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RELATIONSHIP BETWEEN ABUNDANCE OF *Clamydomonas* spp AND *Chlorella* spp ON CLINICAL PERFORMANCE OF RED TILAPIA *Oreochromis niloticus* IN SILVOFISHERY PONDS

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ABSTRACT

Chlamydomonas spp. and *Chlorella* spp. are plankton genera that can be used to determine the level of balance in the aquatic environment. The purpose of this study is to determine the abundance of plankton *Chlamydomonas* spp. and *Chlorella* spp. and its relation to the clinical performance of red tilapia in silvofishery ponds. The research method used is an *ex post facto* causal design with random sampling. The results showed that the water quality in the silvofishery ponds was very good, except for the nitrate parameters of 0.00-1.50 mg/L and salinity of 2-11 gr/L, which exceeded the quality standards. In the silvofishery ponds, 5 plankton classes were found, namely Chlorophyceae, Cyanophyceae, Chrysophyceae, Protozoa, and Dinophysis, consisting of 15 genera. The dominant class Chlorophyceae had an abundance of 2.88E+06 cells/ml. The class Chlorophyceae is dominated by *Chlorella* spp. (2.63E+06 cells/ml) and *Chlamydomonas* spp. (2.20E+05 cells/ml). The abundance of *Chlamydomonas* spp. in silvofishery ponds is closely related to phosphate solubility (0.988). Based on observations of clinical symptoms on the eyes, gills, and fins of fish, no disease infections or physical abnormalities were found in the fish cultured in silvofishery ponds. Fish tend to live well with a growth rate of 0.44 g/day. The aquatic ecosystem of the silvofishery pond, which is dominated by the plankton *Chlorella* spp. and *Chlamydomonas* spp., had a good effect on the performance and condition of the farmed fish. This study concludes that the abundance of *Chlamydomonas* spp. and *Chlorella* spp. was very dominant compared to other plankton genera. The presence of *Chlamydomonas* spp. and *Chlorella* spp. illustrates good and stable environmental conditions in silvofishery ponds, followed by no clinical signs of disease infection in the fish reared during the study period.

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INTRODUCTION

Silvofishery is a multi-organism aquaculture model in mangrove forest ecosystems (Bao et al., 2013; Herrera et al., 2015). The advantages of silvofishery include better harvest productivity levels, low aquaculture waste, and minimal aquaculture inputs (Musa et al., 2020). The concept of silvofishery can be developed in various types of waters with various commodities (Lukman et al., 2021). The silvofishery concept is very suitable to be developed in tropical waters (Ariadi et al., 2019).

Chlamydomonas spp. and *Chlorella* spp. are types of plankton that are widely used for aquaculture activities (Che and Kim, 2023). *Chlamydomonas* spp. and *Chlorella* spp. are microalgae that can be used as bio-indicators of the aquatic environment (Zhou et al., 2022). *Chlamydomonas* spp. is a multicellular microalga that can live in extreme waters (Zhang et al., 2021). *Chlorella* spp. is a plankton that grows continuously in tropical waters (Hernandez et al., 2006; Alagawany et al., 2021).

The existence of *Chlamydomonas* spp. and *Chlorella* spp. is very important as bio-indicator of the aquatic environment (Khalil et al., 2021; Zhou et al., 2022). A good aquatic environment will affect the productivity level of aquaculture (Dong et al., 2022). Conversely, a bad aquatic environment will make fish susceptible to stress and die (Britton et al., 2023). One way to detect the quality level of a water site is to see the abundance of plankton such as *Chlamydomonas* spp. and *Chlorella* spp. (Soeprapto et al., 2023).

Red tilapia is a fish species that is widely used in aquaculture in tropical waters (Ekasari et al., 2023; Arias et al., 2023). Red tilapia is a fish species that responds to changes in aquatic environmental conditions (Nguyen et al., 2023). An indicator of the response of red tilapia to environmental conditions can be seen in the clinical symptoms in the physical body (Wang et al., 2022). The presence of clinical symptoms in the tilapia body indicates a physiological reaction as an adaptation to a changing environmental habitat (Wang et al., 2022; Lukman et al., 2023). Clinical symptoms on the body of red tilapia can be seen from the presence of spots, changes in body structure, color changes, and the presence of wounds (Arias et al., 2022). From this clinical response, the condition of the red tilapia habitat waters can be described (Ariadi and Abidin, 2019).

It is important to understand the existence of *Chlamydomonas* spp. and *Chlorella* spp. as environmental bio-indicators and the clinical symptoms of fish as a physiological response in pathology. Therefore, the purpose of this study is to determine the abundance of plankton *Chlamydomonas* spp. and *Chlorella* spp. and its relation to the clinical performance of red tilapia in silvofishery ponds. Based on the results of this study, we can determine whether there is a correlation between the presence of aquatic bio-indicator microorganisms and clinical symptoms on the fish reared in silvofishery ponds.

MATERIALS AND METHODS

This research was conducted in the silvofishery pond of Slamaran Village, Pekalongan City with the *ex post facto causal* design concept or direct data collection in the field without any treatment engineering. Sampling of fish, water quality, plankton, and fish clinical symptoms was carried out by random sampling. Parameters observed were clinical symptoms on the physical body of the fish, abundance of plankton, and water quality in silvofishery ponds.

The parameters of the clinical symptoms observed were changes in the physical structure of the fish, body deformities, injuries, and changes in the color of the fish. Fish samples were observed by taking fish populations floating on the surface of the water. The water quality parameters observed were pH, dissolved oxygen, salinity, temperature, phosphate, nitrite, nitrate, and ammonia. The plankton parameters observed were the plankton abundance index, which was calculated using the Olympus CX23 microscope and the NEUBEUER© haemocytometer which was then calculated using the formula by APHA, (1998) as follows:

$$N = Z * \frac{x}{y} * \frac{1}{v}$$

where:

- N : Plankton individually abundant (ind/ltr)
- Z : Plankton individual number
- x : Volume of water sample (40 mL)
- y : Volume of 1 water drop (0.06 mL)
- v : Volume of filtered water (100 L).

Furthermore, the data is grouped based on the parameters and research indicators to test the data analysis. Data analysis was carried out descriptively using SPSS 1.16 software.

RESULT AND DISCUSSION

Water quality parameters

The water quality parameters observed in this study are presented in Table 1. Based on a comparison of the measurement results with water quality standards in standard aquaculture, it was found that only nitrate parameters of 0.00-1.50 mg/L and salinity of 2-11 gr/L did not meet the quality standard threshold values for aquaculture activities. This means that the water conditions in silvofishery ponds tend to be low in salinity and eutrophic. The presence of high levels of organic matter and low salinity levels makes aquatic ecosystems tend to be eutrophic (Zhang et al, 2023).

Water quality in silvofishery ponds tends to be better and more stable than in conventional ponds. The existence of a mangrove ecosystem makes the waste absorption process more intense (Musa et al., 2020). The mangrove ecosystem functions as a biofilter and catcher of waste inorganic compounds (Cunha et al, 2019). The mangrove ecosystem in silvofishery cultivation also functions as a natural habitat for crabs and fish roaming places (Sarower et al, 2021).

Table 1. Water quality parameters in silvofishery ponds

Parameter	Measurement Results	Standard*
Phosphate	0.03 – 0.10	< 0.10
Ammonia	0.01 – 0.15	< 0.10
Nitrite	0.00 – 0.10	< 1.00
Nitrate	0.50 – 1.50	< 1.00
Temperature	26.25 – 31.40	24 – 32
Salinity	2 – 11	25 – 35
Dissolved Oxygen	4.50 – 20.00	> 4
pH	7.5 – 8.2	7.5 – 8.5

*Ariadi et al., (2021)

Plankton diversity

The abundance of plankton in silvofishery ponds can be seen in Table 2. Based on the research data, 5 different classes and 15 genera of plankton were found. The most diverse plankton are from the class Dynophyceae in which 5 genera were found (Table 2.). The diversity of Dynophysis is quite diverse because the sampling was carried out during the dry season (Ajani et al., 2022). The genus Dynophyceae found in silvofishery ponds are *Chryptomonas* spp., *Gymnodinium* spp., *Noctiluca* spp., *Peridinium* spp., *Prorocentrum* spp.

The plankton genera Chlorophyceae, Cyanophyceae, Chrysophyceae, and Protozoa were also found in silvofishery ponds. This means that the level of plankton diversity in silvofishery ponds is quite high. The existence of a balance of chemical, physical and biological parameters greatly supports the stability of plankton diversity in the waters (Geng et al., 2022). Plankton experiences seasonal succession which allows the dominance of certain classes (David et al., 2020; Ajani et al., 2022).

Plankton classes

Five classes of plankton were found in silvofishery ponds, namely Chlorophyceae, Cyanophyceae, Chrysophyceae, Dinophyceae, and Protozoa (Figure 1). The existence of plankton in aquatic ecosystems is determined by the abundance of nutrients (Soeprapto et al., 2023). The class Chlorophyceae indicates that silvofishery pond waters are eutrophic. The level of the trophic status of the waters is determined by geographical conditions, wind patterns, and seasons (Valentin et al., 2021). The more fertile the waters, the higher the succession of plankton at the genus and species level (Leles et al., 2021).

Chlorophyceae live in colonies and their life pattern is determined by the level of temperature distribution (Ariadi et al., 2019). The high abundance of Chlorophyceae also allows for more intense dissolved oxygen production (Wafi et al., 2021). These conditions also have an impact on diurnal pH fluctuations (Soeprapto et al., 20.23). Chlorophyceae will carry out photosynthesis during the day and carry out quite intense respiration at night (Yinding et al., 2021).

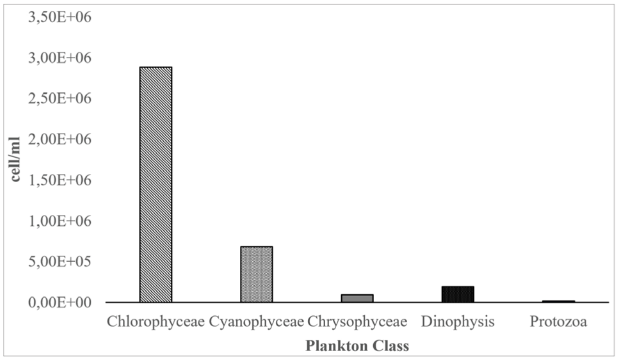

Fig 1. Plankton abundance in silvofishery ponds by class

Chloropyceae

Chlorophyceae in silvofishery ponds have the highest dominance compared to other classes. The plankton genera found in the class Chlorophyceae included *Dicthyospaerium* spp., *Chlamydomonas* spp. and *Chlorella* spp. (Fig. 2).

Table 2. Class and genus of plankton in silvofishery ponds

Classes	Genus
Chlorophyceae	*Dicthyospaerium* sp., *Chlamydomonas* sp., *Chlorella* sp.
Cyanophyceae	*Microcystis* sp., *Oschillatoria* sp.
Chrysophyceae	*Amphora* sp., *Diploneis* sp.
Dinophyceae	*Chryptomonas* sp., *Gymnodinium* sp. *Noctiluca* sp., *Peridinium* sp., *Prorocentrum* sp.
Protozoa	*Acantocystis* sp., *Ciliata* sp., *Euplotes* sp.

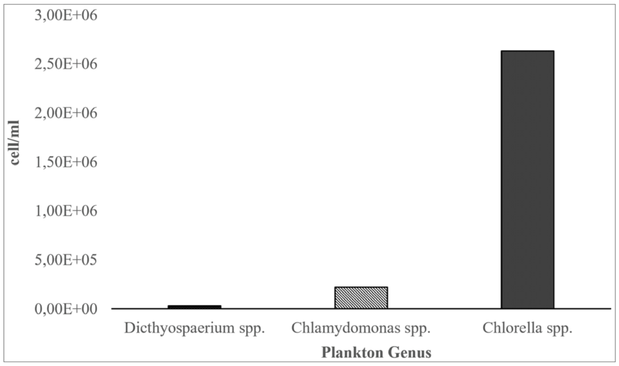

Fig 2. The abundance of the plankton genus Chlorophyceae in silvofishery ponds

Most plankton genera of the class Chlorophyceae have similar morphological and ecological characteristics (Fucikova and Lewis, 2016; Teemraleva and Bukin, 2022). The abundance of Chlorophyceae in waters is influenced by temperature, chlorophyll-a, and light (Rocha et al., 2021).

Chlorella spp. is dominant compared to *Dicthyospaerium* spp. and *Chlamydomonas* spp. *Chlorella* spp. is a cosmopolitan photosynthetic plankton genus (Gong et al., 2021). The life cycle of cosmopolitan plankton is largely determined by temperature distribution and the presence of nutrients as limiting factors (Ariadi et al., 2019; Ariadi et al., 2022). In aquaculture ponds, *Chlorella* spp. can be used as a natural fish food (Saldana et al, 2022).

The high abundance of *Chlorella* spp. (2.63E+06 cell/ml) and *Chlamydomonas* spp. (2.20E+05 cell/ml) can be used as an environmental bio-indicator for aquaculture waters (Che and Kim, 2023). This means that the aquatic ecosystem in silvofishery ponds is very fertile. The high level of primary productivity is caused by the accumulation of aquaculture waste, organic matter, and the presence of trace mineral elements (Ray et al., 2023). Intensive fish farming tends to have a eutrophic impact on the aquatic environment of ponds (Han and Chui, 2016).

Chlorella spp. and Chlamydomonas spp.

Chlorella spp. and *Chlamydomonas* spp. are a genus of Chlorophyceae that often grows in aquaculture waters (Ariadi et al., 2021). The abundance of *Chlorella* spp. and *Chlamydomonas* spp. in silvofishery ponds is presented in Figure 3. The abundance of *Chlorella* spp. and *Chlamydomonas* spp. ranged from 2.80E+05 - 8.70E+05 cell/ml and 2.00E+04 – 9.00E+04 cells/ml, respectively. Increasing the biomass of *Chlorella* spp. and *Chlamydomonas* spp. was caused by a decrease in water salinity levels and an increase in extracellular polymeric substance (EPS) compounds (Vo et al., 2020).

The presence of *Chlorella* spp. and *Chlamydomonas* spp. indicates a low level of solubility of organic matter in aquatic ecosystems (Bilal et al., 2019). *Chlorella* spp. live well in heterotrophic waters (Wang et al., 2016). *Chlamydomonas* spp. is a type of algae that can effectively absorb NH4 in aquatic ecosystems (Zhou et al., 2022). This means that the presence of the two plankton genera indicates a stable silvofishery aquatic ecosystem with moderate nutrient abundance.

The level of abundance ratio between *Chlorella* spp. and *Chlamydomonas* spp. is 12:1. *Chlorella* spp. exists in silvofishery aquatic ecosystems. Differences in life cycles and the effectiveness of nutrient utilization are differentiating factors for plankton growth in the waters (Cao et al., 2021; Zhou et al., 2022). *Chlamydomonas* spp. is a type of plankton that can grow in phototrophic, heterotrophic, and mixotrophic waters (Moon et al., 2013). These conditions allow *Chlamydomonas* spp. to grow in various water conditions. Likewise, *Chlorella* spp. is solitary and its growth is influenced by temperature distribution (Ariadi et al., 2019; Serra-Maia et al., 2016).

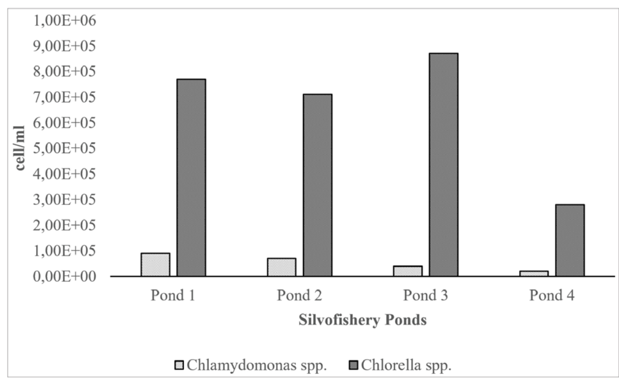

Fig 3. The abundance of *Chlamydomonas* spp. and *Chlorella* spp. in silvofishery ponds

Correlation between the abundance of *Chlorella* spp. and *Chlamydomonas* spp. and water nutrient parameters (NH_3, PO_4, NO_2, NO_3) showed only the phosphate parameter (0.988) correlated with the abundance of *Chlamydomonas* spp. (Table 2). For other parameters, there was no strong correlation. The increase in element *P* causes the metabolic system of *Chlamydomonas* spp. to carry out assimilation more intensely (Plouviez et al., 2023). The excessive abundance of phosphate damages *Chlorella* spp. cells, but in *Chlamydomonas* spp. phosphate becomes a growth-limiting factor (Shilton et al., 2012; Li et al., 2018). The presence of phosphate as a limiting element is very important for aquatic ecosystems (Ray et al., 2023). Phosphate dissolves easily and precipitates if the lime content in the water is relatively high.

Overall, with good water quality parameters in silvofishery ponds, it was found that the highest level of plankton dominance came from the class Chlorophyceae. *Chlorella* spp. and *Chlamydomonas* spp. is the dominant genus in the class Chlorophyceae in silvofishery ponds. Chlorophyceae are cosmopolitan in nutrient-rich environments (Ariadi et al., 2023).

Table 3. Correlation between the abundance of *Chlorella* spp. and *Chlamydomonas* spp. and water nutrient parameters

		***Chlamydomonas* spp.**	***Chlorella* spp.**	**NH_3**	**PO_4**	**NO_2**	**NO_3**
Chlamydomonas spp.	Pearson Correlation	1	.717	.900	.988*	.256	.473
	Sig.(2-tailed)		.283	.100	.012	.744	.527
	N	4	4	4	4	4	4
Chlorella spp.	Pearson Correlation	.717	1	.944	.646	.205	.802
	Sig.(2-tailed)	.283		.056	.354	.795	.198
	N	4	4	4	4	4	4

The high load of feed waste and the accumulation of nutrients triggers the growth of *Chlorella* spp. biomass. and *Chlamydomonas* spp. in aquatic ecosystems (Xie et al., 2019; Ullmann and Grimm, 2021). This is consistent with the results of the correlation test showing a high closeness relationship between the abundance of *Chlamydomonas* spp. and the solubility of phosphate in waters. Another factor that makes *Chlorella* spp. and *Chlamydomonas* spp. dominant is the temperature level and the value of the salinity of the waters (Ma et al., 2020).

Clinical symptoms and fish growth

Clinical symptoms of red tilapia reared in silvofishery ponds is shown in Figure 4. Physically the fish do not experience any symptoms or structural changes in their physical organs. The color of the gills is bright red (4a), the color of the eyes is still bright (4b), and the dorsal fin is intact and has not undergone any structural changes (4c). This means that fish kept in silvofishery ponds are healthy and do not experience clinical symptoms of changes in the structure of their organs. Clinical symptoms in fish are usually caused by infection with pathogenic bacteria and abnormal changes in behavior (Okon et al., 2023; Amminger et al., 2023). Plankton and pathogenic bacteria have complementary properties in the host infection process (Costello et al., 2023).

There are no bad clinical symptoms in fish due to good environmental habitat conditions (Mramba and Kahindi, 2023). Good environmental conditions can be seen from the water quality and the abundance of growing plankton. Plankton and water quality are natural indicators that can stabilize aquaculture ecosystems (Meng et al., 2022). The abundance of *Chlorella* spp. and *Chlamydomonas* spp., a genus of beneficial plankton, affects the performance of farmed fish. *Chlorella* spp. and *Chlamydomonas* spp. are good bioindicators of the aquatic environment, so there is a correlation that good environmental conditions have an impact on the physical performance of fresher fish (Ariadi et al., 2020).

No clinical symptoms were found on the body of the fish, indicating that the silvofishery concept is very feasible. The indicators are the lack of symptoms of physical defects on the body of the farmed fish and the relatively good condition of the water quality in silvofishery ponds (Musa et al., 2020). In addition, the growth of profitable plankton genera greatly supports the productivity of the operational cycle of silvofishery. *Chlorella* spp. and *Chlamydomonas* spp. are types of plankton widely used as natural feed and aquatic bioindicators (Irihimovitch et al., 2008).

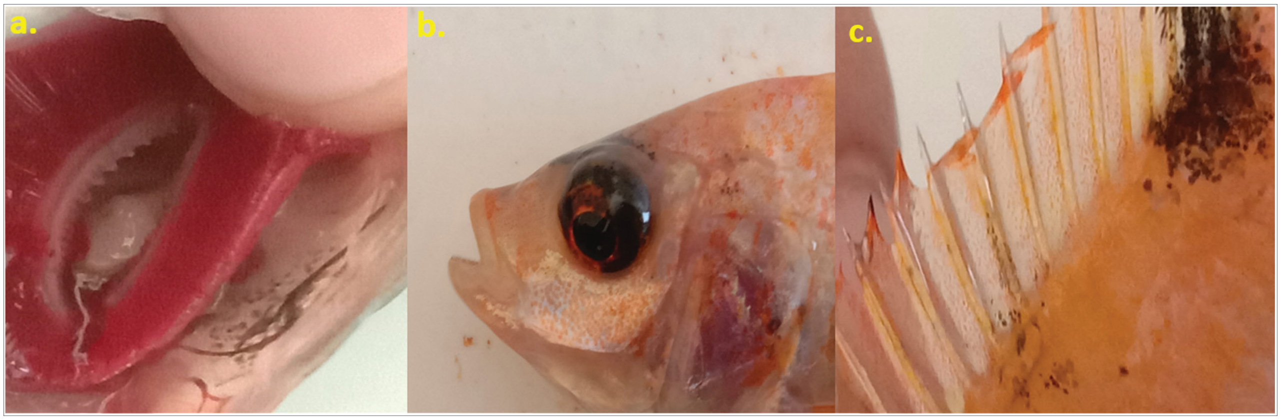

Fig 4. Clinical symptoms on the fish body a) gills, b) eyes, c) dorsal fin

Data from observations of fish growth during the first 40 days of the cultivation period can be seen in Figure 5. Fish growth rates ranged from 3.21-20.76 g and continued to increase (Figure 5.). The growth of the biomass of aquaculture organisms continues to increase as the cultivation period increases (Ariadi et al., 2019). Fish growth factors are influenced by the effectiveness of feeding, feed quality, and supportive environmental habitat conditions (Pouil et al., 2023).

The average growth rate of fish is 0.44 gr/day. This figure is quite high for the development of silvofishery. The average growth rate of fish is a visualization of the effectiveness of feed management during the period (Lee et al., 2023). Effective feeding and good feed quality play an important role in fish growth performance (Madusari et al., 2022). Thus, it can be said that a silvofishery system with a good farm-scale model will have a progressive increase in fish biomass and minimal impact on environmental pollution. The minimal impact of waste on the environment will provide a higher carrying capacity rate (Ariadi et al., 2023).

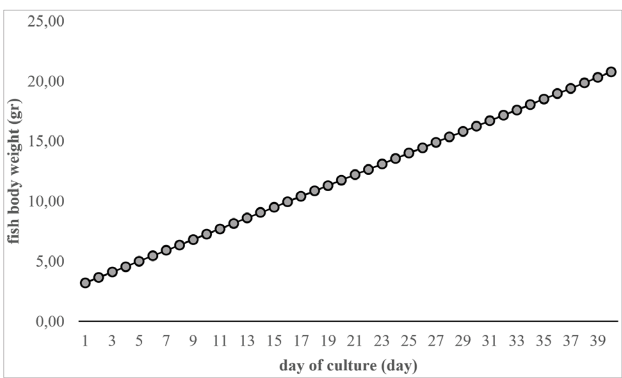

Fig 5. Growth rate of fish in silvofishery ponds during the study

The results of the research study as a whole show that the conditions of water quality and the presence of plankton in silvofishery ponds are ecologically quite good. In addition, the presence of plankton *Chlorella* spp. and *Chlamydomonas* spp. is considered good enough to support aquaculture activities. The concept of silvofishery with minimal waste pollution affects the stability of the ecosystem in pond waters (Wijaya et al., 2019). These good ecological conditions affect the performance of the farmed fish (Ariadi, 2023). Aquaculture in silvofishery ponds is very healthy and there are no clinical symptoms due to disease infections. The more stable ecosystem conditions in silvofishery ponds provide a reduction in the prevalence rate of disease spread (De-Leon-Herrera et al., 2015). In addition, the growth rate of aquaculture in silvofishery ponds tends to increase its biomass progressively every day. This means that the fish cultivated in silvofishery ponds are very good and healthy so they are worth developing further (Mirera, 2011). The healthy condition of the fish can be seen from the lack of clinical symptoms due to diseases acquired during the cultivation period.

CONCLUSION

The abundance of *Chlamydomonas* spp. and *Chlorella* spp. was found to be very dominant compared to other plankton genera. The presence of *Chlamydomonas* spp. and *Chlorella* spp. illustrates good and stable environmental conditions in silvofishery ponds followed by no clinical signs of disease infection in the fish reared during the study period.

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ODNOS IZMEĐU ZASTUPLJENOSTI *Clamydomonas* spp. I *Chlorella* spp. NA KLINIČKU UČINKOVITOST NILSKE TILAPIJE *Oreochromis niloticus* U SILVORIBNJACIMA

SAŽETAK

Chlamydomonas spp. i *Chlorella* spp. su planktonski rodovi koji se mogu koristiti za određivanje razine ravnoteže u vodenom okolišu. Svrha ovog istraživanja je utvrditi zastupljenost planktona *Chlamydomonas* spp. i *Chlorella* spp. i njegov odnos s kliničkim djelovanjem nilske tilapije u silvoribnjacima. Korištena metoda istraživanja je *ex post facto* kauzalni dizajn sa slučajnim uzorkovanjem. Rezultati su pokazali da je kvaliteta vode u silvoribnjacima bila vrlo dobra, osim parametara nitrata od 0,00-1,50 mg/L i saliniteta od 2-11 gr/L, koji su bili ispod standarda kvalitete. U silvoribnjacima pronađeno je 5 razreda planktona, i to Chlorophyceae, Cyanophyceae, Chrysophyceae, Protozoa i Dinophysis, koji se sastoje od 15 rodova. Dominantni razred Chlorophyceae imalo je brojnost od 2,88E+06 stanica/ml. U razredu Chlorophyceae dominiraju *Chlorella* spp. (2,63E+06 stanica/ml) i *Chlamydomonas* spp. (2,20E+05 stanica/ml). Obilje *Chlamydomonas* spp. u silvoribnjacima usko je povezan s topljivošću fosfata (0,988). Na temelju opažanja kliničkih simptoma na očima, škrgama i perajama riba, nisu pronađene nikakve infekcije ili fizičke abnormalnosti u ribama uzgojenim u silvoribnjacima. Ribe imaju tendenciju da žive dobro sa stopom rasta od 0,44 g/dan. Vodeni ekosustav silvoribnjaka u kojem dominira plankton *Chlorella* spp. i *Chlamydomonas* spp, imao je dobar učinak stanje uzgojene ribe. Zaključujemo da je zastupljenost *Chlamydomonas* spp. i *Chlorella* spp. bio vrlo dominantan u usporedbi s drugim planktonskim rodovima. Prisutnost *Chlamydomonas* spp. i *Chlorella* spp. ilustrira dobre i stabilne uvjete okoliša u silvoribnjacima, nakon kojih nema kliničkih znakova infekcije bolesti kod riba u uzgoju.

Ključne riječi: Chlorophyceae, silvoribolov, plankton, simptom.

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