

SHORT COMMUNICATION

Identification of Plankton in the Stomach of Milkfish (*Chanos chanos*) Seeds Obtained from Natural Waters

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ABSTRACT

Milkfish larvae (*Chanos chanos*) are an omnivorous species. Consequently, this study aimed to identify the types of plankton found in the stomachs of milkfish larvae and to determine suitable natural feed. The research was carried out in the coastal area of Slamaran, Pekalongan City, using three sampling sites with different substrate structures. The main data collected included the diversity of plankton species in the stomachs of milkfish larvae and the plankton present in their natural habitat. The results showed that various plankton genera, such as *Tintinnopsis* sp., *Peridinium* sp., *Coscinodiscus* sp., *Brachionus* sp., *Chlorella* sp., *Cryptomonas* sp., *Skeletonema* sp., *Navicula* sp., and *Rhizosolenia* sp., were in the stomachs of the milkfish seeds. In the natural waters, several plankton genera were identified, including *Nitzschia* sp., *Amphora* sp., *Cyclotella* sp., *Chlamydomonas* sp., *Microcystis* sp., *Alexandrium* sp., *Tintinnopsis* sp., *Oocystis* sp., *Skeletonema* sp., *Brachionus* sp., *Coscinodiscus* sp., and *Peridinium* sp. The water quality parameters were relatively stable across the three sampling locations. The types of plankton in the stomachs of milkfish seeds caught from natural waters included green algae, blue-green algae, diatoms, dinoflagellates, and several zooplankton species. Some of the plankton genera in the milkfish seeds' stomachs had similarities with the plankton genera present in the waters where the seeds were caught, specifically *Peridinium* sp., *Coscinodiscus* sp., *Brachionus* sp., *Skeletonema* sp., *Tintinnopsis* sp., and *Microcystis* sp. This finding was in line with the known omnivorous nature of milkfish, which targeted several phytoplankton species as part of their feeding behavior.

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1. INTRODUCTION

Milkfish (*Chanos chanos*) is a highly sought-after commodity within the fishing community (Hussain et al. 2021). Its farming is practiced extensively along the coast of Indonesia, where the production of this fish has consistently increased over time (Prihatiningsih et al. 2016). For instance, in 2013, Central Java produced 64,305 tons of milkfish (Andriyanto 2013). Furthermore, milkfish can be processed into various products, including meatballs, presto milkfish, and *pepes* milkfish (Hsu et al. 2009). The milkfish has a highly beneficial nutritional profile for human consumption, with varying levels of nutrients, specifically proteins ranging from 0.626% to 7.304%, fat from 4.0% to 4.8%, carbohydrates from 37.64% to 41.88%, moisture

content from 12.26% to 14.42%, and ash content from 16.12% to 21.67% (Akhmadi et al. 2019). This fish production is expected to continue growing in line with the rising per capita fish consumption globally. Such growth allows fish farmers to expand their aquaculture activities on a larger scale (Nasmia et al. 2022). Due to the Indonesian warm, stable climate, the country holds substantial potential for developing milkfish farming businesses (Prihatiningsih et al. 2016).

An essential aspect of milkfish cultivation is fish seed nursery activities, which involve enlarging seeds from larvae to a size suitable for cultivation (Estante-Superio et al. 2021). This process involves obtaining larval seeds from parent fish on a continual basis (Shadrack et al. 2021). In some cases, milkfish larvae seeds for nursery cultivation are sourced

directly from nature (Hanke et al. 2019). This natural sourcing is conducted to potentially improve fish performance during growth (Shadrack et al. 2021). Utilizing a seed nursery process makes the fish more resistant to environmental changes and boosts survival rates (Ariadi et al. 2020). Furthermore, milkfish, being euryhaline, can adapt to a wide range of salinity levels, usually between 2–30‰ in nursery settings (Ariadi 2023; Hussain et al. 2021). These factors underscore milkfish suitability for cultivation in coastal regions.

Proper nutrition is essential for milkfish in nursery systems, as the quality of their diet affects their growth and metabolic performance, similar to the metabolic physiology of aquatic organisms (Hanke et al. 2019). Food for milkfish seedlings typically consists of live plankton and powdered feed, given the small size of the fish's mouth opening during the larval phase (Ariadi et al. 2020; Gorospe et al. 2021). As a plankton feeder, milkfish often receive live plankton during nursery cultivation (Hussain et al. 2021). Common plankton used for milkfish seeds feed include *Rotifera*, *Skeletonema sp.*, *Chaetoceros sp.*, *Chlorella sp.*, *Spirulina sp.*, and *Brachionus sp.* (Bera et al. 2019). The choice of plankton species depends on

the origin and characteristics of the milkfish larvae. Utilizing natural feed is both effective and economical in comparison to artificial feed (Madusari et al. 2022).

To optimize milkfish seed cultivation activities, it is necessary to identify the types of natural feed originating from the milkfish seeds. Milkfish in natural waters have diverse feed types due to differing habitats and environmental dynamics (Nasmia et al. 2022). Therefore, this research aims to identify the types of plankton present in the stomachs of naturally obtained milkfish seeds. This will facilitate the selection of appropriate natural feed types, aiming to develop effective feeding strategies for milkfish seed-rearing systems, drawing on the fish's natural feeding behaviors in their aquatic habitats.

The locations for sampling were in the coastal waters of Slamaran, Pekalongan City, Central Java (6°50'42"–6°55'44" S 109°37'55"–109°42'19" E) (Figure 1). Local farmers often use these areas to collect milkfish larvae before starting nursery activities in mini hatcheries. Sampling took place at three points, identified as stations with sand substrates (station 1), rock substrates (station 2), and mud substrates (station 3). The profile of the milkfish seed samples studied based on the data obtained can be seen in Table 1.

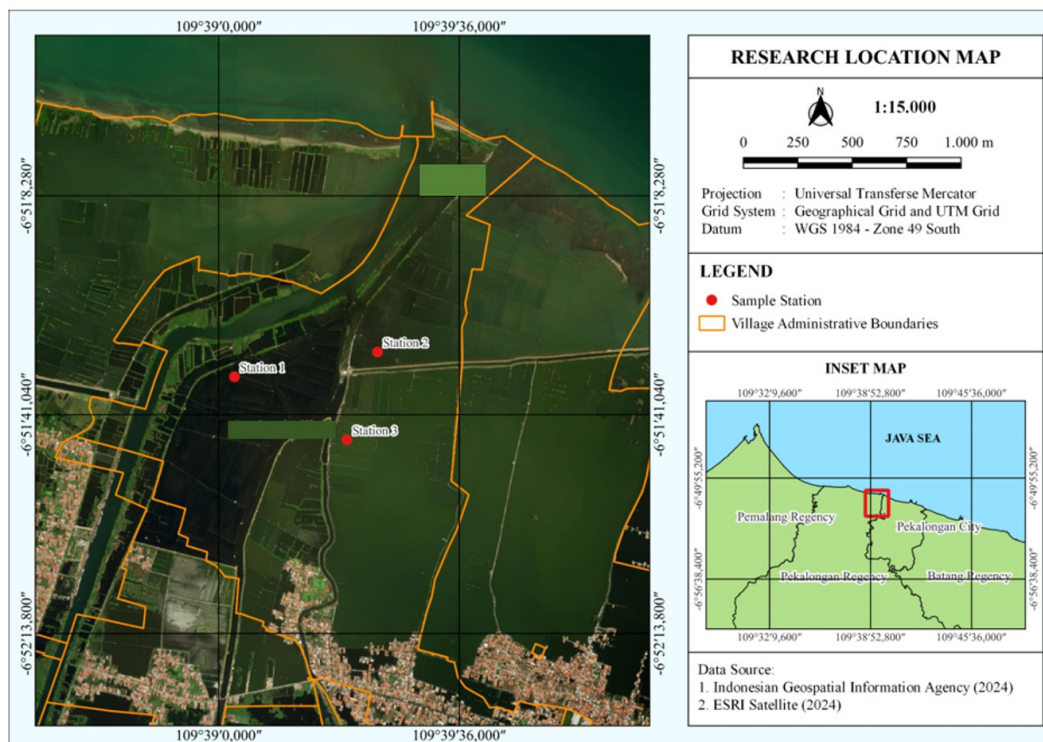


Figure 1. Station of location sampling.

Table 1. Milkfish seed sample research profile.

Location	Total length (cm)	Standard length (cm)	Height length (cm)	Total weight (g)
Station 1	2.8±0.24	2.6±0.12	0.9±0.22	0.02±0.09
Station 2	2.9±0.19	2.7±0.15	0.9±0.25	0.02±0.11
Station 3	3.3±0.23	3.0±0.17	1.0±0.19	0.05±0.09

Sample collection and water sampling

Milkfish seeds sampling took place during the peak fishing season in December 2022. During this time, milkfish larvae commonly appear around the coast, influenced by current distributions associated with the transitional season. The number of milkfish larvae sampled was 75 at each station, with details of 75 fry at station 1, 75 fry at station 2, and 75 fry at station 3, consistently. Sampling of milkfish seeds occurred randomly in the morning, with collected seed samples maintained alive in plastic containers. Concurrently, water samples were collected to identify both the type and abundance of plankton at each location. Water quality parameters were also tested as part of this research. All collected samples were then analyzed and observed at the Integrated Laboratory of Pekalongan University.

The stomach contents of milkfish seeds were examined for plankton by extracting them with a sectional set. The stomach liquid was then collected using a pipette and placed in a Haemocytometer. This sample was covered with a cover glass and observed using an Olympus CX23 microscope. Identification and calculation of plankton species abundance in the stomachs of milkfish seeds were conducted using the following formula:

$$\text{Abundance (\%)} = \frac{\text{cell numbers/individual}}{\text{total cell numbers/total individual}} \times 100\%$$

Identification comparisons were used based on the taxonomic structure of plankton to identify the types of plankton found in fish stomach contents.

Physical and chemical parameters

The water quality parameters observed at the research station include temperature, pH,

dissolved oxygen, salinity, and turbidity (Table 2). Water temperature levels at each sampling station exhibit no significant differences. Station 1, featuring a sand substrate, shows a slightly higher pH value at 8.0. This station also has the highest dissolved oxygen level (6.49 mg.L⁻¹) compared to the other stations. The elevated pH and oxygen solubility can be attributed to the location's more active agitation pattern. Regular agitation fosters homogeneity and water fluctuation (Wezel et al. 2013). Salinity levels are identical at all three stations (15%). Turbidity concentration is highest at station 3 (41.25 mg.L⁻¹), likely due to the mud substrate. Higher concentrations of silt in the water result in increased turbidity and solubility of organic matter (Erbanova et al. 2012).

The uniformity of water quality parameters can be attributed to the proximity of the sampling points within the same coastal water region. Coastal water regions generally show homogeneity due to seawater's mixing and flushing effects (Shaha et al. 2022). The dissolved oxygen level in the coastal area is relatively high (4.63–6.49 mg.L⁻¹) due to the intense flushing rate and relatively low-temperature distribution (Febiyanto 2020). The water quality conditions in Slamaran coastal waters are suitable for milkfish, which favor warm water areas with moderate salinity levels (Hanke et al. 2019).

The turbidity level at each research location station is also relatively stable, with minimal variation in extreme water parameters. The process of current movement and the presence of wind flow have an impact on the agitation process in the water column (Ariadi and Mujtahidah 2022). The stirring process in the water column allows homogenization of the water quality parameters at that location. The intense stirring process is also likely to make the waters more fertile (Wafi and Ariadi 2022). The fertility of these waters is due to a stirring process that occurs naturally and takes place continuously (Ariadi 2019). Aquatic biota

Table 2. Water quality parameters in research location sampling.

Location	Temperature (°C)	pH	Dissolved Oxygen (mg.L ⁻¹)	Salinity (‰)	Turbidity (mg.L ⁻¹)
Station 1	26-29	8.0	6.49	15	33.01
Station 2	27-29	7.8	5.82	15	31.21
Station 3	26-29	7.8	4.63	15	41.25

such as fish and shrimp tend to like water conditions that are homogeneous and rich in various plankton abundances.

Identification of plankton in milkfish seeds stomachs

Figure 2 shows the results of plankton identification in the stomachs of captured milkfish larvae. At station 1, *Peridinium* sp. is the dominant plankton species, accounting for 26.1% of the population. For station 2, *Navicula* sp. is dominant at 24.7%, while Station 3 is dominated by *Tintinnopsis* sp. at 25.3% (Figure 2). Variations in plankton dominance at each station relate to differences in substrate characteristics and water ecosystems. The presence of nutrients and the aquatic ecosystem's dynamics influence the plankton dominance level (Ariadi et al. 2019b).

Several types of plankton were identified at each station. Station 1 revealed a diverse range, including *Tintinnopsis* sp., (17.3%), *Peridinium* sp., (26.1%), *Coscinodiscus* sp., (40%), *Brachionus* sp., (9.3%), and *Chlorella* sp., (7.3%) (Figure 2). Plankton diversity significantly influences aquatic ecosystem dynamics (Ibarbalz et al. 2019).

Station 2 housed several plankton types, including *Tintinnopsis* sp. (10.2%), *Cryptomonas* sp. (4.6%), *Coscinodiscus* sp. (23.6%), *Chlorella* sp. (15.2%), *Skeletonema* sp. (21.7%), and *Navicula* sp. (24.7%). This station featured numerous diatom species, which are beneficial as natural fish food and tend to thrive in waters with balanced nutrients (Sprecher et al. 2023).

At station 3, *Brachionus* sp., (11.5%), *Rhizosolenia* sp., (8.6%), *Peridinium* sp., (9.9%), *Tintinnopsis* sp., (25.3%), *Cryptomonas* sp., (20.4%), and *Microcystis* sp., (24.3%) were found. A variety of plankton genera were present at this location. The ecological structure had a major impact on both the diversity and dominance of plankton in the waters (Ariadi et al. 2019b). *Coscinodiscus* sp., *Navicula* sp., and *Brachionus* sp. are microorganisms resistant to water quality dynamics in brackish water ecosystems (Figure 3).

The various types of plankton at sample locations are due to the water quality dynamics conditions (Duan et al. 2022). At several sampling points, the same plankton genus was found to be very dominant, meaning that the dominant characteristics and distribution of plankton in the research waters tend to be similar. These conditions are quite good

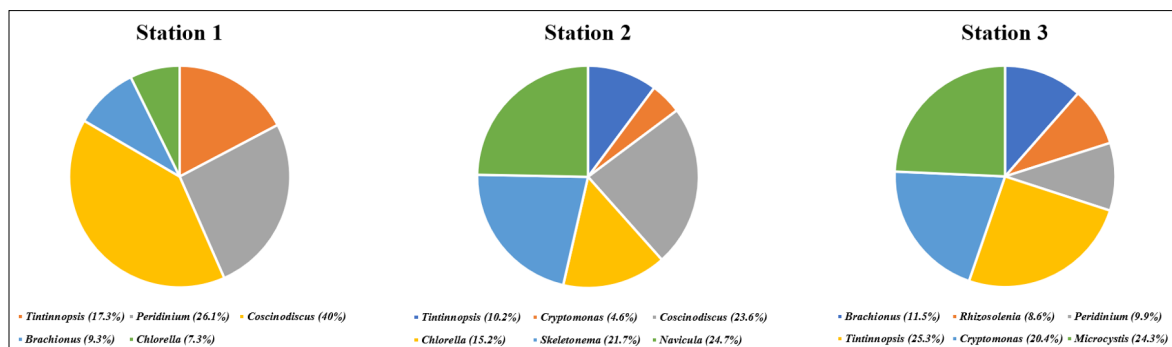


Figure 2. Plankton identified in the stomach of milkfish seeds.

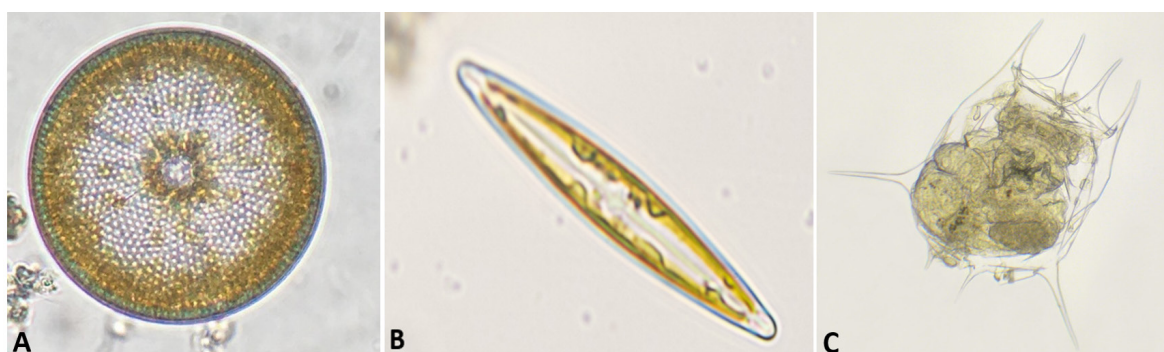


Figure 3. Plankton identification: A.) *Coscinodiscus* sp., B.) *Navicula* sp., C.) *Brachionus* sp.

because the characterization of natural food types for milkfish seeds is easy to do (Vasava et al. 2018). The process of characterizing natural food types should refer to the types of microorganisms consumed by fish while living in the wild (Rong et al. 2022).

Milkfish, being omnivorous, consume various aquatic microorganisms (Hsu et al. 2009). Plankton is widely consumed by milkfish in their natural habitat (Ariadi et al. 2022d). Plankton species such as *Nannochloropsis* sp. and *Brachionus* sp. are commonly found in milkfish larvae diets (Nasukha and dan Aslianti 2019). Milkfish also exhibit opportunistic behavior in the predation cycle, as observed from the variety of abundant plankton species found in their stomachs (Figure 2). This behavior can facilitate using milkfish as a superior commodity for brackish fishery cultivation (Vasava et al. 2018).

Several types of plankton found in the stomach of milkfish seed, such as *Coscinodiscus* sp., *Brachionus* sp., *Chlorella* sp., *Skeletonema* sp., and *Navicula* sp. are good types of plankton used as natural food for fish. Plankton of the diatom genus such as *Skeletonema* sp. and *Navicula* sp. is a genus suitable for use as natural feed for aquaculture commodities (Soeprapto et al. 2023). The existence of *Rhizosolenia* sp., *Tintinnopsis* sp., and several dinoflagellates is possible due to the turbid and nutrient-poor water conditions. Nutrients are a critical factor limiting plankton growth in natural waters (Yang and Zhao 2024).

Milkfish, which are fast-swimming fish, tend to have a much more active metabolic rate. Compensation for active metabolic activity leads to increased levels of predation (Wang et al. 2023). In addition to being caused by the condition of the body's activities, the intensity of active metabolism is also influenced by the conditions of the surrounding environment. An unstable environment will cause aquatic biota to become more easily stressed and susceptible to disease (Zhang et al. 2023). Stressors in waters are caused by cultivation conditions, poor environment, and the presence of disease (Gozdowska et al. 2022).

Plankton in the aquatic habitat of milkfish seeds

The water locations sampled for milkfish seeds collection commonly contain plankton genera such as *Peridinium* sp., *Coscinodiscus* sp., and *Brachionus* sp. (Figure 4 and Figure 5). Comparison with the plankton genera found in fish stomachs in Figure 2 reveals several matches, including *Peridinium* sp., *Coscinodiscus* sp., *Brachionus* sp., *Skeletonema* sp., *Tintinnopsis* sp., and *Microcystis* sp. These findings suggest that milkfish seeds consume about 50% of the plankton in the water through predation. This correlation between water environment plankton and fish gut content stems from the predation process, a part of the grazing cycle within the aquatic ecosystem's food chain (Nakajima et al. 2017).

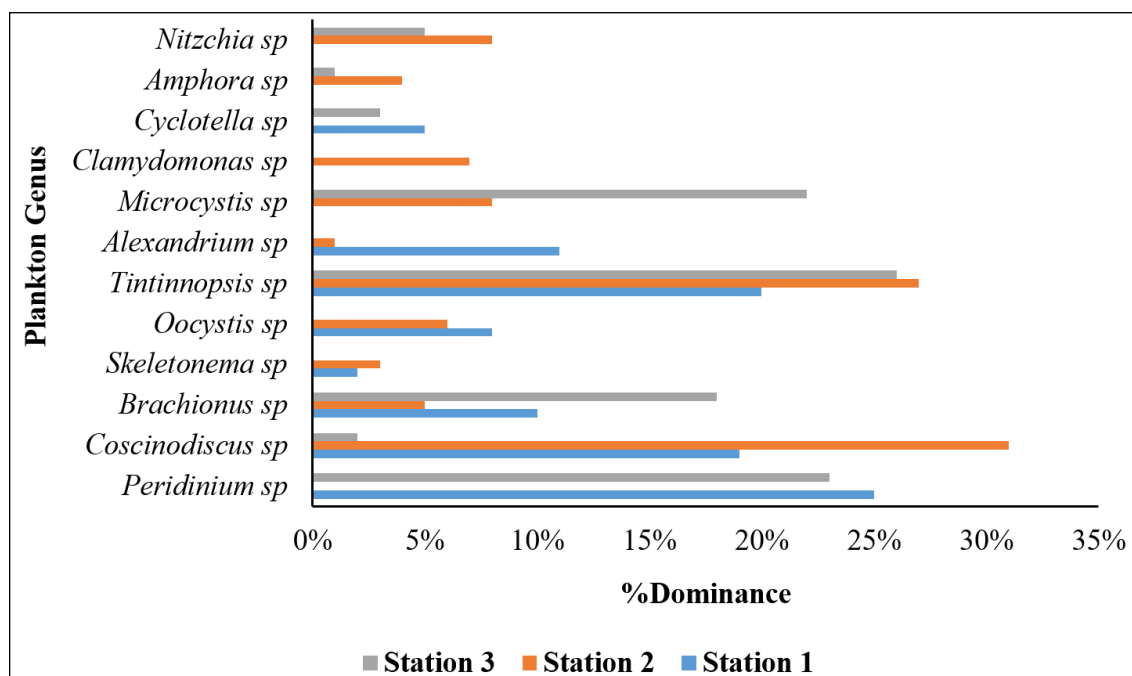


Figure 4. Plankton genus at the location where milkfish seeds were collected.

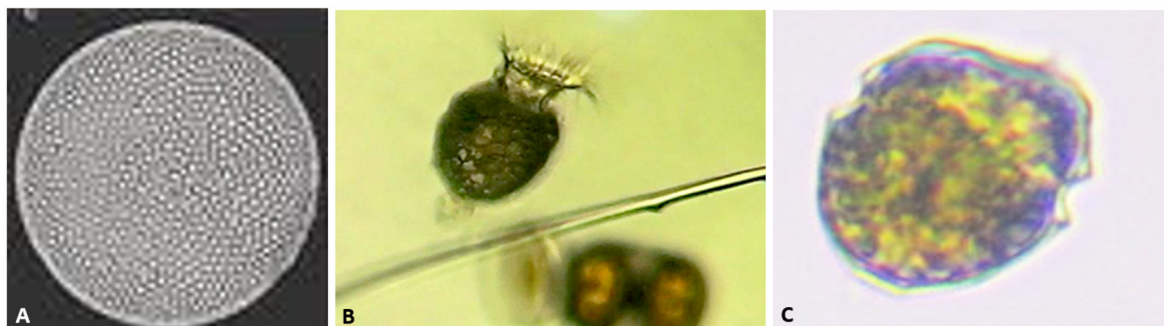


Figure 5. Plankton dominance collected: A.) *Coscinodiscus* sp., B.) *Brachionus* sp., C.) *Peridinium* sp.

In the coastal waters of the research area, plankton diversity is abundant, and their presence is moderate. The term 'moderate plankton status' refers to a condition where no particular plankton species is highly dominant in the water (Ariadi et al. 2022b). The dominance of plankton and the fertility of water are closely linked to the quality of water conditions (Ariadi et al. 2022c). The operational cycle of shrimp aquaculture, through its intensive treatments, can lead to an excessive abundance of nutrients. This abundance significantly influences the pond ecosystem's plankton profile (Ariadi et al. 2019a). As a biological indicator, plankton dominance warrants careful consideration when supporting aquaculture operations.

Certain zooplankton species, such as *Tintinnopsis* sp. and *Brachionus* sp., thrive in the pond ecosystem due to the high organic waste content. Zooplankton favor aquatic ecosystems with a high organic content (Winans et al. 2023). The balanced presence of zooplankton is essential for maintaining the sustainability of the plankton grazing cycle. Grazing optimally occurs when the predation rate remains normal (Pilati et al. 2018). This means grazing can function best when species at each trophic level are balanced in numbers (Chowdhury et al. 2018).

The existence of plankton genera such as *Amphora* sp., *Cyclotella* sp., *Coscinodiscus* sp., and *Skeletonema* sp. is considered good enough to fill the composition of plankton diversity in aquaculture ecosystems. The existence of diatomae genera such as *Amphora* sp., *Coscinodiscus* sp., and *Skeletonema* sp. are very good for use as natural food by fish in aquaculture ecosystems (Bera et al. 2019). The abundance of nutrient balanced in the waters is the key factor determining the level of diatom dominances (Scoe and Cavalier-Smith 2014).

Overall, the plankton dominance level in coastal waters is quite balanced and correlates with the water quality profile. This condition aligns with various case studies demonstrating a relationship

between physical, chemical, and biological parameters in cultivation ecosystems (Ariadi et al. 2022a). Several plankton genera, such as *Amphora* sp., *Cyclotella* sp., *Oocystis* sp., *Peridinium* sp., and *Cyclotella* sp. are found in many pond ecosystems (Ariadi and Mujtahidah 2022). The plankton profile in aquaculture activities in tropical waters tends to be stable throughout its aquaculture cycle (Ariadi et al. 2022d). The conditions in tropical waters feature relatively stable temperatures throughout the year (Wang et al. 2023).

Coastal waters are prone to fluctuating water quality dynamics due to their location at the intersection of freshwater and saltwater ecosystems (Ariadi 2023). These dynamic water conditions benefit aquaculture activities (Ibarbalz et al. 2019). Adapting to surrounding aquatic environment conditions, aquaculture has become a productive activity that has often developed in such areas—iconic coastal areas with aquatic resource utilization activities for aquaculture and fishing capture activities.

Data identifying plankton species greatly assists farmers in determining suitable natural feed types for fish in their habitat. Natural feeds widely used by cultivators, such as *Brachionus* sp., *Skeletonema* sp., and *Chaetocheros* sp., are essential for fish during the larval phase (Hussain et al. 2021). Milkfish also prey on various algae or wild microorganisms in the water. However, they prioritize natural food types derived from plankton due to continuous propagation and nursery opportunities. Milkfish also prefer water conditions that are stable and rich in various plankton (Sumagaysay-Chavoso and Diego-McGlone 2023).

In conclusion, the research findings showed that milkfish seeds caught from natural waters consumed various types of plankton, including green algae, blue-green algae, diatomite, dinoflagellates, and zooplankton species. The specific plankton genera found in the stomachs of these milkfish seeds, such as *Peridinium* sp., *Coscinodiscus* sp., *Brachionus* sp., *Skeletonema* sp., *Tintinnopsis* sp., and *Microcystis* sp.,

exhibited similarities to the plankton present in the surrounding waters where the fry was caught. This correspondence strongly suggests the omnivorous nature of milkfish, demonstrating their preference for feeding on multiple species of phytoplankton. For future research, field studies on the dominance of plankton in the stomach of milkfish fry and their environment can be further developed across different seasons. This can be done with more varied research variables, such as different sampling site conditions, plankton preferences in the stomachs of similar fish species, and spatio-temporal analyses related to the distribution of milkfish fry and plankton in the aquatic ecosystem of Pekalongan.

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AUTHOR CONTRIBUTIONS

Ariadi H: Conceptualization, Investigation, Data analysis, Supervision. **Mardiana TY:** Conceptualization, Writing – Original draft preparation, Data analysis. **Linayati L:** Writing – Reviewing and Editing, Writing – Original draft preparation. **Syakirin MB:** Investigation, Data analysis, Writing – Original draft preparation. **Madusari BD:** Writing – Reviewing and Editing, Supervision.

CONFLICTS OF INTEREST

We declare no conflict of interest in doing this work.

ETHICS STATEMENT

This study did not deal with live animals nor humans as subjects.

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