~~ aquatic organism, for example, polyculture between milkfish and prawn or shrimp~~.~~ (Soedibya 2013; Rachmawati and Samidjan 2014; ~~Nuryanto et al. 2017~~; Soedibya et al. 2017). Other studies also reported polyculture between Milkfish and Nile Tilapia (Soedibya 2013; Mutia et al. 2018; Muyot et al. 2018). Some other studies also reported brackish water polyculture between prawn or shrimp and Nile Tilapia (Yang and Fitzsimmons 2004; Mutia et al. 2018; Prabu et al. 2019).

Natural food is essential to support brackish water fish polyculture. Fish natural food consists of plankton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacteria associated with detritus (Sukardjo 2004; Rougier et al. 2005; Nagelkerken et al. 2008; Mendoza and Henkel 2017; Andriyani et al. 2018). Generally, plankton is divided into phytoplankton and zooplankton (Henmi et al. 2017; Mendoza and Henkel 2017; Andriyani et al. 2020; Alam et al. 2021).~~ Besides natural food, phytoplankton is the primary producer in the aquatic ecosystem (Ishii and Kamikawa 2017; Karl and Church 2017; Alam et al. 2021)~~. Previous studies proved plankton availability and abundance are essential for fish growth and fisheries productivity (Henmi et al. 2017; Andriyani et al. 2020; Alam et al. 2021).

Plankton diversity and abundance have a high potential to support Nile Tilapia and other species in polyculture ~~production~~productivity, which has been reported from several regions (Yang and Fitzsimmons 2004; Nagelkerken et al. 2008; Soedibya 2013; Mutia et al. 2018; Kusuma et al. 2019). Some researchers reported plankton diversity and abundance in the polyculture pond of Nile Tilapia, *Oreochromis niloticus (*Sihombing et al. 2017; Muyot et al. 2018). Some other studies also found similar data in Milkfish *Chanos chanos* (Soedibya 2013; Ekasari et al. 2015; Kusuma et al. 2019). Currently, no report about plankton diversity and abundance in the polyculture of saline Nile Tilapia ~~milkfish~~ and ~~Nile Tilapia~~ milkfish in the brackish water pond in Tritih Kulon Village, District of North Cilacap, Cilacap Regency. These data are vital for developing the brackish water polyculture of both species in the Tritih Kulon Village.

Saline Nile Tilapia, *Oreochromis niloticus (Linnaeus, 1758)* (Yang and Fitzsimmons 2004; Soedibya 2013; Ekasari et al. 2015; Kusuma et al. 2019) is a tropical fish that lives in shallow waters. This species lives in an aquatic ecosystem with various salinities (Suresh and Lin 1992; Basuki and Rejeki 2015; Kusuma et al. 2019; Prabu et al. 2019). This species can adapt and live in brackish water with a salinity of up to 25°/₀₀ (Japse and Caipang 2011). Nile tilapia consume a broad spectrum of feed and are classified as omnivorous fish. These species consume phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacteria associated with detritus (Mendoza and Henkel 2017; Neal et al. 2018; Takenaka et al. 2018; Teoh et al. 2018; Setyawati et al. 2019; Rahman et al. 2021). The ecological ~~characteristics~~ advantages of Saline Nile Tilapia have been ~~developed among~~utilized to develop brackish water aquaculture and had been polycultured with many fisheries commodities (Yang and Fitzsimmons 2004; Soedibya 2013; Ekasari et al. 2015; Muyot et al. 2018). Polyculture between Saline Nile Tilapia and Milkfish, Chanos chanos (Forsskal, 1775) ~~polycultures with the Nile~~ have long been ~~cultivated~~ carried out intensively in ponds. The feeding habits of milkfish, including herbivores, are the advantages of this species. This species is used as a superior product for cultured animals kept in polyculture with omnivores or carnivores. This condition causes the production of milkfish to be stable. Besides that, the specific taste causes milkfish to be in great demand. This species has been cultivated in a polyculture system with Tiger Prawn, milkfish, shrimp, and others (Soedibya 2013; Muyot et al. 2018)

The Tritih Kulon Village resides in North Cilacap District, Cilacap Regency Central Java, Indonesia. The village has an area of 503.43 hectares consisting of land and swampy brackish water areas, including the mangrove and lagoon ecosystems (Hilmi et al. 2019, 2021c, d, e). The previous study utilized most saline water areas for shrimp aquacultures. At that time, shrimp farming has become a profitable business for the Tritih Kulon villagers due to high prices. Currently, shrimp production tends to decline and lead to business failure. This condition has persisted in South Java for the last four years, from Bantul to Cilacap Regencies. The defeat led to the shrimp pond in the Tritih Kulon Village becoming abandoned and unproductive. This study tried to provide a solution by providing information about the opportunities for using abandoned shrimp farming ~~land~~ brackish water pond in the Tritih Kulon Village as a polyculture area between saline Nile ~~t~~Tilapia and milkfish. A preliminary need is to study plankton diversity in a polyculture pilot pond of Saline Nile Tilapia, *O.* ~~*italics*~~*niloticus*, and Milkfish, *C. chanos*. This study aimed to analyze

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Materials and Methods

Study area and time

The brackish water polyculture pilot pond ~~was~~ is located in the Tritih Kulon Village North Cilacap District, Cilacap Regency, Central Java, Indonesia (**Figure 1**) (Hilmi et al. 2021c, a). The pilot pond was a traditional earthen pond with an area of 2000 m^{-2} width. The research was conducted from August to October 2018.

Figure 1. Location of Tritih Kulon Village, North Cilacap District, Cilacap Regency Central Java, indicating the polyculture pond of *Oreochromis niloticus (Linnaeus, 1758)* and *Chanos chanos*

Procedures and data analysis

Pilot pond preparation for polyculture

Inflow and outflow of water occur naturally following tidal currents (Yang and Fitzsimmons 2004; Soedibya 2013; Muyot et al. 2018; Prabu et al. 2019). This study used a pilot earthen brackish water pond with an area of 2000 square meters (m^2). Pond preparation was initially by reversing the bottom of the pond for soil oxidation and killing fish disease-causing organisms. Neutralize soil acidity by adding ten sacks of lime to the pond surfaces and~~,~~ leaving the pool for two weeks without water. Afterward, the pond was fertilized using chicken manure and filled with water as deep as approximately 10 cm to allow plankton to grow. Then, the water depth was increased to about 1.5 m. We kept the depth at 1.5 m by adjusting the inlet and outlet holes as high as 1.5 meters.

Preparation, stocking, and cultivation of fish seeds

The seeds of *Oreochromis niloticus (Linnaeus, 1758)* ~~-~~and *Chanos chanos* were obtained from a governmental agency, namely the Center for Brackish Water Aquaculture Research (BBPBAP) Jepara, Central Java, Indonesia. The average length of Saline Nile Tilapia seeds was 3.1 ± 0.241 cm, with average body weight was 5.36 ± 0.21 g ~~(Yang and Fitzsimmons 2004)~~.

Both fish seed species were acclimatized for 2 hours before stocking in a pilot earthen pond. The stocking density for Saline Nile Tilapia was five individuals per m^2, with the total stocked fish being 10,000 seeds. In contrast, the stocking density of milkfish was set up for three seeds per square meter, meaning the total number of milkfish seeds was 6,000 individuals. During the first two weeks of rearing, the fish were fed with fine pellets and continued with ~~the number of~~the pellets size of 1000 after reaching a body length of 5 cm. After six weeks, we provided the fish with pellet number 2000 (Soedibya 2013).

~~Phytoplankton~~ Plankton collection and identification

~~Phytoplankton~~ Plankton samples were collected weekly for ten weeks of polyculture periods. Water samples were taken using a 10 liters bucket and filtered using plankton net number 25. We filtered the water samples ten times to have a total volume of filtered water of approximately 100 liters. The plankton was collected and remained in the collection bottle. Afterward, we removed the collection bottle from the plankton net. The filtered plankton was transferred into sample bottles and preserved using formalin until the final concentration became 4% and two drops of pure Lugol (Zafar 2004; Tsuji and Montani 2017; Andriyani et al. 2020).

~~Phytoplankton~~ Plankton identification was conducted under a binocular microscope with a magnification of 10 x 40. The ~~phytoplankton~~ plankton identification processes were as follows. One drop of sample water is placed on an object glass and covered. We carried out microscopic observations in 20 fields of view with up-down and left-right macrometer directions. ~~Phytoplankton~~ Plankton identification according to the figure in identification keys (Henmi et al. 2017; Sihombing et al. 2017). ~~Phytoplankton~~ Plankton abundance was calculated using the following formula (Arumugam et al. 2016; Andriyani et al. 2020; Asmarany et al. 2022).

Abundance (F) = [(A/B) x (C/D) x (E/F)] cell/litre

Note:
- A= Water volume in a bottle sample (30 mL)
- B= Water volume observed (0.06 mL)
- C= Cover glass width (484 mm^2)
- D= view field number (20)
- E= number of ~~phytoplankton~~ pankton individual
- F= total volume of filtered water (100 L)

Nile Tilapia growth

Saline Nile Tilapia growth was measured as the absolute growth of body weight (W_G), measured weekly for ten weeks. Body weight was measured using a balance with an accuracy of 0.01 g. Absolute body weight growth was calculated as follows (Palada-de Vera and Eknath 1993; Soedibya 2013; Muyot et al. 2018).

W_G= Wt – Wi

Note:
- W_G= absolute weight growth
- Wt= body weight after t-time
- Wi= initial body weight

Specific growth rate

The specific growth rate (μ) was calculated from the initial and final densities during the cultivation period, according to the method of Njouondo.

$$\mu = \frac{\ln(x2) - \ln(X1)}{t2 - t1}$$

Where X2 and X1 are the final and initial densities, respectively, t2 is the final cultivation time, and t1 is the initial cultivation time. The specific growth rate was noted as cells/day. This study calculated phytoplankton doubling time-based on the specific growth rate according to the equation

$$g = \frac{\ln 2}{\mu}$$

The relation between ~~phyto~~plankton to Nile Tilapia growth

This study estimated the potential of plankton to support saline Nile Tilapia growth based on the weekly plankton abundance and weekly saline Nile Tilapia growth. This study analyzed data about ~~phyto~~plankton diversity and abundance descriptively. Then we compared the result to the information available in previous publications. The potential of plankton to support Nile Tilapia growth was analyzed using Pearson correlation and regression (Karl and Church 2017; Hilmi et al. 2020)

Result and Discussion

Natural food ~~in polyculture system using the indicator of plankton~~ diversity and abundance

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Natural food, especially plankton, in the polyculture system is required to support fish productivity, including in the saline Nile Tilapia cultivation. The ~~observation~~ diversity and abundance of natural ~~f~~food~~eeding~~ in the polyculture of saline Nile Tilapia and milkfish is summarized in Figure 1~~ showed plankton in aquatic ecosystems~~. ~~Based on the microscopic identification of phytoplankton samples from the polyculture pond of Nile Tilapia and Milkfish can be shown in **Figure 2**.~~ The total number of natural foods was 21 plankton species, consisting of 12 phytoplankton species and nine zooplankton groups (**Table 1**~~ **and Figure 2**~~). ~~Whereas **Table 1** also describes the phytoplankton diversity and absolute abundance.~~

Table 1 showed that phytoplankton consisted of Cyanophyceae, Dinophyceae, and Bacillariophyceae with abundance between 455 cells/liter – 908 cells/liter and diversity between 1.44 – 2.23. Zooplankton consisted of Codonillidae, Arthropoda, Protozoa, Mollusca, and Crustacea with abundance between 253 cells/liter – 463 cells/liter and diversity between 1.88 – 2.6. The data indicated low plankton abundance were observed during study. The potential phytoplankton species were *Oscillatoria, Peridinium, Prorocentrum, Navicula, Nitzia, Rhizosolenia, Diatoma, Striatella, Pleurosigma, Chaetoceros, Coscinodiscus,* and *Fragillaria.* The total zooplankton abundance in Lampung and South Sulawesi ranges between 32,745 individuals.m^{-3} and 652,925 individuals.m-3, dominated by *Calanoida* and *Cyclopoida nauplii (Duggan et al. 2011).* This study found that *Tintinnopsis*, *Lapadella*, *Brachionus*, *Cephalodella*, and *Colurella* dominated the zooplankton. Some researchers also report that Indonesia has five new records of plankton species that are *Oithona decipiens, Oithona hebes, Oncaea atlantica group, Oncaea zernovi group, and Spinoncaea* spp (references?).

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Different with Tigris River Iraq, which has 106 taxonomy units of zooplankton, including 65 taxa belonging to rotifers, 25 taxa to copepod, and 16 taxa to Cladocera with The Shannon–Weiner index of Rotifera varied from 0.67 to 3, 0.50–1.72 for Cladocera and from 0.91 to 2.51 for Copepoda. (Abdulwahab and Rabee 2015). The other condition shows that Delta Mahakam has 48 taxa phytoplankton belonging to Bacillariophyceae (35), Dinophyceae (6), Chlorophyceae (4), and Cyanophyceae (3) (Effendi et al. 2016). Lake Burdur Turkey has six zooplankton taxa: Hexarthra fennica, Brachionus plicatilis from Rotifera, and Arctodiaptomus burduricus from Copepoda. The average zooplankton density in Lake Burdur shows that 399,074 ind.m^{-3} is distributed by 51% H. fennica, 9% B. plicatilis, and 40% A. burduricus..(Gülle et al. 2010). Pearl River estuary, China, has 132 species of zooplankton (Honggang et al. 2012). In River Yeúilırmak, Amasya, Turkey has plenty of divisions, which are *Navicula cincta, N. cryptocephala,* and *N. rhyncocephala* (Soylu and

Gönülol 2003). (Fang et al. 2012) also reports that the Xiaoqing River estuary has seven plankton species: *Skeletonema costatum, Tribonema affine,* and *Chlorella* sp.

The data showed that the abundance of Bacillariophyceae > Dinophyceae > Cyanophyceae with *Nitzia, Diatoma,* and *Chaetoceros* had high potency in the polyculture system. Whereas Rotifera, Chodonillidae, and Arthropoda had high potency as natural feeding. The distribution of natural feed of both phytoplankton and zooplankton (diversity and abundance) can be shown in **Figure 2.** Based on **Figure 2** showed that the diversity and abundance of phytoplankton > zooplankton. The variety of phytoplankton on stations 6, 7, 8, and 10 was greater than on other stations, whereas the diversity of zooplankton station 1 was the highest.

Table 1. The diversity and abundance of plankton in the polyculture system

Classis/Species	abundance (cell/liter)										confidence interval
	1	2	3	4	5	6	7	8	9	10	
Phytoplankton											
Cyanophyceae											
Oscillatoria	822	121	251	311	184	286	384	611	167	282	341.9±217.29
Dinophyceae											
Peridinium	1.302	212	114	221	377	211	306	288	601	719	435.1±357.50
Prorocentrum		412	218	174	89	281	241	418	291	339	246.3±133.42
Bacillariophyceae											
Navicula	481	504	417	385	1.298	738	781	552	828	829	681.3±275.43
Nitzia	781	1.027	818	1.219	3.036	1.833	2.077	1.865	2.199	3.071	1,792.6±838.09
Rhizosolenia	241	438	221	371	218	638	721	811	719	693	507.1±234.34
Diatoma	1.799	2.385	1.755	1.544	311	1.539	2.190	3.082	2.710	2.804	2,011.9±808.14
Striatella	422	390	271	315	278	529	481	365	391	380	382.2±82.38
Pleurosigma	251	622	315	128	276	383	485	391	588	429	386.8±152.79
Chaetoceros	204	281	316	285	3.138	1.892	926	819	692	539	909.2±928.53
Coscinodiscus	151	189	326	618	261	522	419	791	839	549	466.5±239.83
Fragillaria		765	433	221	141	523	409	591	285	263	363.1±226.94
total	6454	7346	5455	5792	9607	9375	9420	10584	10310	10897	
abundance (cell/liter)	645	612	455	483	801	781	785	882	859	908	
Diversity	1,44	2,14	2,18	2,18	1,81	2,23	2,22	2,20	2,17	2,09	
Zooplankton											
Codonillidae											
Tintinnopsis	219	316	467	361	363	412	504	629	523	528	432.2±120.99
Arthropoda		263	329	442	388	327	628	721	572	422	409.2±204.20
Protozoa	241	421	329	289	207	284	286	275	319	318	296.9±57.15
Mollusca	352	327	287	391	319	321	319	428	329	409	348.2±45.85
Crustacea	252	316	337	209	281	255	777	489	417	379	371.2±165.69
Rotifera											
Lapadella	174	215	255	186	228	347	429	519	438	379	317±121.34
Brachionus	306	218	203	408	391	436	389	328	372	351	340.2±78.13
Cephalodella	231	242	429	291	207	230	281	361	369	328	296.9±72.97

Colurella	250	362	274	422	501	427	389	421	408	367	382.1±74.35
Total	2025	2680	2910	2999	2885	3039	4002	4171	3747	3481	
abundance (cell/liter)	253	298	323	333	321	338	445	463	416	387	
Diversity	2,06	1,95	1,92	1,88	1,88	1,94	1,85	1,85	1,89	1,93	

The species domination of zooplankton and phytoplankton is highly correlated with eutrophication (Xiong et al. 2003). Climate change also has an impact on potential zooplankton. During spring and summer, zooplankton was irregularly distributed through the water profile, where the highest average density was recorded at 10–5 m depth (66,007 and 66,734 Ind. m^{-3} (Khalifa et al. 2015). The seasonal potential of zooplankton dynamics in the southern Chukchi Sea is less influenced by the local growth of zooplankton and spring phytoplankton bloom and more influenced by the advection of zooplankton abundance (Kitamura et al. 2017).

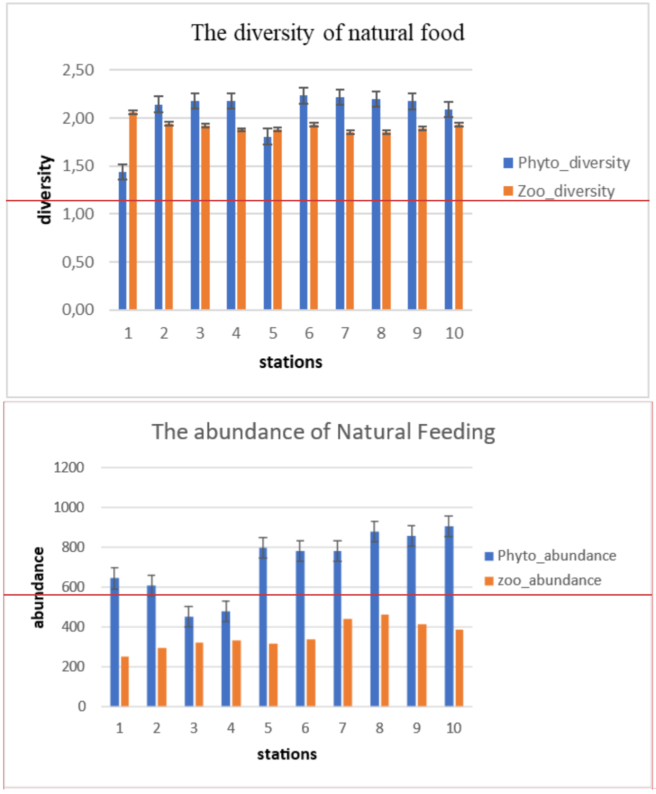

Figure 2. The potential (diversity and abundance) of ~~Natural feeding~~natural food (phytoplankton and zooplankton)

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The other condition, the species composition of zooplankton, determined using hierarchical cluster analysis and indicator species analysis, showed the influencing of season condition. The winter community, characterized by warm water indicator species including Mesocalanus tenuicornis, Calanus pacificus, and Corycaeus anglicus, diverged into four communities throughout the spring and summer (McKinstry and Campbell 2018). The other location also shows the total potential 48 taxa phytoplankton from 26 genera and three classes, namely Bacillariophyceae (37 taxa), Cyanophyceae (10 taxa), and Schizomycetes (1 taxon) (Onyema 2007) which is influenced by variations in the physical environments (Ormańczyk et al. 2017). A previous study in Tanggerang coastal water also reported that Crustacean dominated 12 groups of zooplankton. The Morisita Index shows that the zooplankton in Tangerang coastal waters have been grouped in a patchy pattern distribution (Pratiwi et al. 2016). The distribution of zooplankton and phytoplankton is influenced by phosphate, nitrate, nitrite, dissolved oxygen, salinity, and depth water. (Putri et al. 2019)

The impact ~~of the abundance of zooplankton and phytoplankton as natural feeding~~of natural food diversity and abundance ~~toward~~on saline ~~-~~Nile Tilapia growth in the brackish water Polyculture system

This study showed the effect of natural ~~feeding~~ food diversity and abundance ~~support~~ on Nile Tilapia ~~Growth~~ poductivity in the ~~Polycalutrure~~polyculture system **(Table 2**). The potential natural ~~feeding~~food to support saline Nile Tilapia productivity was indicated by ~~, growth, and gut content of the Nile of Tilapia showed the abundance and diversity~~the diversity and abundance of zooplankton and phytoplankton~~, as proved by~~in the saline Nile Tilapia gut content~~and fish growth~~. The potential natural ~~feeding~~food of phytoplankton 721 ± 163,2 ind/l (abundance) and 2,07 ± 0,25 (diversity) and zooplankton with value 358 ± 67,6 ind/l (abundance) and 1,91± 0,06 (diversity). The impact of natural feeding to support Nile Tilapia ~~growth~~ productivity was shown by low mortality (17,5 ±8,59 %), high weight growth rate ~~(the fish mortality),~~ (208.2 ± 22,5 gr) (weight rate), and hight specific growth rate (2,28 ± 0.77 %/day) ~~(specific growth)~~. The ~~indicator~~ potential of natural ~~feeding~~ food to support saline Nile Tilapia productivity was also showed by the present of natural food in Nile Tilapia gut. Natural food abundance in Saline Nile Tilapian gut was~~can be shown by gut content, that is~~, 43-168 individuals/ml (phytoplankton) and 23-65 individuals/ml (zooplankton).

Table 2. The potential natural ~~feeding~~food, growth, and gut content of Nile of Tilapia

Station	Phytoplankton		Zooplankton		Nile Tilapia						
					Fish Growth			Potential of Gute content		Percent of Gute content	
	Abundance (ind/l)	Diversity	Abundance (ind/l)	Diversity	Mortality (%)	Weight absolute (gr)	Specific growth	Phytoplakton in gute ~~content~~	Zooplankton in gut ~~content~~	Percent of n gut content of phytoplankton	Percent of n gut content of zooplankton
							(%/day)	Ind/ml	Indv/ml	(%)	(%)
1.	645	1,44	253	2,06	28,67	194,03	1,48	46	34	7,1 (46/645)	13,4
2.	612	2,14	298	1,95	25,33	187,04	1,37	44	23	7,2	7,7
3.	455	2,18	323	1,92	27,33	171,3	1,3	43	31	9,5	9,6
4.	483	2,18	333	1,88	25,33	187,04	1,51	63	22	13,0	6,6
5.	801	1,81	321	1,88	10,67	223,1	2,71	64	35	8,0	10,9
6.	781	2,23	338	1,94	16,67	201,3	2,51	52	33	6,7	9,8
7.	785	2,22	445	1,85	15,33	229,4	2,94	33	36	4,2	8,1
8.	882	2,2	463	1,85	10,67	230,2	2,95	126	56	14,3	12,1
9.	859	2,17	416	1,89	7,33	224,6	2,82	99	65	11,5	15,6
10.	908	2,09	387	1,93	6,67	233,53	3,23	168	24	18,5	6,2
average	721	2,07	358	1,91	17,4	208,15	2,28			100,0	100,0
Stdev	163,1	0,25	67,59	0,06	8,59	22,54	0,77				

Fish growth and gut content ~~can show~~proves the impact of natural ~~feeding~~food. Base on the data in **Table 2**, ~~shows~~ there was the positive correlation between natural food ~~with~~ and Nile Tilapia growth and gut content because the potential natural feeding (phytoplankton and zooplankton) has a positive impact on supporting Nile Tilapia growth. The gut content consisted of two parameters; potential and percentage of gut content. Both natural supply feeding from zooplankton and phytoplankton showed potential gut content in Nile tilapia 33-168 individuals/ml (phytoplankton) and 23 – 65 individuals/ml (zooplankton). The indicator of gut content also showed that the percent of gut content in Nile Tilapia was 4.2-18.5 % (phytoplankton) and 7.7 – 15.6 % (zooplankton).

Soedibya (2013), Soedibya et al. (2021), and Soedibya et al. (2017) stated that the increase in natural feeding has a positive impact on specific growth, survival rate, and gut content. The gut content indicates positive growth and survival rate of Nila Tilapia. The increasing gut content demonstrates the high ability of Nila tilapia to utilize natural feeding to support Nile tilapia growth in the fishpond. The Nile Tilapia, an adaptive species from a tropical region, can grow in salinities less than 18-20 pp (Soedibya 2013; Basuki and Rejeki 2015; Ekasari et al. 2015; Kusuma et al. 2019). The specific growth, weight absolute, and mortality of Nile tilapia are influenced by natural feeding, saline media, and diseases (Soedibya 2013; Basuki and Rejeki 2015)

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The Relation Between Natural ~~Feeding~~ Food with Nile Tilapia Growth in Polyculture system

The Relation Between Phytoplankton with Nile Tilapia Growth

The impact of ~~natural feeding (phytoplankton)~~phytoplankton on Nile tilapia growth is shown in **Figure_3**. The data showed that the relation between phytoplankton abundance with specific growth rate Y = 0.0044x – 0,8869 (linear) and Y = 0.000008X^2 – 0.0058 x +2.39 (best equation), with weight absolute, was Y = 0.1292 x + 115,02 (linear) and y = 0.0001X^2 – 0,010 x + 159,72 (best equation) and Nila Mortality Y = 51.88 -0,0478 x (linear) and Y = -0.0001X^2 + 0.1249 x -3,55 (best equation)

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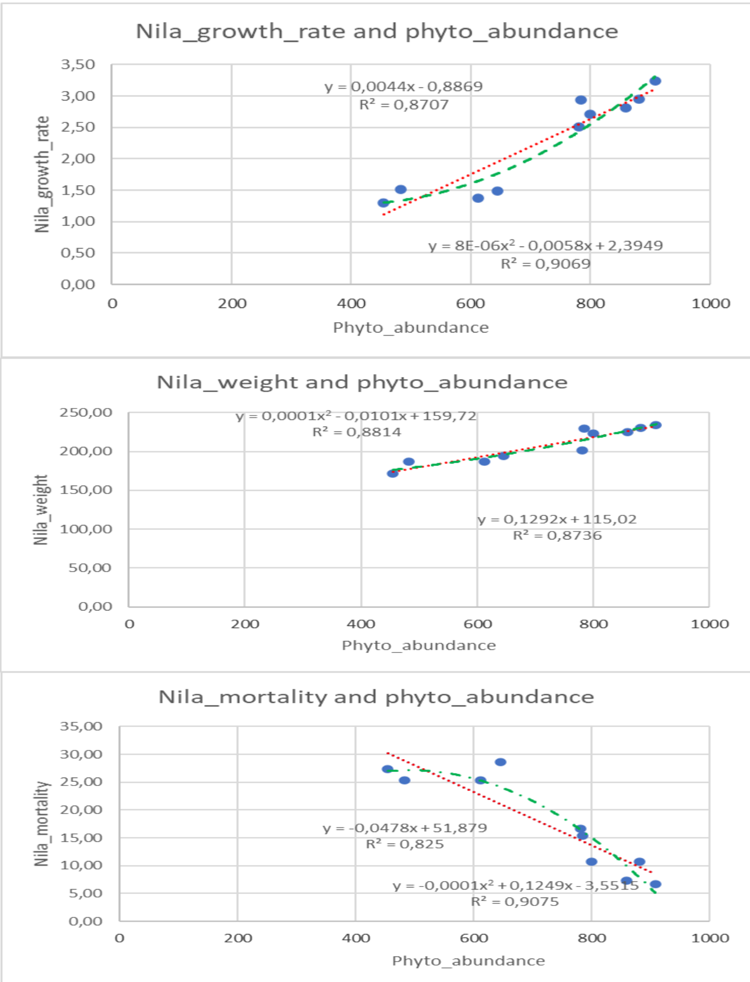

Figure 3. The relation between the abundance of phytoplankton with Nile Tilapia growth (mortality, growth rate, and weight

The Relation Between Zooplankton with Nile Tilapia Growth

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The impact of natural feeding (zooplankton) on Nilapia growth is illustrated in **Figure 4**. The data showed that the relation between zooplankton abundance with specific growth rate $Y = 0{,}0087x – 0{,}8167$ (linear) and $Y = 0.00000001X^4 – 0.010\ x^2 - 2.433\ x + 215{,}24$ (best equation), with weight absolute $Y = 0.2429\ x + 121{,}26$ (linear) and $y = 0.0000001\ X^4 + 0{,}00002X^3 +0{,}133\ x - 245{,}73$ (best equation) and Nila Mortality $Y = 49{,}75 -0{,}0904\ x$ (linear) and $Y = -0.000000006\ X^3 + 0{,}0087X^2 +2{,}73\ x^2 -34{,}23x +3385{,}8$ (best equation)

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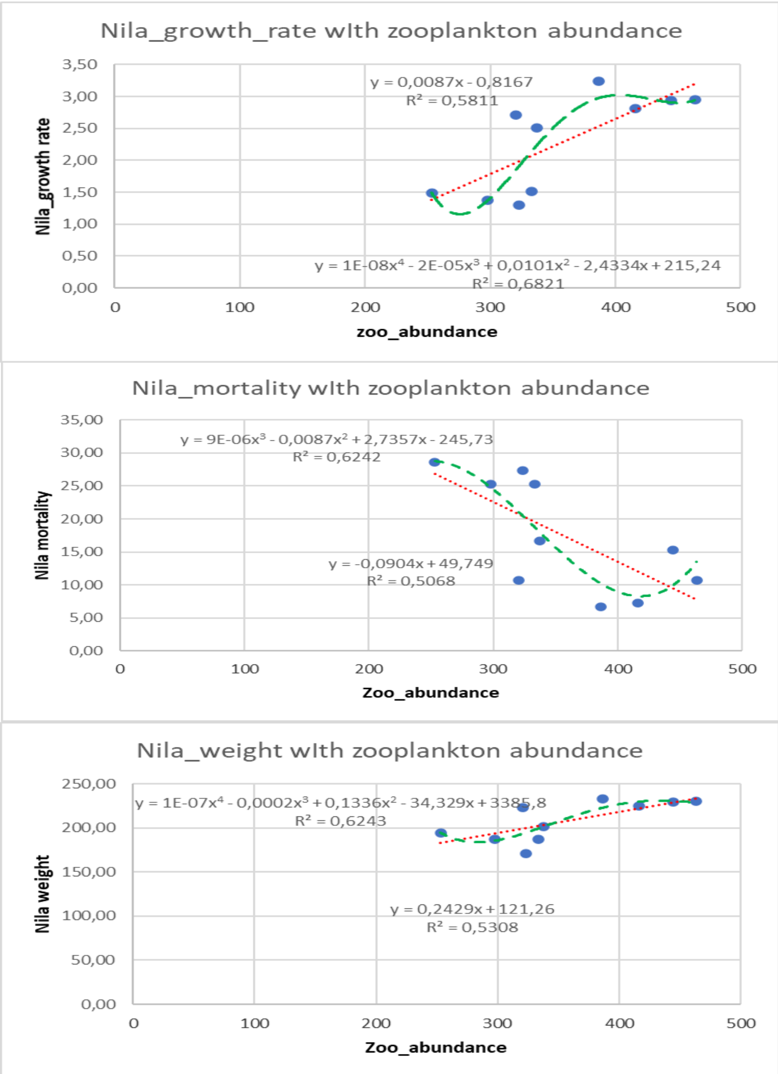

Figure 4. The relation between the abundance of zooplankton with Nile Tilapia growth (mortality, growth rate, and weight

The data in **Figure 4** showed that the abundance of phytoplankton had a positive correlation with specific growth and weight growth absolute and had a negative correlation with the mortality of

Nile Tilapia. Similar to the impact of phytoplankton to support Nila growth, zooplankton also had a positive correlation with specific growth and weight growth absolute and had a negative correlation with the mortality of Nile Tilapia (Figure 5). The relation between zooplankton and phytoplankton with Nile tilapia growth shows that Nile tilapia is Herbivore / Omnivore, a Low trophic level feeder). It can be grown by adding Algae, bacteria, and detritus (bio flocs). Moreover, Nile tilapia has a fast growth rate, adaptable to different conditions, including high stocking densities, is highly disease resistant, and is tolerant of poor water quality (Prabu et al. 2019). Besides the natural feeding, Nila tilapia also was influenced by water salinity, water fertility, pH, DO, and pollution condition (McKinstry and Campbell 2018; Hilmi et al. 2021b, 2022b)

Conclusion

Phytoplankton and zooplankton are the potential natural food supporting Nile tilapia growth in polyculture in the Tritih Kulon Cilacap, as indicated by a positive impact on Nile Tilapia growth. A total number of 21 plankton species consisted of 12 phytoplankton species and nine zooplankton groups. Increasing weight and rate of growth of Nile tilapia and Milkfish in Tritih Kulon Aquaculture proves the potential of plankton. Nile Tilapia growth was 17,5 ±8,59 % (the fish mortality), 208.2 ± 22,5 gr (weight rate), and 2,28 ± 0.77 %/day (specific growth). The indicator of percent of gut content also showed that the percent of gut content in Nile Tilapia was 4,2-18,5 % (phytoplankton) and 7,7 – 15,6 % (zooplankton). Natural feeding (phytoplankton and zooplankton) positively supports the growth of Nila Tilapia, both specific growth, absolute weight, and mortality.

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The natural food to support the growth rate of Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) for the polyculture system in the mangrove ecosystem, Segara Anakan

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Abstract

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The polyculture of Nile Tilapia and Milkfish requires natural feeding to support fish production. Currently, no study has been done about the potential of natural food to support the productivity of the Nile Tilapia and Milkfish polyculture system in Tritih Kulon, Cilacap, Central Java. The potential natural food can be assessed through the abundance and diversity of plankton (phyto and zooplankton) analysis in the water body. This research analyzed the correlation between plankton diversity and abundance as natural feeding with Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) productivity in the polyculture system in the Tritih Kulon Village, Cilacap Regency, Central Java. The results showed that 21 plankton was identified from the research location, comprising 12 phytoplankton species and nine zooplankton groups. The plankton supports Nile tilapia and Milkfish productivity in the polyculture system, as indicated by low mortality (R^2= 0.825-0.908), high weight gain (R2=0.881-0.874) and high specific growth (0.87-0.91). In addition, the gut content in Nile Tilapia was 4.2-18.5 % (phytoplankton) and 7.7 – 15.6% (zooplankton). It could be concluded that natural food availability significantly supports Nile Tilapia productivity.

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Keywords: abundance, brackish water pond, fishery, monoculture, phytoplankton

Introduction

Aquaculture technology is divided into two different systems, i.e., monoculture and polyculture systems (Carbone and Accordi 2000; Cochard 2017; Hu et al. 2020; Zhu et al. 2022; Jansen et al. 2023). Aquaculture can be conducted in the mangrove ecosystem because mangrove has a good supply of natural feeding (Hilmi et al. 2021c, 2022a; Murniasih et al. 2022) to support aquaculture activity. A polyculture system was developed to increase fish productivity per unit area and to maintain water quality (Ekasari et al. 2015; Rose et al. 2015). Polyculture is designed to support the aquaculture system between two or more fish species or organism aquatic, for example, polyculture between milkfish and prawn or shrimp.(Soedibya 2013; Rachmawati and Samidjan 2014; Nuryanto et al. 2017; Soedibya et al. 2017). Other studies also reported polyculture between Milkfish and Nile Tilapia (Soedibya 2013; Mutia et al. 2018; Muyot et al. 2018). Some other studies also reported polyculture between prawn or shrimp and Nile Tilapia (Yang and Fitzsimmons 2004; Mutia et al. 2018; Prabu et al. 2019).

Fish natural food consists of plankton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacteria associated with detritus (Sukardjo 2004; Rougier et al. 2005; Nagelkerken et al. 2008; Mendoza and Henkel 2017; Andriyani et al. 2018). Generally, plankton is divided into phytoplankton and zooplankton (Henmi et al. 2017; Mendoza and Henkel 2017; Andriyani et al. 2020; Alam et al. 2021). Besides natural food, phytoplankton is the primary producer in the aquatic ecosystem (Ishii and Kamikawa 2017; Karl and Church 2017; Alam et al. 2021). Previous studies proved plankton availability and abundance are essential for fish growth and fisheries(Henmi et al. 2017; Andriyani et al. 2020; Alam et al. 2021).

Plankton diversity and abundance have a high potential to support Nile Tilapia and other species in polyculture production, which has been reported from several regions (Yang and Fitzsimmons 2004; Nagelkerken et al. 2008; Soedibya 2013; Mutia et al. 2018; Kusuma et al. 2019). Some researchers reported plankton diversity and abundance in the polyculture pond of Nile Tilapia, *Oreochromis niloticus (*Sihombing et al. 2017; Muyot et al. 2018). Some other studies also found similar in Milkfish *Chanos chanos* (Soedibya 2013; Ekasari et al. 2015; Kusuma et al. 2019). Currently, no report about plankton diversity and abundance in the polyculture of milkfish and Nile Tilapia in the Tritih Kulon Village, District of North Cilacap, Cilacap Regency. These data are vital for developing the polyculture of both species in the Tritih Kulon Village.

Nile Tilapia, *Oreochromis niloticus (Linnaeus, 1758)* (Yang and Fitzsimmons 2004; Soedibya 2013; Ekasari et al. 2015; Kusuma et al. 2019) is a tropical fish that lives in shallow waters. This species lives in an aquatic ecosystem with various salinities (Suresh and Lin 1992; Basuki and Rejeki 2015; Kusuma et al. 2019; Prabu et al. 2019). This species can adapt and live in brackish water with a salinity of up to 25‰ (Japse and Caipang 2011). Nile tilapia consume a broad spectrum of feed and are classified as omnivorous fish. These species consume phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacteria associated with detritus (Mendoza and Henkel 2017; Neal et al. 2018; Takenaka et al. 2018; Teoh et al. 2018; Setyawati et al. 2019; Rahman et al. 2021). The ecological characteristics of Nile Tilapia have been developed among polyculture fisheries commodities (Yang and Fitzsimmons 2004; Soedibya 2013; Ekasari et al. 2015; Muyot et al. 2018). Milkfish, Chanos chanos (Forsskal, 1775) polycultures with the Nile have long been cultivated intensively in ponds. The feeding habits of milkfish, including herbivores, are the advantages of this species. This species is used as a superior product for cultured animals kept in polyculture with omnivores or carnivores. This condition causes the production of milkfish to be stable. Besides that, the specific taste causes milkfish to be in great demand. This species has been cultivated in a polyculture system with Tiger Prawn, milkfish, shrimp, and others (Soedibya 2013; Muyot et al. 2018)

The Tritih Kulon Village resides in North Cilacap District, Cilacap Regency Central Java, Indonesia. The village has an area of 503.43 hectares consisting of land and swampy brackish water areas, including the mangrove and lagoon ecosystems (Hilmi et al. 2019, 2021c, d, e). The previous study utilized most saline water areas for shrimp aquacultures. At that time, shrimp farming has become a profitable business for the Tritih Kulon villagers due to high prices. Currently, shrimp production tends to decline and lead to business failure. This condition has persisted in South Java for the last four years, from Bantul to Cilacap. The defeat led to the shrimp pond in the Tritih Kulon Village becoming abandoned and unproductive. This study tried to provide a solution by providing information about the opportunities for using abandoned shrimp farming land in the Tritih Kulon Village as a polyculture area between tilapia and milkfish. A preliminary need is to study plankton diversity in a polyculture pilot pond of Nile Tilapia, *O.* italics, and Milkfish, *C. chanos*. This study aimed to analyze plankton diversity and abundance as natural feeding in the polyculture pond of Nile Tilapia, *Oreochromis niloticus (Linnaeus, 1758)* with Milkfish, *Chanos chanos* in the Tritih Kulon Village, District of North Cilacap, Cilacap Regency, Central Java.

Materials and Methods

Study area and time

The polyculture pilot pond was in the Tritih Kulon Village North Cilacap District, Cilacap Regency, Central Java, Indonesia (**Figure 1**) (Hilmi et al. 2021c, a). The pilot pond was a traditional earthen pond with an area of 2000 m^{-2} width. The research was conducted from August to October 2018.

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Figure 1. Location of Tritih Kulon Village, North Cilacap District, Cilacap Regency Central Java, indicating the polyculture pond of *Oreochromis niloticus (Linnaeus, 1758)* and *Chanos chanos*

Procedures and data analysis

Pilot pond preparation for polyculture

Inflow and outflow of water occur naturally following tidal currents (Yang and Fitzsimmons 2004; Soedibya 2013; Muyot et al. 2018; Prabu et al. 2019). This study used a pilot earthen brackish water pond with an area of 2000 square meters (m^2). Pond preparation was initially by reversing the bottom of the pond for soil oxidation and killing fish disease-causing organisms. Neutralize soil acidity by adding ten sacks of lime to the pond surfaces, leaving the pool for two weeks without water. Afterward, the pond was fertilized using chicken manure and filled with water as deep as approximately 10 cm to allow plankton to grow. Then, the water depth was increased to about 1.5 m. We kept the depth at 1.5 m by adjusting the inlet and outlet holes as high as 1.5 meters.

Preparation, stocking, and cultivation of fish seeds

The seeds of *Oreochromis niloticus (Linnaeus, 1758)* and *Chanos chanos* were obtained from a governmental agency, namely the Center for Brackish Water Aquaculture Research (BBPBAP) Jepara, Central Java, Indonesia. The average length of Nile Tilapia seeds was 3.1 ± 0.241 cm, with average body weight was 5.36 ± 0.21 g (Yang and Fitzsimmons 2004).

Both fish seed species were acclimatized for 2 hours before stocking in a pilot earthen pond. The stocking density for Nile Tilapia was five individuals per m^2, with the total stocked fish being 10,000 seeds. In contrast, the stocking density of milkfish was set up for three seeds per square meter, meaning the total number of milkfish seeds was 6,000 individuals. During the first two weeks of rearing, the fish were fed with fine pellets and continued with the number of pellets 1000 after reaching a body length of 5 cm. After six weeks, we provided the fish with pellet number 2000 (Soedibya 2013).

Phytoplankton collection and identification

Phytoplankton samples were collected weekly for ten weeks of polyculture periods. Water samples were taken using a 10 liters bucket and filtered using plankton net number 25. We filtered the water samples ten times to have a total volume of filtered water of approximately 100 liters. The plankton was collected and remained in the collection bottle. Afterward, we removed the collection bottle from the plankton net. The filtered plankton was transferred into sample bottles and preserved using formalin until the final concentration became 4% and two drops of pure Lugol (Zafar 2004; Tsuji and Montani 2017; Andriyani et al. 2020).

Phytoplankton identification was conducted under a binocular microscope with a magnification of 10 x 40. The phytoplankton identification processes were as follows. One drop of sample water is placed on an object glass and covered. We carried out microscopic observations in 20 fields of view with up-down and left-right macrometer directions. Phytoplankton identification according to the figure in identification keys (Henmi et al. 2017; Sihombing et al. 2017). Phytoplankton abundance was calculated using the following formula (Arumugam et al. 2016; Andriyani et al. 2020; Asmarany et al. 2022).

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Abundance (F) = [(A/B) x (C/D) x (E/F)] cell/litre

Note:
- A= Water volume in a bottle sample (30 mL)
- B= Water volume observed (0.06 mL)
- C= Cover glass width (484 mm^2)
- D= view field number (20)
- E= number of phytoplankton individual
- F= total volume of filtered water (100 L)

Nile Tilapia growth

Nile Tilapia growth was measured as the absolute growth of body weight (W_G), measured weekly for ten weeks. Body weight was measured using a balance with an accuracy of 0.01 g. Absolute body weight growth was calculated as follows (Palada-de Vera and Eknath 1993; Soedibya 2013; Muyot et al. 2018).

W_G= Wt – Wi

Note:
- W_G= absolute weight growth
- Wt= body weight after t-time
- Wi= initial body weight

Specific growth

The specific growth rate (μ) was calculated from the initial and final densities during the cultivation period, according to the method of Njouondo.

$$\mu = \frac{\ln(x2) - \ln(X1)}{t2 - t1}$$

Where X2 and X1 are the final and initial densities, respectively, t2 is the final cultivation time, and t1 is the initial cultivation time. The specific growth rate was noted as cells/day. This study calculated phytoplankton doubling time-based on the specific growth rate according to the equation

$$g = \frac{\ln 2}{\mu}$$

The relation between phytoplankton to Nile Tilapia growth

This study estimated the potential of plankton to support Nile Tilapia growth based on the weekly plankton abundance and weekly Nile Tilapia growth. This study analyzed data about phytoplankton diversity and abundance descriptively. Then we compared the result to the information available in previous publications. The potential of plankton to support Nile Tilapia growth was analyzed using Pearson correlation and regression (Karl and Church 2017; Hilmi et al. 2020)

Result and Discussion

Natural food in polyculture system using the indicator of plankton diversity and abundance

Natural food, especially plankton, in the polyculture system is required to support fish productivity, including in the Nile Tilapia cultivation. The observation of natural feeding in Figure 1 showed plankton in aquatic ecosystems. Based on the microscopic identification of phytoplankton samples from the polyculture pond of Nile Tilapia and Milkfish can be shown in **Figure 2**. The total number of natural food was 21 plankton species, consisting of 12 phytoplankton species and nine zooplankton groups (**Table 1 and Figure 2**). Whereas **Table 1** also describes the phytoplankton diversity and absolute abundance.

Table 1 showed that phytoplankton consisted of Cyanophyceae, Dinophyceae, and Bacillariophyceae with abundance between 455 cells/liter – 908 cells/liter and diversity between 1.44 – 2.23. Zooplankton consisted of Codonillidae, Arthropoda, Protozoa, Mollusca, and Crustacea with abundance between 253 cells/liter – 463 cells/liter and diversity between 1.88 – 2.6. The potential phytoplankton species were *Oscillatoria, Peridinium, Prorocentrum, Navicula, Nitzia, Rhizosolenia, Diatoma, Striatella, Pleurosigma, Chaetoceros, Coscinodiscus,* and *Fragillaria.* The total zooplankton abundance in Lampung and South Sulawesi ranges between 32,745 individuals.m^{-3} and 652,925 individuals.m-3, dominated by *Calanoida* and *Cyclopoida nauplii (Duggan et al. 2011).* This study found that Tintinnopsis, Lapadella, Brachionus, Cephalodella, and Colurella dominated the zooplankton. Some researchers also report that Indonesia has five new records of plankton species that are *Oithona decipiens, Oithona hebes, Oncaea atlantica group, Oncaea zernovi group, and Spinoncaea* spp.

Different with Tigris River Iraq, which has 106 taxonomy units of zooplankton, including 65 taxa belonging to rotifers, 25 taxa to copepod, and 16 taxa to Cladocera with The Shannon–Weiner index of Rotifera varied from 0.67 to 3, 0.50–1.72 for Cladocera and from 0.91 to 2.51 for Copepoda. (Abdulwahab and Rabee 2015). The other condition shows that Delta Mahakam has 48 taxa phytoplankton belonging to Bacillariophyceae (35), Dinophyceae (6), Chlorophyceae (4), and Cyanophyceae (3) (Effendi et al. 2016). Lake Burdur Turkey has six zooplankton taxa: Hexarthra fennica, Brachionus plicatilis from Rotifera, and Arctodiaptomus burduricus from Copepoda. The average zooplankton density in Lake Burdur shows that 399,074 ind.m^{-3} is distributed by 51% H. fennica, 9% B. plicatilis, and 40% A. burduricus..(Gülle et al. 2010). Pearl River estuary, China, has 132 species of zooplankton (Honggang et al. 2012). In River Yeúilırmak, Amasya, Turkey has plenty of divisions, which are *Navicula cincta, N. cryptocephala,* and *N. rhyncocephala* (Soylu and Gönülol 2003). (Fang et al. 2012) also reports that the Xiaoqing River estuary has seven plankton species: *Skeletonema costatum, Tribonema affine,* and *Chlorella* sp.

The data showed that the abundance of Bacillariophyceae > Dinophyceae > Cyanophyceae with *Nitzia, Diatoma,* and *Chaetoceros* had high potency in the polyculture system. Whereas Rotifera, Chodonillidae, and Arthropoda had high potency as natural feeding. The distribution of natural feed of both phytoplankton and zooplankton (diversity and abundance) can be shown in **Figure 2.** Based on **Figure 2** showed that the diversity and abundance of phytoplankton > zooplankton. The variety of phytoplankton on stations 6, 7, 8, and 10 was greater than on other stations, whereas the diversity of zooplankton station 1 was the highest.

Table 1. The diversity and abundance of plankton in the polyculture system

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Classis/Species	abundance (cell/liter)										confidence interval
	1	2	3	4	5	6	7	8	9	10	
Phytoplankton											
Cyanophyceae											
Oscillatoria	822	121	251	311	184	286	384	611	167	282	341.9±217.29
Dinophyceae											
Peridinium	1.302	212	114	221	377	211	306	288	601	719	435.1±357.50
Prorocentrum		412	218	174	89	281	241	418	291	339	246.3±133.42
Bacillariophyceae											
Navicula	481	504	417	385	1.298	738	781	552	828	829	681.3±275.43
Nitzia	781	1.027	818	1.219	3.036	1.833	2.077	1.865	2.199	3.071	1,792.6±838.09
Rhizosolenia	241	438	221	371	218	638	721	811	719	693	507.1±234.34
Diatoma	1.799	2.385	1.755	1.544	311	1.539	2.190	3.082	2.710	2.804	2,011.9±808.14
Striatella	422	390	271	315	278	529	481	365	391	380	382.2±82.38
Pleurosigma	251	622	315	128	276	383	485	391	588	429	386.8±152.79
Chaetoceros	204	281	316	285	3.138	1.892	926	819	692	539	909.2±928.53
Coscinodiscus	151	189	326	618	261	522	419	791	839	549	466.5±239.83
Fragillaria		765	433	221	141	523	409	591	285	263	363.1±226.94
total	6454	7346	5455	5792	9607	9375	9420	10584	10310	10897	
abundance (cell/liter)	645	612	455	483	801	781	785	882	859	908	
Diversity	1,44	2,14	2,18	2,18	1,81	2,23	2,22	2,20	2,17	2,09	
Zooplankton											
Codonillidae											
Tintinnopsis	219	316	467	361	363	412	504	629	523	528	432.2±120.99
Arthropoda		263	329	442	388	327	628	721	572	422	409.2±204.20
Protozoa	241	421	329	289	207	284	286	275	319	318	296.9±57.15
Mollusca	352	327	287	391	319	321	319	428	329	409	348.2±45.85
Crustacea	252	316	337	209	281	255	777	489	417	379	371.2±165.69
Rotifera											
Lapadella	174	215	255	186	228	347	429	519	438	379	317±121.34
Brachionus	306	218	203	408	391	436	389	328	372	351	340.2±78.13
Cephalodella	231	242	429	291	207	230	281	361	369	328	296.9±72.97
Colurella	250	362	274	422	501	427	389	421	408	367	382.1±74.35
Total	2025	2680	2910	2999	2885	3039	4002	4171	3747	3481	
abundance (cell/liter)	253	298	323	333	321	338	445	463	416	387	
Diversity	2,06	1,95	1,92	1,88	1,88	1,94	1,85	1,85	1,89	1,93	

The species domination of zooplankton and phytoplankton is highly correlated with eutrophication (Xiong et al. 2003). Climate change also has an impact on potential zooplankton. During spring and summer, zooplankton was irregularly distributed through the water profile, where the highest average density was recorded at 10–5 m depth (66,007 and 66,734 Ind. m^{-3} (Khalifa et al. 2015). The seasonal potential of zooplankton dynamics in the southern Chukchi Sea is less influenced by the local growth of zooplankton and spring phytoplankton bloom and more influenced by the advection of zooplankton abundance (Kitamura et al. 2017).

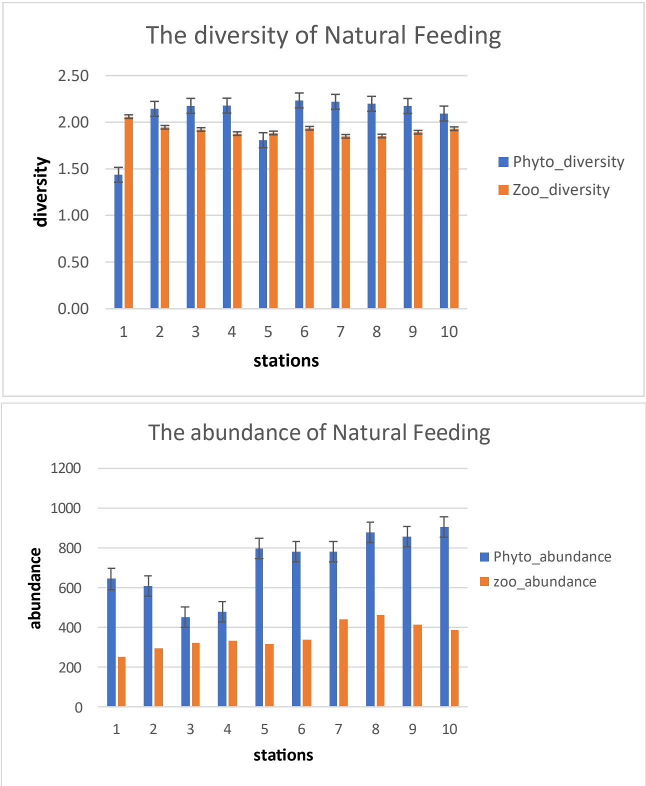

Figure 2. The potential (diversity and abundance) of Natural feeding (phytoplankton and zooplankton)

The other condition, the species composition of zooplankton, determined using hierarchical cluster analysis and indicator species analysis, showed the influencing of season condition. The winter community, characterized by warm water indicator species including Mesocalanus tenuicornis, Calanus pacificus, and Corycaeus anglicus, diverged into four communities throughout the spring and summer (McKinstry and Campbell 2018). The other location also shows the total potential 48 taxa phytoplankton from 26 genera and three classes, namely Bacillariophyceae (37 taxa), Cyanophyceae (10 taxa), and Schizomycetes (1 taxon) (Onyema 2007) which is influenced by variations in the physical environments (Ormańczyk et al. 2017). A previous study in Tanggerang coastal water also reported that Crustacean dominated 12 groups of zooplankton. The Morisita Index shows that the zooplankton in Tangerang coastal waters have been grouped in a patchy pattern distribution (Pratiwi et al. 2016). The distribution of zooplankton and phytoplankton is influenced by phosphate, nitrate, nitrite, dissolved oxygen, salinity, and depth water. (Putri et al. 2019)

The impact of the abundance of zooplankton and phytoplankton as natural feeding toward Nile Tilapia growth in the Polyculture system

his study showed the effect of natural feeding support on Nile Tilapia Growth in the Polycalutrure system **(Table 2**). The potential natural feeding, growth, and gut content of the Nile of Tilapia showed the abundance and diversity of zooplankton and phytoplankton, as proved by Nile

Tilapia gut content and fish growth. The potential natural feeding of phytoplankton 721 ± 163,2 ind/l (abundance) and 2,07 ± 0,25 (diversity) and zooplankton with value 358 ± 67,6 ind/l (abundance) and 1,91± 0,06 (diversity). The impact of natural feeding to support Nile Tilapia growth was 17,5 ±8,59 % (the fish mortality), 208.2 ± 22,5 gr (weight rate), and 2,28 ± 0.77 %/day (specific growth). The indicator of natural feeding can be shown by gut content, that is, 43-168 individuals/ml (phytoplankton) and 23-65 individuals/ml (zooplankton).

Table 2. The potential natural feeding, growth, and gut content of Nile of Tilapia

Station	Phytoplankton		Zooplankton		Nile Tilapia						
					Fish Growth			Potential of Gute content		Percent of Gute content	
	Abundance (ind/l)	Diversity	Abundance (ind/l)	Diversity	Mortality (%)	Weight absolute (gr)	Specific growth	Phytoplakton in gute content	Zooplankton in gut content	Percent of n gut content of phytoplankton	Percent of n gut content of zooplankton
							(%/day)	Ind/ml	Indv/ml	(%)	(%)
1.	645	1,44	253	2,06	28,67	194,03	1,48	46	34	7,1 (46/645)	13,4
2.	612	2,14	298	1,95	25,33	187,04	1,37	44	23	7,2	7,7
3.	455	2,18	323	1,92	27,33	171,3	1,3	43	31	9,5	9,6
4.	483	2,18	333	1,88	25,33	187,04	1,51	63	22	13,0	6,6
5.	801	1,81	321	1,88	10,67	223,1	2,71	64	35	8,0	10,9
6.	781	2,23	338	1,94	16,67	201,3	2,51	52	33	6,7	9,8
7.	785	2,22	445	1,85	15,33	229,4	2,94	33	36	4,2	8,1
8.	882	2,2	463	1,85	10,67	230,2	2,95	126	56	14,3	12,1
9.	859	2,17	416	1,89	7,33	224,6	2,82	99	65	11,5	15,6
10.	908	2,09	387	1,93	6,67	233,53	3,23	168	24	18,5	6,2
average	721	2,07	358	1,91	17,4	208,15	2,28			100,0	100,0
Stdev	163,1	0,25	67,59	0,06	8,59	22,54	0,77				

Fish growth and gut content can show the impact of natural feeding. Base on the data in **Table 2** shows the positive correlation between natural food with Nile Tilapia growth and gut content because the potential natural feeding (phytoplankton and zooplankton) has a positive impact on supporting Nile Tilapia growth. The gut content consisted of two parameters; potential and percentage of gut content. Both natural supply feeding from zooplankton and phytoplankton showed potential gut content in Nile tilapia 33-168 individuals/ml (phytoplankton) and 23 – 65 individuals/ml (zooplankton). The indicator of gut content also showed that the percent of gut content in Nile Tilapia was 4.2-18.5 % (phytoplankton) and 7.7 – 15.6 % (zooplankton).

Soedibya (2013), Soedibya et al. (2021), and Soedibya et al. (2017) stated that the increase in natural feeding has a positive impact on specific growth, survival rate, and gut content. The gut content indicates positive growth and survival rate of Nila Tilapia. The increasing gut content demonstrates the high ability of Nila tilapia to utilize natural feeding to support Nile tilapia growth in the fishpond. The Nile Tilapia, an adaptive species from a tropical region, can grow in salinities less than 18-20 pp (Soedibya 2013; Basuki and Rejeki 2015; Ekasari et al. 2015; Kusuma et al. 2019). The specific growth, weight absolute, and mortality of Nile tilapia are influenced by natural feeding, saline media, and diseases (Soedibya 2013; Basuki and Rejeki 2015)

The Relation Between Natural Feeding with Nile Tilapia Growth in Polyculture system

The Relation Between Phytoplankton with Nile Tilapia Growth

The impact of natural feeding (phytoplankton) on Nile tilapia growth is shown in **Figure3**. The data showed that the relation between phytoplankton abundance with specific growth rate Y = 0.0044x – 0,8869 (linear) and Y = 0.000008X^2 – 0.0058 x +2.39 (best equation), with weight absolute, was Y = 0.1292 x + 115,02 (linear) and y = 0.0001X^2 – 0,010 x + 159,72 (best equation) and Nila Mortality Y = 51.88 -0,0478 x (linear) and Y = -0.0001X^2 + 0.1249 x -3,55 (best equation)

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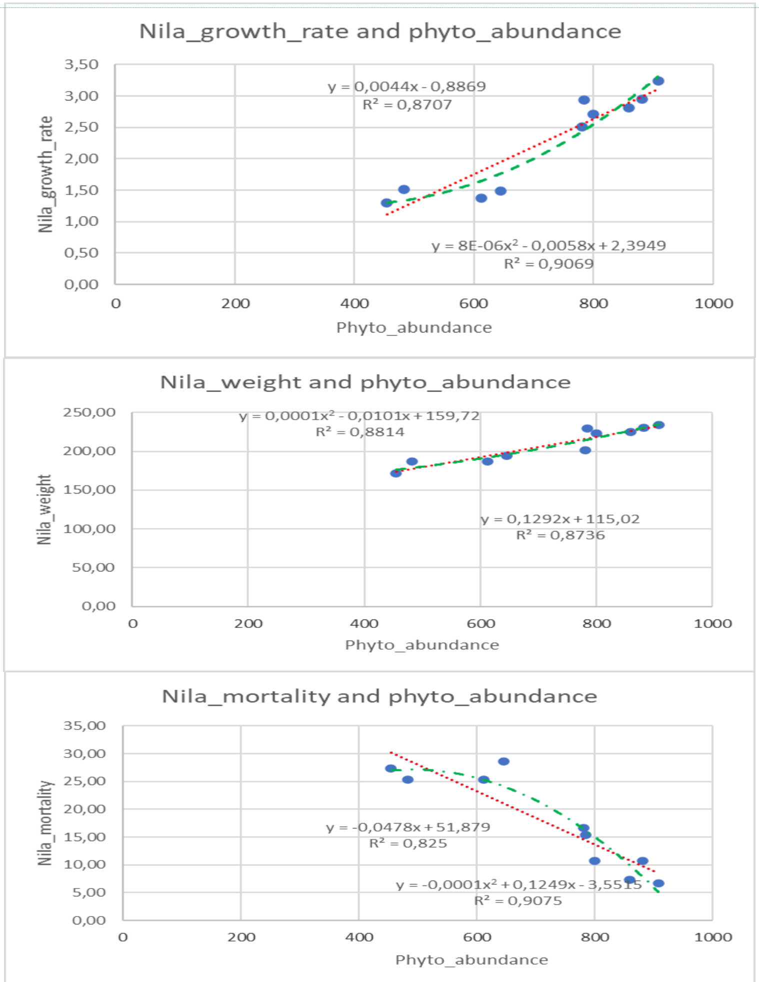

Figure 3. The relation between the abundance of phytoplankton with Nile Tilapia growth (mortality, growth rate, and weight

The Relation Between Zooplankton with Nile Tilapia Growth

The impact of natural feeding (zooplankton) on Nilapia growth is illustrated in **Figure 4**. The data showed that the relation between zooplankton abundance with specific growth rate Y = 0,0087x

– 0,8167 (linear) and $Y = 0.00000001X^4 – 0.010 x^2 - 2.433 x + 215,24$ (best equation), with weight absolute $Y = 0.2429 x + 121,26$ (linear) and $y = 0.0000001 X^4 + 0,00002X^3 +0,133 x -245,73$ (best equation) and Nila Mortality $Y = 49,75 -0,0904 x$ (linear) and $Y = -0.000000006 X^3 + 0,0087X^2 +2,73 x^2 -34,23x +3385,8$ (best equation)

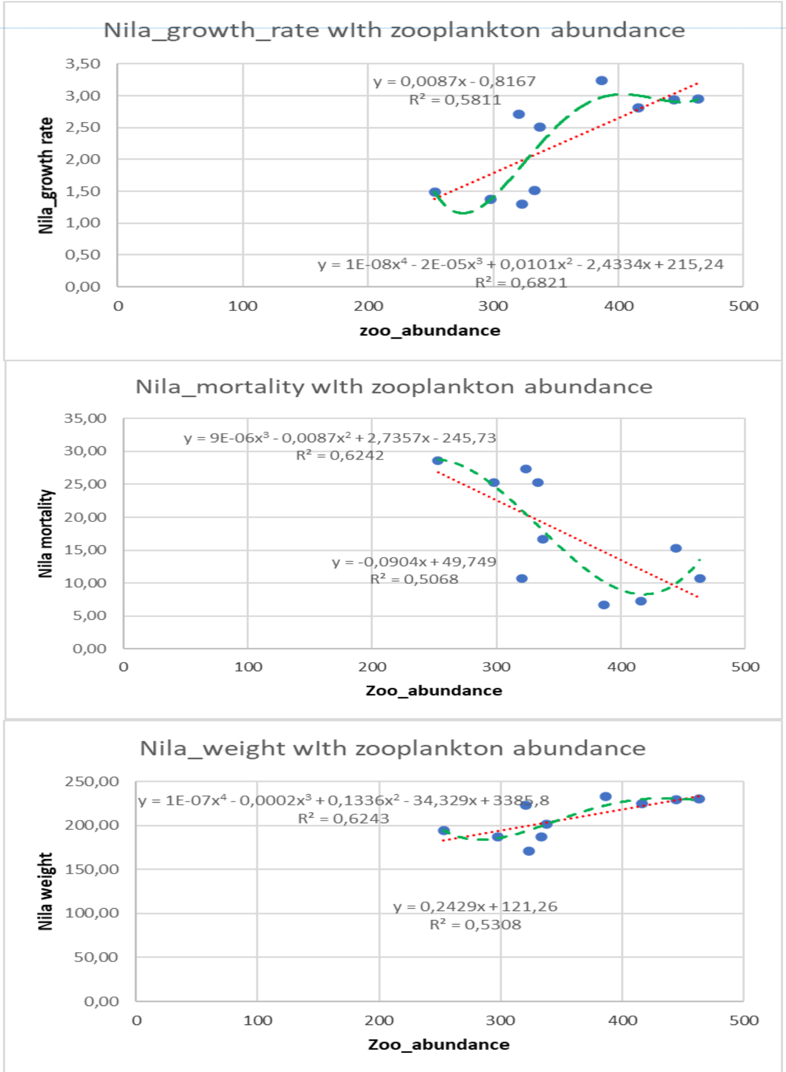

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Figure 4. The relation between the abundance of zooplankton with Nile Tilapia growth (mortality, growth rate, and weight

The data in **Figure 4** showed that the abundance of phytoplankton had a positive correlation with specific growth and weight growth absolute and had a negative correlation with the mortality of Nile Tilapia. Similar to the impact of phytoplankton to support Nila growth, zooplankton also had a positive correlation with specific growth and weight growth absolute and had a negative correlation with the mortality of Nile Tilapia (Figure 5). The relation between zooplankton and phytoplankton with Nile tilapia growth shows that Nile tilapia is Herbivore / Omnivore, a Low trophic level feeder). It can be grown by adding Algae, bacteria, and detritus (bio flocs). Moreover, Nile tilapia has a fast growth rate, adaptable to different conditions, including high stocking densities, is highly disease resistant, and is tolerant of poor water quality (Prabu et al. 2019). Besides the natural feeding, Nila tilapia also was influenced by water salinity, water fertility, pH, DO, and pollution condition (McKinstry and Campbell 2018; Hilmi et al. 2021b, 2022b)

Conclusion

Phytoplankton and zooplankton are the potential natural food supporting Nile tilapia growth in polyculture in the Tritih Kulon Cilacap, as indicated by a positive impact on Nile Tilapia growth. A total number of 21 plankton species consisted of 12 phytoplankton species and nine zooplankton groups. Increasing weight and rate of growth of Nile tilapia and Milkfish in Tritih Kulon Aquaculture proves the potential of plankton. Nile Tilapia growth was 17,5 ±8,59 % (the fish mortality), 208.2 ± 22,5 gr (weight rate), and 2,28 ± 0.77 %/day (specific growth). The indicator of percent of gut content also showed that the percent of gut content in Nile Tilapia was 4,2-18,5 % (phytoplankton) and 7,7 – 15,6 % (zooplankton). Natural feeding (phytoplankton and zooplankton) positively supports the growth of Nila Tilapia, both specific growth, absolute weight, and mortality.

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Exploring the Role of Natural Food in Enhancing the Productivity of Saline Nile Tilapia in the Mangrove Ecosystem of Segara Anakan Lagoon, Brackish Water Culture

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Abstract

Saline Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) and Milkfish, *Chanos chanos* (Forskal, 1775) polyculture systems require natural food to sustain their brackish water fish production. Brackish water polyculture of Saline Nile Tilapia is developed to improve the productivity of abandoned shrimp pond. At present, there are no studies examining the potential of natural food to improve the productivity of these polyculture systems in the brackish water pond located in Tritih Kulon Village, Cilacap, Central Java. To assess the availability of this food source, the diversity and abundance of plankton in the water body were evaluated through analysis. Therefore, this study aimed to analyze plankton diversity in the water body and the relationship between plankton abundance as a natural feed and saline Nile Tilapia productivity in the brackish water polyculture. Phytoplankton and zooplankton were identified according the plankton characteristic available in the references. The results showed that 21 planktons, comprising 12 phytoplankton species and 9 zooplankton groups, were identified. In spite of low plankton diversity, however, this study proved that the plankton abundance positively supported Saline Nile tilapia productivity in the polyculture system with milkfish, as indicated by low mortality (17,5 ±8,59 %), high weight gain (208.2 ± 22,5 gr), and high specific growth (2,28 ± 0.77 %/day) with an R^2 of 0.825-0.908, 0.881-0.874, and 0.87-0.91, respectively. The productivity of Saline Nile Tilapia in brackish water polyculture with milkfish is significantly supported by the availability of natural food, with 5.95 to 18.50% of their gut content obtained from plankton.
Keywords: abundance, brackish water pond, fishery, monoculture, phytoplankton

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Introduction

The Brackish water culture is divided into two different systems, monoculture and polyculture (Cochard 2017; Hu et al. 2020; Zhu et al. 2022; Jansen et al. 2023). Additionally, brackish water aquaculture can be carried out in areas with abundant natural food sources, such as estuaries and mangrove ecosystems (Duncan et al. 2016; Hilmi et al. 2021c, a; Soedibya et al. 2021; Murniasih et al. 2022). The development of a brackish water polyculture system enhances fish productivity per unit area and maintain water quality (Ekasari et al. 2015; Rose et al. 2015). A brackish water polyculture is an aquaculture system between two or more fish species or aquatic organisms, such as milkfish and prawn or shrimp polyculture (Soedibya 2013; Rachmawati and Samidjan 2014; Nuryanto et al. 2017; Soedibya et al. 2017). Some research reported polyculture between Milkfish and Nile Tilapia (Soedibya 2013; Mutia et al. 2018; Muyot et al. 2018), while others described a polyculture between prawn or shrimp and Nile Tilapia in brackish water (Yang and Fitzsimmons 2004; Mutia et al. 2018; Prabu et al. 2019). (Hilmi et al. 2021c, 2022; Murniasih et al. 2022)(Hilmi et al. 2021c, 2022; Murniasih et al. 2022)(Hilmi et al. 2021c, 2022; Murniasih et al. 2022)(Hilmi et al. 2021c, 2022; Murniasih et al. 2022)(Hilmi et al. 2021c, 2022; Murniasih et al. 2022)(Hilmi et al. 2021c, 2022; Murniasih et al. 2022)(Hilmi et al. 2021c, 2022; Murniasih et al. 2022)

Natural food is necessary to support fisheries productivity (Albertson et al. 2018; Pusey et al. 2020; Thoral et al. 2021), including in brackish water fish (Karna et al. 2014; Mondal and Chakravortty 2015; Mondal and Mitra 2016). The natural food of fish includes plankton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacteria associated with detritus (Mendoza and Henkel 2017; Andriyani et al. 2018). Additionally, plankton is divided into phytoplankton and zooplankton (Henmi et al. 2017; Mendoza and Henkel 2017; Andriyani et al. 2020; Alam et al. 2021). Previous studies showed that plankton availability and abundance are essential for fish growth and productivity(Henmi et al. 2017; Andriyani et al. 2020; Alam et al. 2021).

Plankton diversity and abundance support polyculture productivity, as reported by several regions (Soedibya 2013; Mutia et al. 2018; Kusuma et al. 2019). Some studies reported these characteristics in the polyculture pond of Nile Tilapia, *Oreochromis niloticus* (Soedibya 2013; Ahsan et al. 2014; Ekasari et al. 2015; Kusuma et al. 2019; Samidjan et al. 2020). Also, other studies found similar data in Milkfish *Chanos chanos* (Sihombing et al. 2017; Muyot et al. 2018). There are no reports on plankton diversity and abundance in saline Nile Tilapia and milkfish polyculture of in the brackish water pond. The data are essential for developing the brackish water polyculture of both species.

Saline Nile Tilapia (Soedibya 2013; Ekasari et al. 2015; Kusuma et al. 2019) is a tropical fish in shallow waters that thrives in an aquatic ecosystem with varying salinities (Basuki and Rejeki 2015; Kusuma et al. 2019; Prabu et al. 2019), hence, they can adapt and live in brackish water with a salinity of about 25$^{o}/_{oo}$ (Ninh et al. 2014). Furthermore, these species are classified as omnivorous fish because they consume a broad spectrum of feed including phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus, and bacteria associated with detritus (Mendoza and Henkel 2017; Neal et al. 2018; Takenaka et al. 2018; Teoh et al. 2018; Setyawati et al. 2019; Rahman et al. 2021). The ecological benefits of Saline Nile Tilapia have been used to develop brackish water aquaculture and it has been polycultured with various fisheries products (Soedibya 2013; Ekasari et al. 2015; Muyot et al. 2018). Therefore, the intensive polyculture of saline Nile Tilapia and Milkfish, Chanos chanos *Forsskal*, 1775 has been carried out in ponds overtime (An and Anh 2020; Samidjan et al. 2020).

Tritih Kulon Village is located in North Cilacap District, Cilacap Regency, Central Java, Indonesia. Furthermore, the village has an area of 503.43 hectares consisting of land and swampy brackish water areas, including the mangrove and lagoon ecosystems (Hilmi et al. 2019, 2021c, d, e). The saline water areas were used by previous studies for shrimp aquacultures, as this was a profitable business for the villagers at that time, due to high prices. However, shrimp production has currently declined, leading to business failure. This condition has persisted in South Java, from Bantul to Cilacap Regencies, for the past four years. The shrimp pond in Tritih Kulon Village was abandoned and unproductive due to the defeat. Therefore, this study offered a solution by outlining the potential for using an abandoned brackish water shrimp farming pond in Tritih Kulon Village as a polyculture area between Saline Nile Tilapia and Milkfish. Studying plankton diversity in a polyculture pilot pond of Saline Nile Tilapia, *O. niloticus*, and Milkfish, *C. chanos* is necessary. Therefore, this study aimed to analyze plankton diversity and abundance as natural food in the brackish water polyculture pond of Saline Nile Tilapia with Milkfish in Tritih Kulon Village.

Materials and Methods

Study area and time

The pond w located in the Tritih Kulon Village, North Cilacap District, Cilacap Regency, Central Java, Indonesia (**Figure 1**) (Hilmi et al. 2021c, b). Furthermore, it is a traditional earthen pond with an area of 2000 m^{-2} width. This study was conducted from August to October 2018.

Figure 1. Location of Tritih Kulon Village, North Cilacap District, Cilacap Regency Central Java indicating the polyculture pond of *Oreochromis niloticus (Linnaeus, 1758)* and *Chanos chanos*

Procedures and data analysis

Pilot pond preparation for polyculture

Water Inflow and outflow occur naturally in response to tidal currents (Soedibya 2013; Muyot et al. 2018; Prabu et al. 2019) . This study used a pilot earthen brackish water pond with an area of 2000 square meters (m^2). This pond was prepared by reversing the bottom for soil oxidation and killing fish disease-causing organisms. The soil acidity was neutralized by adding ten sacks of lime to the surfaces and draining the pool for two weeks. Then, the pond was fertilized using chicken manure and filled with water to a depth of approximately 10 cm, to allow plankton growth. The water level was elevated to a height of approximately 1.5 meters and kept at that level by adjusting the inlet and outlet openings accordingly.

Preparation, stocking, and cultivation of fish seeds

The seeds of *Oreochromis niloticus (Linnaeus, 1758)* and *Chanos chanos* were obtained from a governmental agency, namely the Center for Brackish Water Aquaculture Research (BBPBAP) Jepara, Central Java, Indonesia. The average length of Saline Nile Tilapia seeds was 3.1 ± 0.241 cm, with an average body weight of 5.36 ± 0.21 g (Yang and Fitzsimmons 2004).

The fish seed species were acclimatized for 2 hours before stocking in a pilot earthen pond, with a density of five individuals per m^2 for Saline Nile Tilapia and a total stocked fish of 10,000 seeds. The fish were fed fine pellets for the first two weeks of rearing and then 1000-size pellets once they had grown to a length of 5 cm. After six weeks, they were provided with pellet number 2000 (Soedibya 2013).

Plankton collection and identification

Weekly samples of plankton were collected for the ten weeks of polyculture periods. Also, 10 liters bucket was used to collect water samples, which were filtered using plankton net number 25. The water samples were filtered ten times to attain a total volume of approximately 100 liters. The plankton was obtained in a collection bottle and then removed from the plankton net. The filtered plankton was transferred into sample bottles and preserved using formalin until the final concentration was 4% and two drops of pure Lugol were added (Tsuji and Montani 2017; Andriyani et al. 2020).

Plankton identification was carried out under a binocular microscope with a magnification of 10 x 40. The identification processes were as follows, one drop of sample water was placed on a glass object and covered, then microscopic observations were conducted in 20 fields of view with up-down and left-right macrometer directions. Furthermore, plankton identification was achieved using the figure in the identification keys (Henmi et al. 2017; Sihombing et al. 2017), and plankton abundance was calculated using the formula (Arumugam et al. 2016; Andriyani et al. 2020; Asmarany et al. 2022).

Abundance (F) = [(A/B) x (C/D) x (E/F)] cell/liter

Note:

A= Water volume in a bottle sample (30 mL)
B= Water volume observed (0.06 mL)
C= Cover glass width (484 mm^2)
D= view field number (20)
E= number of plankton individual
F= total volume of filtered water (100 L)

Saline Nile tilapia growth

The absolute growth of body weight (WG) in Saline Nile Tilapia was measured weekly for ten weeks. In addition, a balance with an accuracy of 0.01 g was used to measure the body weight. Absolute body weight growth was calculated as follows (Palada-de Vera and Eknath 1993; Soedibya 2013; Muyot et al. 2018):

W_G= Wt – Wi

Note:

W_G= absolute weight growth
Wt= body weight after t-time
Wi= initial body weight

Specific growth rate

According to the method of Rachmawati and Samidjan (2014), Ekasari et al. (2015), Soedibya et al. (2017) and Soedibya et al. (2021) the specific growth rate (μ) was calculated from the initial and final densities during the cultivation period.

$$\mu = \frac{\ln(x2) - \ln(X1)}{t2 - t1}$$

Where X2 and X1 are the final and initial densities, t2 and t1 are the final and initial cultivation time, respectively. The specific growth rate was noted as cells/day, and this study calculated phytoplankton doubling time based on the specific growth rate according to the following equation:

$$g = \frac{\ln 2}{\mu}$$

The relation between plankton to Saline Nile tilapia growth

Based on weekly plankton abundance and Aaline Nile Tilapia growth, this study estimated the potential of plankton to support saline growth. The data on plankton diversity and abundance were descriptively analyzed in this study, and the result was compared to the information available in previous publications. Additionally, Pearson correlation and regression were used to analyze the potential of plankton to support Saline Nile tilapia growth (Karl and Church 2017; Hilmi et al. 2020).

Result and Discussion

Natural food diversity and abundance

Plankton identification from the brackish water polyculture pond of Saline Nile Tilapia and Milkfish found about 21 plankton species, comprising 12 phytoplankton and 9 zooplankton species. Table 1 showed the weekly plankton diversity, diversity index, and absolute abundance.

Table 1. The diversity and abundance of plankton in the polyculture system

Classis/Species	abundance (cell/litre)										confindence interval
	1	2	3	4	5	6	7	8	9	10	
Phytoplankton											
Cyanophyceae											
Oscillatoria	822	121	251	311	184	286	384	611	167	282	341.9±217.29
Dinophyceae											
Peridinium	1.302	212	114	221	377	211	306	288	601	719	435.1±357.50
Prorocentrum		412	218	174	89	281	241	418	291	339	246.3±133.42
Bacillariophyceae											
Navicula	481	504	417	385	1.298	738	781	552	828	829	681.3±275.43
Nitzia	781	1.027	818	1.219	3.036	1.833	2.077	1.865	2.199	3.071	1,792.6±838.09
Rhizosolenia	241	438	221	371	218	638	721	811	719	693	507.1±234.34
Diatoma	1.799	2.385	1.755	1.544	311	1.539	2.190	3.082	2.710	2.804	2,011.9±808.14
Striatella	422	390	271	315	278	529	481	365	391	380	382.2±82.38
Pleurosigma	251	622	315	128	276	383	485	391	588	429	386.8±152.79
Chaetoceros	204	281	316	285	3.138	1.892	926	819	692	539	909.2±928.53
Coscinodiscus	151	189	326	618	261	522	419	791	839	549	466.5±239.83
Fragillaria		765	433	221	141	523	409	591	285	263	363.1±226.94
total	6454	7346	5455	5792	9607	9375	9420	10584	10310	10897	
abundance (cell/litre)	645	612	455	483	801	781	785	882	859	908	
Diversity	1,44	2,14	2,18	2,18	1,81	2,23	2,22	2,20	2,17	2,09	
Zooplankton											
Codonillidae											
Tintinnopsis	219	316	467	361	363	412	504	629	523	528	432.2±120.99
Arthropoda		263	329	442	388	327	628	721	572	422	409.2±204.20
Protozoa	241	421	329	289	207	284	286	275	319	318	296.9±57.15
Mollusca	352	327	287	391	319	321	319	428	329	409	348.2±45.85
Crustacea	252	316	337	209	281	255	777	489	417	379	371.2±165.69
Rotifera											
Lapadella	174	215	255	186	228	347	429	519	438	379	317±121.34
Brachionus	306	218	203	408	391	436	389	328	372	351	340.2±78.13

Cephalodella	231	242	429	291	207	230	281	361	369	328	296.9±72.97
Colurella	250	362	274	422	501	427	389	421	408	367	382.1±74.35
Total	2025	2680	2910	2999	2885	3039	4002	4171	3747	3481	
abundance (cell/litre)	253	298	323	333	321	338	445	463	416	387	
Diversity	2,06	1,95	1,92	1,88	1,88	1,94	1,85	1,85	1,89	1,93	

Table 1 demonstrated that the phytoplankton obtained consisted of three classes, including Cyanophyceae, Dinophyceae, and Bacillariophyceae. Similarly, six zooplankton classes, namely Codonillidae, Arthropoda, Protozoa, Mollusca, Crustacea, and Rotifera, were observed during the study. The results showed that the diversity of plankton classes was lower than in other studies. Specifically, a smaller number of plankton classes was recorded compared to the studies conducted by Nugroho et al. (2020) and Sastranegara et al. (2020) in the Segara Anakan estuary. The difference is that this study was conducted in a semi-closed ecosystem and narrow areas, while Nugroho et al. (2020) and Sastranegara et al. (2020) were carried out in open water and broader areas covering across Segara Anakan estuary. Previous studies reported that wider and more variable habitats may result in phytoplankton diversity (Wiyarsih et al. 2019; Priska et al. 2020).

Table 1 showed that 12 phytoplankton and 9 zooplankton species were observed during the study. The data indicated low species diversity of phytoplankton which was also supported by a low phytoplankton diversity index, ranging between 1.44 and 2.23 and 1.85 to 2.06 for zooplankton (**Table 1**). This study was conducted in a pilot brackish water polyculture pond with an area of 2000 m^{-2}, with a homogenous condition. However, previous studies were carried out in wider areas across Segara Anakan esturay (Nugroho et al. 2020; Sastranegara et al. 2020); Tidung Island (Rachman 2020), and with heterogenous ecological conditions (Wiyarsih et al. 2019). Previous studies noted that different ecosystems have varying plankton diversity due to differences in ecological factors and nutrient availability. Therefore, a lower phytoplankton diversity than those previous studies is required.

Table 1 also showed that phytoplankton and zooplankton abundance ranged from 455 to 908 cells/liter and 253 to 463 cells/liter, respectively, indicating low plankton abundance. The data also indicated that the brackish water polyculture pond was less fertile or at the mesotrophic level. Therefore, further study on the trophic level of the pond is necessary to estimate the trophic index (Ismail et al. 2018).

This study obtained lower phytoplankton and zooplankton abundance than previous studies in the Segara Anakan estuary (Wiyarsih et al. 2019; Nugroho et al. 2020; Sastranegara et al. 2020) due to differences in sampling locations width and variability. Furthermore, this study was conducted in a narrow brackish water pond, while previous studies were carried out in wide areas across the Segara Anakan estuary. The difference in sampling coverages and variability results in different phytoplankton diversity and abundance because each sampling site has their fertility (Wiyarsih et al. 2019; Priska et al. 2020). Moreover, variations in the physical environments influence plankton diversity and abundance (Pratiwi et al. 2016; Ormańczyk et al. 2017; Putri et al. 2019).

The abundance of natural food, particularly plankton, can be used to estimate the potential to support Saline Nile Tilapia polyculture with Milkfish. Furthermore, Bacillariophyceae provided the highest support because it was the most abundant phytoplankton in the brackish water polyculture pond, followed by Dinophyceae, and Cyanophyceae had the lowest potential (**Table 1**). For the zooplankton groups, *Nitzia, Diatoma, Chaetoceros*, Rotifera, Chodonillidae, and Arthropoda supported Saline Nile Tilapia productivity in polyculture with Milkfish. Also, the support of natural food on Saline Nile Tilapia productivity is strengthened by comparing the abundance and gut content analysis. Additional support was also obtained from correlation analysis between natural food abundance and productivity parameters.

The Potential of Natural Food To Support Saline Nile Tilapia Productivity in Brackish Water Polyculture

The potential of natural food to support Saline Nile Tilapia productivity is indicated by the abundance of zooplankton and phytoplankton in the gut compared to the natural abundance. Furthermore, the abundance of phytoplankton and zooplankton in natural food were 721 ± 163,2 cells/l and 358 ± 67,6 cells/l, respectively. **Table 2** showed that the amount of phytoplankton and zooplankton inside the gut of saline Nile Tilapia were 73.8 ± 43.71 and 35.9 ± 8.59, respectively.

Table 2. The potential natural feeding, growth and gute content of Nile of Tilapia

Station	Phytoplankton		Zooplankton		Nile Tilapia						
					Fish Growth			Potential of Gute content		Percent of Gute content	
	Abundance (ind/l)	Diversity	Abundance (ind/l)	Diversity	Mortality (%)	Weight absolute (gr)	Specific growth (%/day)	Phytoplakton in gute content Ind/ml	Zooplankton in in gute content Indv/ml	Percent of n gute content of phytolankton (%)	Percent of n gute content of zooplankton (%)
1.	645	1,44	253	2,06	28,67	194,03	1,48	46	34	7,1 (46/645)	13,4
2.	612	2,14	298	1,95	25,33	187,04	1,37	44	23	7,2	7,7
3.	455	2,18	323	1,92	27,33	171,3	1,3	43	31	9,5	9,6
4.	483	2,18	333	1,88	25,33	187,04	1,51	63	22	13,0	6,6
5.	801	1,81	321	1,88	10,67	223,1	2,71	64	35	8,0	10,9
6.	781	2,23	338	1,94	16,67	201,3	2,51	52	33	6,7	9,8
7.	785	2,22	445	1,85	15,33	229,4	2,94	33	36	4,2	8,1
8.	882	2,2	463	1,85	10,67	230,2	2,95	126	56	14,3	12,1
9.	859	2,17	416	1,89	7,33	224,6	2,82	99	65	11,5	15,6
10.	908	2,09	387	1,93	6,67	233,53	3,23	168	24	18,5	6,2
average	721	2,07	358	1,91	17,4	208,15	2,28			100,0	100,0
Stdev	163,1	0,25	67,59	0,06	8,59	22,54	0,77				

Table 2 showed that an increase in natural food abundance had a positive effect on the absolute growth and specific growth rate of Saline Nile Tilapia. However, plankton abundance had negative impact on mortality. The data depicted that natural food abundance had a positive impact on productivity, as shown by low mortality, high absolute weight growth, and high specific growth rate with a value of 17,5 ±8,59 %, 208.2 ± 22,5 gr, and 2,28 ± 0.77%/day, respectively. In addition, Saline Nile Tilapia productivity is affected by several factors, including food abundance and ecological characteristics. Previous studies showed that natural food had a positive impact on specific growth and survival rate (Soedibya 2013; Soedibya et al. 2017; Soedibya et al. 2021). Other studies also reported that water salinity, diseases, and natural food influenced absolute growth, specific growth rate, and fish survival (Soedibya 2013; Basuki and Rejeki 2015; Ekasari et al. 2015; Kusuma et al. 2019). This support was strengthened by the correlation analysis between plankton abundance and productivity parameters.

The Correlation between Natural Food Abundance and Saline Nile Tilapia Productivity in Brackish Water Polyculture system

The Correlation between Phytoplankton Abundance and Saline Nile Tilapia Growth

According to R^2 values ranging from 0.8707 to 0.9069, there is a strong positive correlation between phytoplankton abundance and specific growth rate. Similar correlations were observed between phytoplankton abundance and absolute growth, with R^2 values ranging from 0.8736 to 0.8814. These values illustrated that an increase in phytoplankton abundance was significantly followed by Saline Nile Tilapia growth. However, a negative correlation was observed between phytoplankton abundance and the mortality of the fish, with R^2 values ranging between 0.8250 to 0.9075 **(Figure 2)**. The R^2-values of mortality proved that an increase in phytoplankton abundance was followed by low mortality. Furthermore, the correlation data demonstrated that phytoplankton abundance supported Saline Nile Tilapia productivity in the brackish water polyculture system with Milkfish.

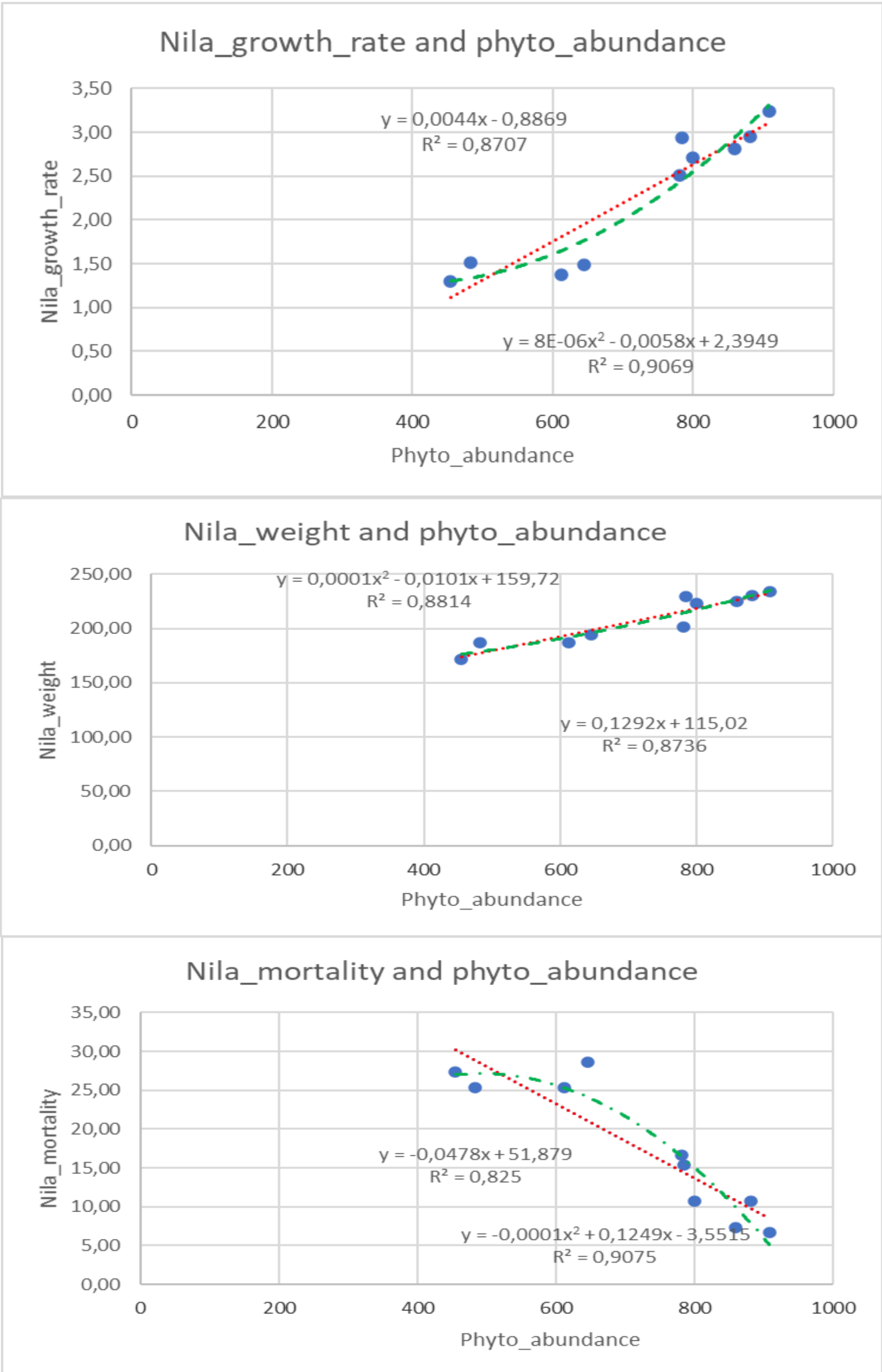

Figure 2 The relation between abundance of phytoplankton with Nila Tilapia growth (mortality, growth rate and weight

The Correlation between Zooplankton Abundance and Saline Nile Tilapia Productivity

Zooplankton abundance correlated positively with absolute and specific growth rates, with R^2 values ranging from 0.5811 to 0.6821 and 0.5308 to 0.6243, respectively. This indicated that a rise in zooplankton was followed by an increase in Saline Nile Tilapia growth. However, a negative correlation was observed between zooplankton abundance and Saline Nile Tilapia mortality, with R^2 values ranging from 0.5068 to 0.6242 (**Figure 3**), where the increase of zooplankton abundance is accompanied by lower mortality. The data demonstrated that zooplankton, as a natural food, promotes Saline Nile Tilapia productivity in a brackish water polyculture system.

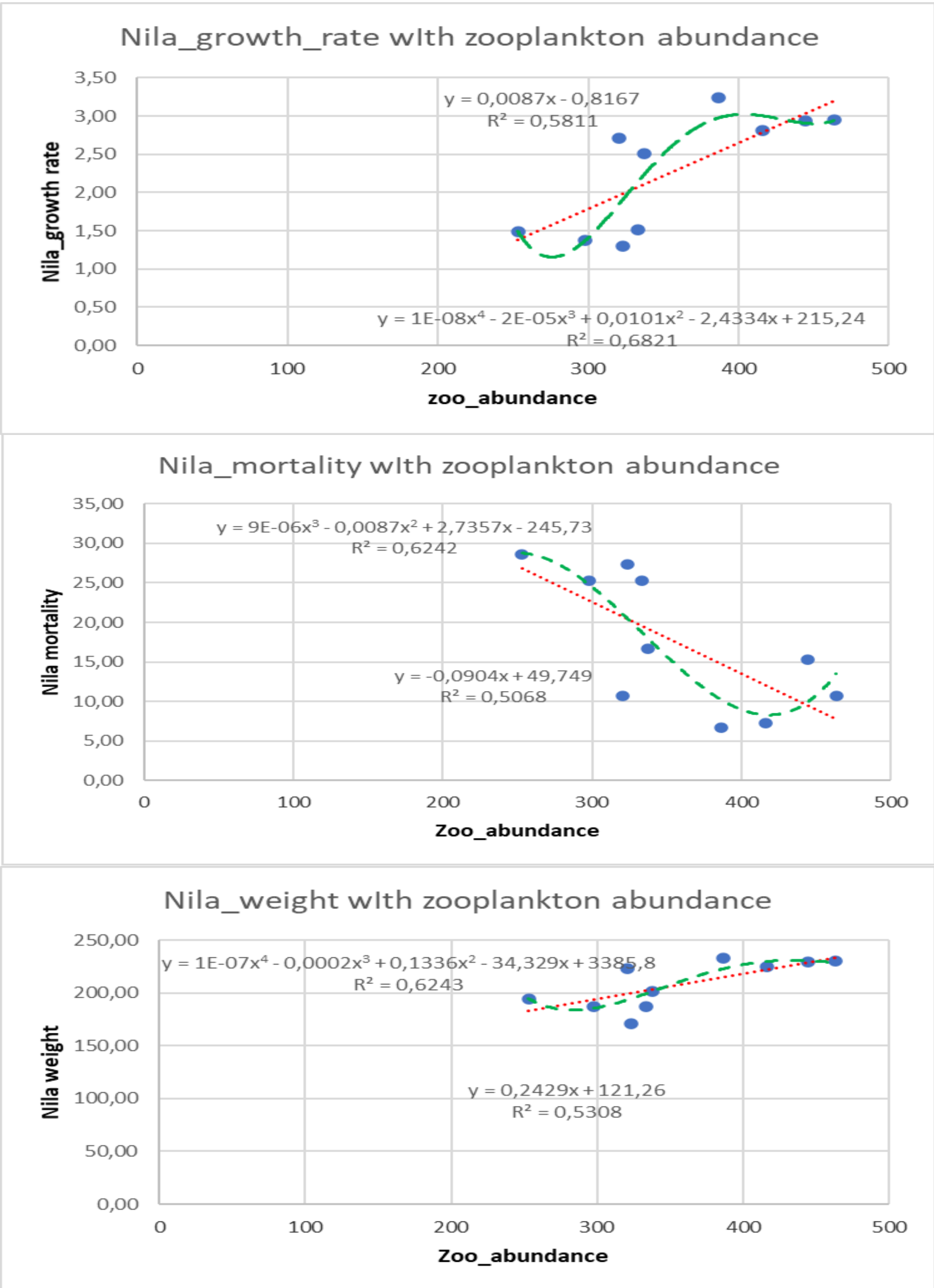

Figure 3. The relation between abundance of zooplankton with Nila Tilapia growth (mortality, growth rate and weight

The comparison of R^2 values in **Figures 2** and **3** showed that zooplankton has a lower support for Saline Nile Tilapia productivity than phytoplankton. Additionally, zooplankton abundance significantly supports Saline Nile Tilapia productivity, because R^2 values between zooplankton abundance and productivity parameters were above 0.5. Previous studies emphasized the importance of natural food sources in fisheries productivity (Albertson et al. 2018; Thoral et al. 2021; Wibowo et al. 2022).

Conclusion

A total of 21 plankton species, which consisted of 12 phytoplankton and 9 zooplankton species were found in a brackish water polyculture pond in Tritih Kulon Village. The parameters and correlation analysis provided strong evidence that natural food abundance significantly supports Saline Nile Tilapia productivity, as indicated by low mortality, high absolute growth, and specific growth rate.

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Petrus Hary Tjahja Soedibya, Endang Hilmi, Isdy Sulistyo, Florencius Eko Dwi Haryono, Hanan Hassan Alsheikh Mahmoud

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Petrus Hary Tjahja Soedibya, Endang Hilmi, Isdy Sulistyo, Florencius Eko Dwi Haryono, Hanan Hassan Alsheikh Mahmoud

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