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Temporal and spatial distribution of plankton community in three Indonesian salt pond environments

RINI SUSILOWATI[1,♥], DIETRIECH G. BENGEN[2], MAJARIANA KRISANTI[3], HEDI INDRA JANUAR[4], IMAN RUSMANA[5]

[1]Graduate School of Marine Sciences, Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor. Jl. Rasamala, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-622908, Fax.: +62-251-622907, ♥email: rini.susilowati@brin.go.id

[2]Department of Marine Science and Technology, Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor. Jl. Rasamala, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia

[3]Program of Conservation Biology, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor. Jl. Rasamala, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia

[4]Ecology and Ethnobiology Research Center, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor 16911, West Java, Indonesia

[5]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia

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Abstract. *Susilowati R, Bengen DG, Krisanti M, Januar HI, Rusmana I. 2023. Temporal and spatial distribution of plankton community in three Indonesian salt pond environments. Biodiversitas 24: 1833-1844.* Plankton is a biological community that plays an important role in biological systems during salt crystallization. The correlation between plankton communities and water quality parameters was examined in three salt pond center areas (Brebes, Tuban, and Sampang). The values of nutrient content in Brebes were higher compared to other locations, with phosphate (5.38-5.66 mg/L), nitrate (0.17-0.74 mg/L), nitrite (0.07-0.1 mg/L), and total organic matter (0.03-0.07 mg/L). Based on multivariate analysis, there was no significant difference in the abundance of plankton among the three salt pond locations. Furthermore, Spearman's correlation test indicated a negative correlation (Sig<0.05) between salinity and the abundance and diversity of plankton. The highest abundance of phytoplankton (127,721±11.227 cells/L) and zooplankton (3,339±391 idvs/L) was found in the Brebes. Phytoplankton from the genera Bacillarophyceae Bacillarophyceae was dominant in the three locations. In Brebes and Sampang, the dominant classes of zooplankton were Litostomatea, whereas, Oligotrichea was dominant in Tuban, The phytoplankton community structure has a diversity index (H') of 0.91-2.05, and Evenness index (E) ranging from 0.12-0.20, and a dominance index (C) showing a level of 0.14-0.34. At the same time, the zooplankton community has a diversity index (H') of 0.81-1.64, an evenness index (E) that has ranged from 0.18-0.22, and a dominance index (C) that has ranged from 0.03-0.20. The research examines plankton communities in varying salinity and their response to environmental changes. This data is useful for managing salt pond productivity, vital for sustainable and high-quality salt production.

Keywords: Phytoplankton, salt ponds, water quality, zooplankton

INTRODUCTION

Traditional salt ponds are semi-enclosed coastal ecosystems that receive seawater and gradually evaporate, resulting in a salinity gradient from reservoir areas (low salinity) to crystallization ponds (high salinity). Apart from being a source of salt production, salt ponds are also one of the integrated coastal environments with a diverse range of microorganisms and constant salinity (Martínez et al. 2022). According to Rodrigues et al. (2011), the salt pond ecosystem has two ecological cycles, including periods of salt production and non-production. Generally, the salt pond ecosystem has a biological community similar to coastal ecosystems during non-salt production periods. However, the ecosystem develops distinct physicochemical and biological characteristics once salt production begins due to the salinity gradient (Wasserman et al. 2022). Salt pond habitats are typically heterogeneous based on salinity, which strongly influences organism community dynamics, chemical compositions, and genetic structures (Soares et al. 2018).

Seawater exchanges, hydrology, salinity, and nutrients influence the development of biological communities in salt pond environments (Soares et al. 2018). Furthermore, according to Rodrigues et al. (2011), the biological community living in salt water is an important ecosystem for salt production. They are valuable in salt production using biological systems due to their functions to increase evaporation, reduce organic matter, and improve salt quality (Asencio 2013). According to Davis (2000), phytoplankton is one of the biological communities in salt ponds that contributes to salt crystallization. Phytoplanktonic organisms influence the salt quality and play an ecological role in salt pond environments (Costa et al. 2015). Davis (2009) stated that the characteristics of biological ecosystems are responsible for the quality of salt products. Several studies on plankton diversity and abundance in salt lakes and salt ponds have been conducted around the world in recent decades, including phytoplankton community structure in Kenyan salt ponds

(Hinzano et al. 2022), Brazil (Soares et al. 2018), Spain (Asencio 2013), India (Stephen et al. 2013), Tunisia (Ghannay et al. 2015), and Greece (Hotos 2021). Nonetheless, the available information on the works mentioned above in South East Asian salt is restricted. Only a handful of researchers have investigated plankton communities within salt ponds. For instance, previous studies have examined such communities in Indonesian salt ponds (Fitriyah et al. 2016; Sukmayati et al. 2013) and Thailand (Chatcawan et al. 2011).

The problem of salt production in Indonesia is influenced by production using traditional systems and increasing the quantity and quality of Indonesian salt (Ramly et al. 2022). The Indonesian government has promoted and developed physical technologies such as geomembrane and filter thread techniques as salt-making technology to improve salt production, quality, and quantity (Supriyo et al. 2022; Chasanah et al. 2022). Meanwhile, geomembrane technology is costly for salt farmers; thus, not all farmers use this technology (Yaqin and Setiani 2017). In addition to these methods, some biological methods are less commonly known and were not developed by Indonesian salt farmers. This system involves biological communities in salt production, especially in traditional salt ponds. However, biological systems can potentially improve the quality and production of traditional salt. This system is inexpensive, environmentally friendly, and capable of producing high-quality salt continuously (Chasanah et al. 2020)

Brebes, Tuban, and Sampang Regencies are three areas of traditional salt production centers in Indonesia. These districts are on the Northern Coast route, including Java Island (Tuban, Brebes) and Madura Island (Sampang). Madura Island is located in northeastern Java Island and is well-known as a leading salt-producing area (Nurif and Hermanto 2019; Gani and Gitayuda 2020). This study examines the environmental characteristics of salt ponds in the three salt center areas and the correlation between environmental characteristics and the biological community (phytoplankton and zooplankton). These environmental characteristics and biological communities can be the initial study for developing biological systems to improve the quality of traditional salt ponds in Indonesia.

MATERIALS AND METHODS

Study site

Traditional salt ponds were studied in three districts: (i) Brebes (BSR, BSS, BST) of Central Java, (ii) Tuban (TSR, TSS, TST), and (iii) Sampang (SSR, SSS, SST) of East Java, Indonesia. The pond is divided into three sections, each with 50-60 cm water depths and low salinities of less than 100 psu (BSS, TSR, and SSR). The medium salinity was between 100-200 psu (BSS, TSS, SSS), and the water depth was 15-20 cm. Finally, due to the highest salinity of > 200 psu, the water depth ranged from 5-10 cm (BST, TST, and SST). Furthermore, traditional salt ponds have an area of 1-1.5 ha, and each pond has an area of 100-150 m^2. The salt ponds of Sampang District are located at coordinates 113º08'21.67" E -7º12'33.76" S; Brebes District was found at 109º01'07.0" E -6º48'06.0" S; while Tuban District was found at 112º09'44.8" E -6º54'24.3" S. In addition, the selection of sampling points is based on the availability of reservoir ponds (low salinity), evaporation ponds (medium salinity), and salt crystallization ponds (high salinity). As most salt farms are traditional and their production process is not uniform among salt farmers, sampling points are selected from those with all three types of ponds or who have at least completed one cycle of salt harvest. The sample was taken in August-September 2021. The selection of sampling time is based on the monthly prediction of low rainfall from Meteorological, Climatological, and Geophysical Agency from (BMKG) data and adjusted according to field conditions (harvesting time, daily rainfall conditions). Figure 1 illustrates the research locations.

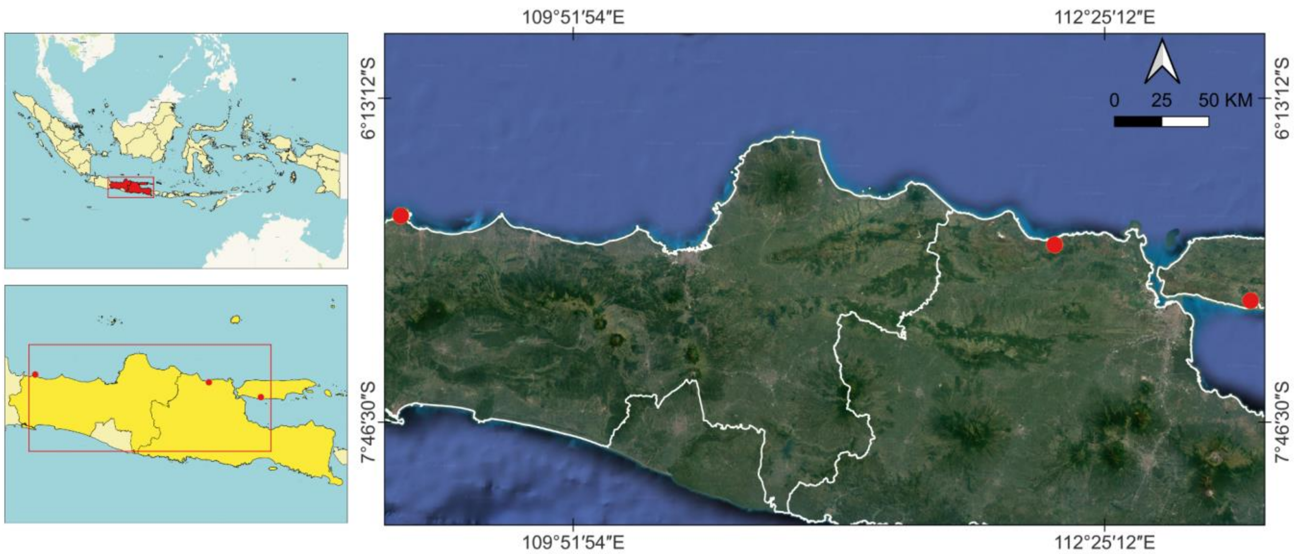


Figure 1. Research location in Brebes (Central Java), Tuban and Sampang (East Java), Indonesia

Sampling protocols

A 25-mesh plankton net was used to collect plankton in three replications in randomly selected ponds. A total of ten liters of water are filtered using a phytoplankton net. Furthermore, 100 mL of filtered water was mixed with 1 mL of 5% Lugol's solution for phytoplankton storage. Moreover, adding 1 mL of 4% formalin was used for storing zooplankton (Ghannay et al. 2015; Yunandar et al. 2020).

Sampling was conducted once during the salt harvest season, with the analysis of parameters and collection of samples between 8:00 am and 12:00 pm each day. Three replicated measurements were taken at each sampling point. Temperature, dissolved oxygen (DO), and pH were measured in situ using a Multi-parameter IP67/C/8603 instrument. The water salinity was measured using a Be meter and a salinometer; water salinity measurements were also calibrated. Therefore, the water samples with salinity greater than 100 psu must be diluted with distilled water to fall within the refractometer or salinometer scale (Alió et al. 2000). Also, the nitrogen, nitrate, phosphate, and ammonia levels were measured using HACH reagents, and procedures with the HACH DR890 colorimeter. Total organics were analyzed using a titration method based on the SNI 06-.6989.22-2004 standard method (BSN 2016)

Sampling analysis

Plankton identification was carried out in the Macro Biology laboratory of the Department of Fisheries Resource Management, Faculty of Fisheries and Marine Sciences, IPB University, using a microscope and a Sedgwick rafter. The identification of plankton is based on references from the books of Davis (1995), Bold and Wyne (1985), and Edmonson (1959). Plankton abundance, the diversity index (H'), the evenness index (E), and the dominance index (C) use the following formula:

The abundance index based on the formula (APHA 2005) is as follows:

$$N=\frac{1}{Vd}\,x\,\frac{Vt}{Vs}\,x\;n$$

Where: N: plankton abundace (cells/m^3 or ind/m^3); Vd: volume of filtered water sample (m^3); Vt: filtered sample volume (ml); Vs: sample volume in Sedgwick Rafter Counting Cell (ml); n: the number of observed plankton

The diversity index (H') is calculated based on the Shannon-Wiener index and the evenness index (Odum and Barrett 2005) using the following formula:

$$H' = -\sum_{i=1}^{s}(Pi)(\ln Pi)$$

Where: H': diversity index, Pi = ni: the number of individuals in a species, N: the number of species. The criteria for the diversity index are as follows: $H' \leq 1$ = Low diversity; $1 < H' \leq 3$ = moderate diversity; $H' > 3$ = high diversity

The following formula calculates the uniformity index:

$$E=\frac{H'}{H\,max}$$

Where: E: Evenness index, H': diversity index, Hmax: ln S, S: the number of species found. The evenness index values range from 0-1. Furthermore, the evenness index, according to Krebs (1989), is categorized as follows: $0 < E \leq 0.5$ = depressed community, $0.5 < E \leq 0.75$ = unstable community, $0.75 < E \leq 1$ = stable community

The following formula calculates the dominance index:

$$C = \sum\left(\frac{ni}{N}\right)^2$$

Where: C: dominance index, ni: the number of individuals of each species, and n: the total number of individuals. Dominance index values are categorized as follows: $0 < C < 0.5$ = low dominance. $0.5 < C \leq 0.75$ = moderate dominance. $0.75 < C \leq 1.0$ = high dominance (Krebs 1989).

Data analysis

The relationship between variables was evaluated using Spearman's correlation coefficients. First, to analyze the physicochemical water quality parameters between study sites, a one-way ANOVA with SPSS 2016 was used (Cronk 2018). Next, the characteristics of both water and plankton at the study site were analyzed using Multivariate Linear Discriminant Analysis (LDA). Finally, the correlation between multivariable water characteristics and plankton abundance was established using Correlation Canonical Analysis (CCA). All statistical tests were performed with PAST Statistical Software V4.03 (Hammer 2022).

RESULTS AND DISCUSSION

Environmental characteristic

Based on the water quality parameters in Table 1, it can be observed that the three salt ponds in three locations (Brebes, Tuban, and Sampang) have various environmental conditions. Nutrient concentrations (phosphate, nitrate, and nitrite) and total organic matter in Brebes ponds had higher values than in the two other locations. Values of nutrient content were 5.38-5.66 mg/L (phosphate), 0.17-0.74 mg/L (nitrate), 0.07-0.1 mg/L (nitrite), and 0.03-0.07 mg/L (total organic matter). Water temperatures at the three pond locations ranged from 30-36ºC, with the highest temperatures in high-salinity ponds (BST, TST, and SST). Generally, low phosphate concentrations were observed in high-salinity ponds (BST, TST, and SST). This is due to changes in seawater mass caused by evaporation, which could change seawater composition. The pH value decreased, accompanied by a decrease in nutrient concentrations (nitrate, ammonia, and phosphate), which characterizes the medium salinity ponds (BSS, TSS, and SSS). In addition, pH values ranged from 7.09±0.92 to 8.42±0.01. The salinity of the water ranged from 61.22±7.18 to 243.00±17.35 psu, with the highest salinity in the Sampang salt ponds.

Distribution of plankton communities

Table 2 shows the abundance of phytoplankton at low salinity ponds (BSS, TSR, SSR) ranged from 89,573±5,694 to 127,721±11,227cells/L and at high salinities from 1,450±134 to 2,306±333 cells/L (BST, TST, SST). However, *Trichodesmium* sp. was the dominant phytoplankton at low salinity in three salt ponds (BSR, TSR, SSR). Meanwhile, *Pleurisigma* sp. (11,092±661 cells/L), *Nitzschia* sp. (544±164 cells/L), and *Trichodesmium* sp. (551±181 cells/L) were dominant phytoplankton that survived in high salinity. There are 13 genera of phytoplankton found in three salt pond locations, including: *Amphora* sp., *Cocconeis* sp., *Coscinodiscus* sp., *Melosira* sp., *Navicula* sp., *Nitzschia* sp., *Pleurosigma* sp., *Rhizosolenia* sp., *Surirella* sp., *Biddulphia* sp., *Trichodesmium* sp., *Ceratium* sp., and *Peridinium* sp.

Figure 2 illustrates that the relative abundance of the phylum Bacillariophyceae group dominates at three salt pond locations, with approximately 64.73% in Brebes, 53.42% in Tuban, and 69.38% in Sampang. Furthermore, zooplankton abundance ranged from low to medium salinity, ranged from 2,017±246 to 3,339±391 idvs/L (BSS, TSR, SSR), and 755±149 to 1,111±250 idvs/L (BSS, TSS, SSS). *Didinium* sp. dominated Brebes salt ponds at low-mid salinities of 1,076±367 idv/L (BSR) and 457±203 idv/L (BSS). Sampang salt ponds also dominated at low-mid salinities of 942±374.77 idv/L (SSR) and 363±66 idv/L (SSS). Meanwhile, *Tintinnopsis* sp. dominated Tuban salt ponds at low-mid salinities of 841±218 (TSS) and 508±109 idv/L (TSS).

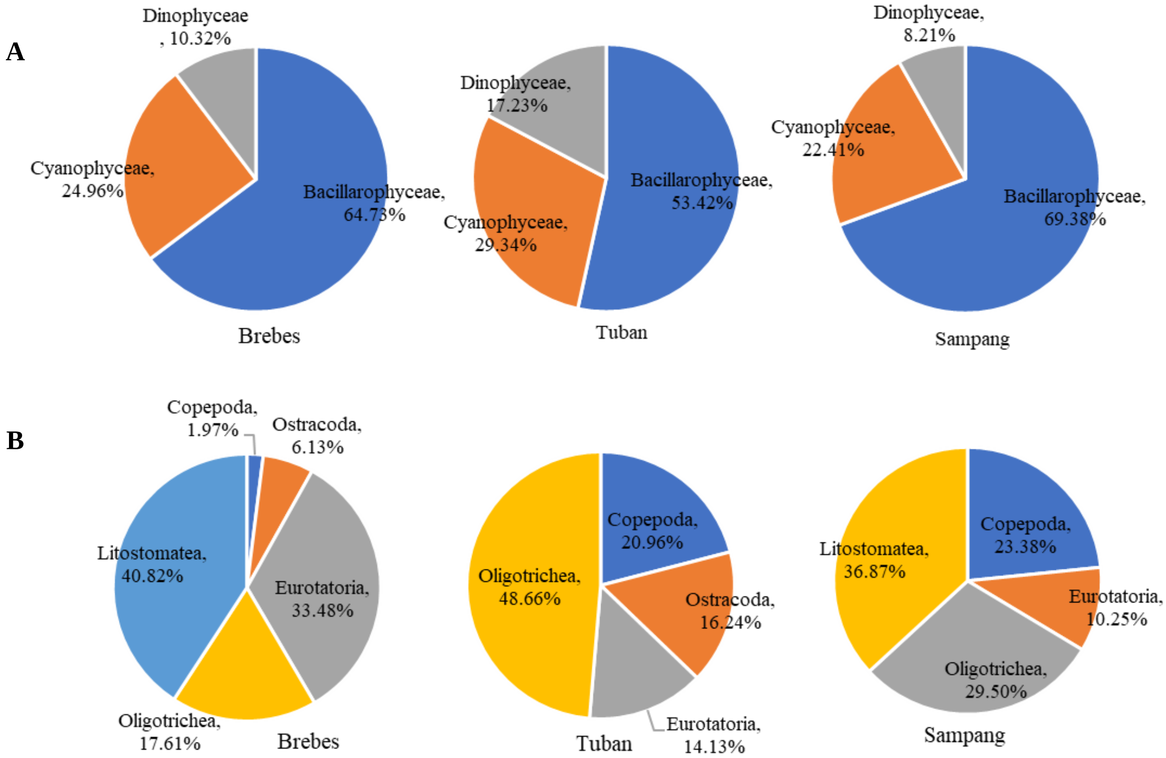


Figure 2. The relative abundance percentage of different phyla of phytoplankton (A) and zooplankton (B) were measured at three salt pond locations (Brebes, Tuban, and Sampang)

Table 1. Salt-pond water quality parameters at three salt ponds locations

Location	Sample	Temp. (°C)	DO	Salinity (psu)	pH	PO_4^{3-} (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	NH_4^+ (mg/L)	TOM
Brebes	BSR	30.67±0.38	11.82±0.88	61.22±7.18	8.18±0.26	5.62±0.08	0.17±0.09	0.08±0.02	0.08±0.02	0.03±0.00
	BSS	31.92±0.20	10.29±0.90	117.56±9.14	7.13±0.16	7.99±0.52	0.74±0.51	0.07±0.08	0.07±0.08	0.07±0.01
	BST	33.49±0.47	10.89±0.74	241.11±16.78	7.09±0.92	5.38±0.87	0.47±0.32	0.10±0.03	0.13±0.05	0.07±0.01
Tuban	TSR	33.13±0.72	7.76±0.31	47.63±9.54	8.42±0.01	0.38±0.02	0.00±0.00	0.00±0.00	0.11±0.01	0.02±0.01
	TSS	33.13±0.06	9.50±0.13	165.67±27.79	7.87±0.07	0.31±0.05	0.05±0.02	0.01±0.00	0.27±0.05	0.03±0.01
	TST	34.40±0.53	8.37±1.14	239.33±15.04	7.52±0.09	0.34±0.06	0.02±0.00	0.00±0.00	0.10±0.08	0.03±0.01
Sampang	SSR	33.07±0.51	9.45±0.26	71.00±5.22	8.04±0.32	0.64±0.03	0.13±0.01	0.01±0.00	0.08±0.04	0.05±0.01
	SSS	33.60±0.61	9.02±0.49	169.67±41.31	7.83±0.09	0.62±0.04	0.21±0.03	0.01±0.00	0.52±0.15	0.02±0.00
	SST	36.10±0.95	8.60±0.28	243.00±17.35	7.46±0.07	0.54±0.07	0.27±0.03	0.00±0.00	0.35±0.03	0.03±0.01
F value		9.691	2.522	0.231	1.988	468.532	6.729	36.736	2.966	7.877
p		0.001*	0.101	0.795	0.159	0.000*	0.005*	0.000*	0.071	0.002*

Note: *$p < 0.05$

Table 2. Distribution of phytoplankton and zooplankton in salt ponds

Class	Genera	Code	Brebes			Tuban			Sampang		
			BSR	BSS	BST	TSR	TSS	TST	SSR	SSS	SST
Phytoplankton											
Bacillarophyceae	*Amphora* sp.	Amp	-	-	-	-	-	-	4,725±1,048	-	-
	Cocconeis sp.	Coc	-	-	-	2,087±570	812±280	174±87	1,421±153	715±266	116±50
	Coscinodiscus sp.	Cos	5,935±1,222	1,131±371	392±173	13,276±5,305	4,319±2,097	319±50	7,682±2,646	2,667±1,890	261±151
	Melosira sp.	Mel	-	-	-	-	-	-	6,059±779	1,044±348	-
	Navicula sp.	Nav	13,131±7,864	4,218±1,111	261±235	9,276±2,088	1,479±522	145±50	6,841±629	2,000±348	58±50
	Nitzschia sp.	Nit	26,588±6,946	4,631±629	544±164	16,116±11,345	4,319±1,303	145±50	16,261±4,983	3,798±784	261±87
	Pleurosigma sp.	Ple	16,631±7,018	4,218±1,691	1,109±261	7,508±1,609	4,841±875	145±50	8,262±6,019	2,001±712	87±87
	Rhizosolenia sp.	Rhi	17,500±9,602	1,761±985	-	-	-	-	-	-	-
	Surirella sp.	Sur	-	-	-	-	-	-	5,682±1,783	1,218±543	-
	Biddulphia sp.	Bid	-	-	-	-	-	-	6,261±1,768	-	-
Cyanophyceae	*Trichodesmium* sp.	Tri	32,848±1.603	4,957±791	-	29,189±9,581	5,943±2.589	551±181	19,798±8.987	5,218±1.110	464±219
Dinophyceae	*Ceratium* sp.	Cer	1,326±679	-	-	-	-	-	-	-	-
	Peridinium sp.	Per	13,761±2.268	544±164	-	14,580±3,596	6,377±1,234	-	6,580±2,514	2,377±494	203±133
Number of Types			8	7	4	7	7	6	11	9	7
Total Number of Individuals (N) (cells/L)			127,721±11,227	21.460±2.058	2,306±333	93,032±9,251	28,090±2,590	1,476±169	89,573±5,694	21,048±1,618	1,450±134
Diversity (H')			1.38±0.18	1.40±0.09	1.19±0.10	1.33±0.04	1.34±0.03	0.91±0.46	2.05±0.20	1.26±0.14	1.43±0.17
Evenness Index (E)			0.12±002	0.14±001	0.15±0.01	0.12±0.00	0.13±0.00	0.12±0.06	0.18±0.02	0.13±0.01	0.20±0.02
Dominance Index (D)			0.18±000	0.20±002	0.34±004	0.22±0.01	0.19±0.01	0.24±0.05	0.14±0.01	0.16±0.01	0.24±0.08
Zooplankton											
Copepoda	Nauplii	Nau	66±33	-	-	116±44	-	-	-	-	-
	Oithona sp.	Oit	-	-	-	131±109	87±44	-	348±115	58±25	-
	Corycaeus sp	Cor	-	-	-	-	-	-	290±110	131±44	-
	Paracalanus sp.	Par	-	-	-	116±8	-	-	-	-	-
	Eucalanus sp.	Eug	22±19	-	-	-	-	-	-	-	-
	Euterpina sp.	Eut	-	-	-	131±25	-	-	-	-	-
Ostracoda	*Conchoecia* sp.	Con	207±68	66±33	-	290±47	160±67	-	-	-	-
Eurotatoria	*Brachionus* sp.	Bra	272±82	185±94	-	392±75	-	-	261±87	102±66	-
	Lepadella sp.	Lep	1,033±294	-	-	-	-	-	-	-	-
Oligotrichea	*Tintinnopsis* sp.	Tin	479±322	305±82	-	841±218	508±109	-	827±189.5	218±76	-
Litostomatea	*Didinium* sp.	Did	1,076±367	457±203	-	-	-	-	942±374.77	363±66	-
	Loxophyllum sp.	Lox	185±82	98±33	-	-	-	-	-	-	-
Number of Types			8	5	0	7	3	0	5	5	0
Total Number of Individuals (N) (Indv/L)			3,339±391	1,111±150	-	2,017±246	755±149	-	2,669±377	871±115	-
Diversity (H')			1.64±0.04	1.40±0.01	-	1.60±0.18	0.81±0.16	-	1.45±0.09	1.39±0.12	-
Evenness Index (E)			0.20±0.01	0.20±0.01	-	0.21±0.18	0.12±0.02	-	0.18±0.02	0.22±0.02	-
Dominance Index (D)			0.24±0.01	0.28±0.00	-	0.08±0.06	0.03±0.05	-	0.26±0.04	0.30±0.06	-

Meanwhile, no zooplankton groups were found at high-salinity ponds (BST, TST, and SST). The zooplanktons found in three locations were from 12 genera, including Nauplii, *Oithona* sp., *Corycaeus* sp., *Paracalanus* sp., *Eucalanus* sp., *Euterpina* sp., *Conchoecia* sp., Brachionus sp., *Lepadella* sp., *Tintinnopsis* sp., *Didinium* sp., and *Loxophyllum* sp. Figure 2 shows the Litostomatea group (*Didinium* sp.) dominated in the Brebes salt ponds, with a relative abundance of 40.82%, and Sampang with 36.87%. On the other hand, Oligotrichea (*Titintinnopsis* sp.) dominated Tuban salt ponds, with a relative abundance of 48.66%.

According to Table 2, the phytoplankton community structure had a diversity index (H) of 0.92-1.05, an evenness index (E) of 0.12-0.20, and a dominance index (C) of 0.14-0.34. Furthermore, the zooplankton community had a diversity index (H') of around 0.81-1.64; an evenness index (E) ranged from 0.18-0.22, and a dominance index (C) ranged from 0.03-0.30 at three pond locations.

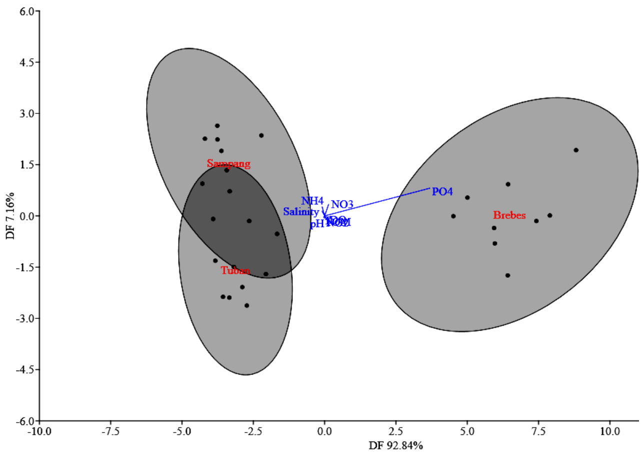


Figure 3. Biplot of water quality parameters at three salt ponds locations

Discussion

Environmental characteristics of salt ponds

Multivariate discriminant analysis in Figure 3 shows that Brebes ponds have different environmental characteristics than Sampang and Tuban ponds. Nutrient parameters, such as phosphate and inorganic nitrogen (nitrate and nitrite), and total organic matter are the main characteristics that distinguish the water conditions of the Brebes salt ponds, as shown in Table 1 ($P < 0.05$). Differences in nutrient concentrations in salt ponds depend on geographical factors (such as distance to river flow, human settlements, and sources of pollution), climate differences, and nutrient content of seawater as raw material for salt ponds (Pandey 2011). Fresh water from the river estuaries is one of the sources of nutrients that enter the sea. Tao et al. (2021) suggest that waste from human activities carried by rivers is one of the main sources of nutrient pollution in the sea. The high levels of nutrients in Brebes salt ponds are likely due to the salt ponds' location, near the river estuary, where small fishing boats were anchored, and residential areas, which resulted in high nutrient concentrations. According to Costa et al. (2015), nutrient concentrations are similar to other bodies of water; in salt, ponds can be classified as oligo-, meso-, or eutrophic. Furthermore, Costa et al. (2015) reveal that nutrient concentration, temperature, and light intensity limit phytoplankton growth.

The phosphorus concentration at the study site ranged from 0.34 to 7.99 mg/L, higher than observed in Brazilian salt ponds, ranging from 0.04 to 1.40 mg/L (Costa et al. 2015) and Chinese salt ponds ranged from 0.07 to 0.63 mg/L (Li et al. 2018). Girsang et al. (2021) suggest that estuaries, which transport particulate and other waste into the sea, are one source of phosphate. Additionally, the resuspension of sediment can contribute nutrients such as phosphate from the sediment to the water column (Guo et al. 2019). Finally, the bacterial decomposition of phosphorus compounds in sediments can also produce dissolved phosphate compounds that diffuse into the water bodies (Rustiah et al. 2019).

Generally, nutrient concentrations in salt ponds, especially nitrates, and phosphates, increase with increasing salinity (Masmoudi et al. 2015). Costa et al. (2015) suggest that the increase in nutrient concentration at high salinity is caused by evaporation and bacterial activity. However, autotrophic nitrification is not the primary source of nitrate in high-salinity ponds. Because it does not occur at salinity levels of 100-150 psu, it is also responsible for the oxidation of ammonium from nitrate to nitrite in low-salinity ponds (Costa et al. 2015). For example, the observations of nitrate concentrations in Brazilian salt ponds showed an average value of 0.165 mg/L. In contrast, the nitrite value was lower than the nitrate and ammonia parameters, ranging from 0.04-0.5 mg/L (Costa et al. 2015). Meanwhile, in this study, the nitrate concentration ranged from 0.10 to 0.74 mg/L, and the nitrite concentration ranged from 0 to 0.10 mg/L.

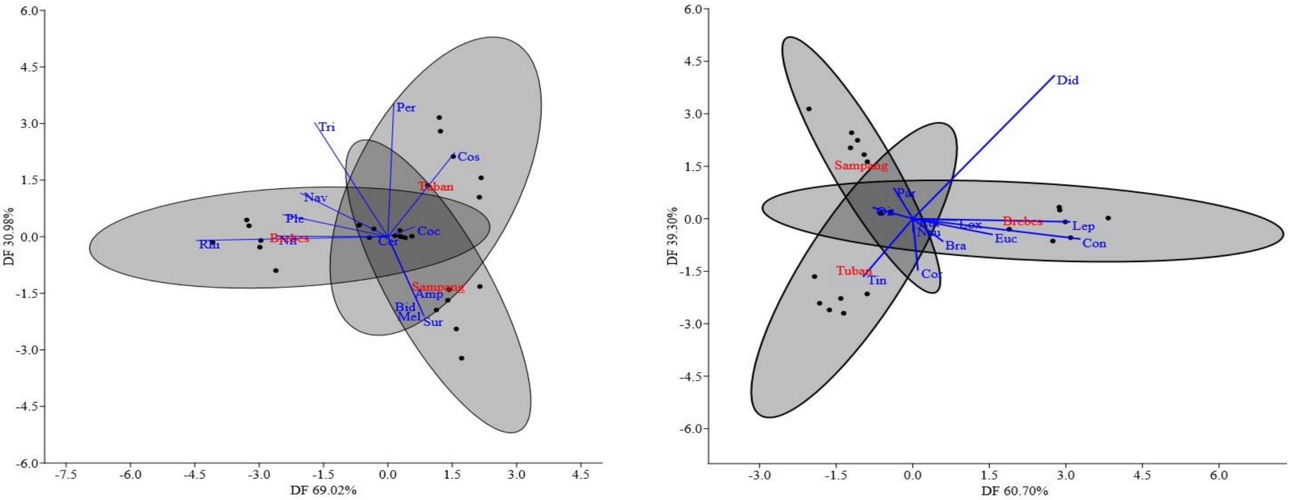
In salt ponds, decreasing pH and nutrient content were influencing organism distribution. pH values and nutrient concentrations are two factors that influence the distribution of organisms in water, particularly phytoplankton (Humphreys et al. 2018). Li et al. (2018) stated that the pH value of water is determined by its ionic composition, including the interactions between anions and cations and water temperature. Moreover, the presence of strong alkalinity will form an alkaline environment. pH values of water are also influenced by the relative content of free CO_2, carbonate, and bicarbonate, as well as the water's biological activity. Water tends to be more alkaline when carbonate or bicarbonate concentrations are high, and conversely, it tends to be less alkaline when it contains more free CO_2 (Li et al. 2018). pH values at the three salt pond locations tended to decrease as the salinity value increased. The decrease in pH values at high salinity is due to changes in the mineral composition of the brine induced by evaporation and possibly the involvement of biological activity (Li et al. 2018; Liu et al. 2021). pH values in this research ranged from 7.09±0.92 to 8.42±0.01. Similarly, pH values in Pemekasan salt pond, one of Madura Island's areas, ranged from 7.4-7.5 (salinity: 20 ºBe), 5.7-6.4 (29 ºBe), and 8.5 (2.5 ºBe) (Jayanthi et al. 2020). Meanwhile, the pH values of salt ponds in China ranged from 7.38 to 8.94, and they correlated with the concentration of Mg^+ and CO_2 ions at different salinity gradients (Liu et al. 2021).

The temperature significantly impacts phytoplankton photosynthesis, respiration, and physiology (Seifert et al. 2020; Trombetta et al. 2019). High temperatures can increase carbon assimilation during photosynthesis (Waal and Litchman 2020). Moreover, the phytoplankton growth rate increases with every 10°C temperature rise (Trombetta et al. 2019). Additionally, under unlimited nutrient conditions, phytoplankton nutrient uptake increases at high temperatures (González and Maraón 2021). However, the accumulation of phytoplankton in crystallization ponds during the salt-making process is undesirable as it affects seawater evaporation. In this study, the Sampang salt ponds had the highest temperature (36ºC). In addition, increased light intensity is one of the factors contributing to the rise in water temperatures. Generally, Sampang has a longer dry season (about 4-5 months) and less rainfall (about 1,200 mm/year) than Java Island (Prasetyo et al. 2017), which is conducive to the seawater evaporation process. Based on BMKG data, the average rainfall in the three salt pond locations during August-September 2021 was 10-80 mm/month (Tuban), (60-150 mm/month (Brebes), and 0-27 mm/month (Sampang) (Badan Pusat Statistik 2021) Therefore, temperature affects the rate of water evaporation and salt formation. In addition, salinity increases in direct proportion to temperature rise as the evaporation reduces the mass of water, resulting in a rise in salinity.

Distribution of plankton communities

Based on the discriminant test in Figure 4, it has been determined that there is no significant difference between the abundance of phytoplankton and zooplankton in salt ponds at the three study locations. That showed the plankton communities were spatially distributed in the three study locations, and there was no significant increase in plankton communities at certain locations. As illustrated in Table 2, the highest abundance of phytoplankton in Brebes salt pond occurred at a low salinity of 127,721±37,202 and a high salinity of 2,306±333. According to Spearman's correlation, the abundance of zooplankton in the three pond locations significantly affected the salinity factor (sig<0.01). Generally, phytoplankton abundance decreased as salinity increased in the three study locations (Table 2). For example, according to Sukmawati et al. (2013), the abundance of phytoplankton in the Demak salt ponds, Indonesia, is 44,250 cells/L at a salinity of 40 psu and decreases by 2,204 cells/L at a salinity of 275 psu, with the dominance of the plankton *Microcystis* sp. at low salinity. According to Pandey and Yeragi (2020), plankton growth in hypersaline environments is limited by their inability to tolerate high salinity, high temperatures due to water evaporation, and limited nutrient availability.

Moreover, the composition of the phytoplankton found in the three locations was made up of 13 genera, i.e., Bacillarophyceae (10 genera), Cyanophyceae (1 genus), and Dinophyceae (2 genera) (Table 2.). Several observations of phytoplankton in salt ponds indicated the dominance of Bacillariophyceae in the salt pond environment. For example, Fitriyah et al. (2016) discovered the dominance of Bacillarophyceae (9 genera) in Demak salt pond, Indonesia. Furthermore, the observations of Sukmawati et al. (2013) found 9 genera of phytoplankton that dominant in the crystallization process in Jepara salt ponds, Indonesia, i.e., Ascidiacea (1 genus), Cyanophyceae (3 genera), Bacillarophyceae (4 genera), and Trebouxiophyceae (1 genus). The phytoplankton community found in Pati traditional salt ponds, Indonesia, is divided into 4 genera, i.e., *Nitzschia* sp., *Trachelomonas* sp., *Oscillatoria* sp., and *Tetraselmis* sp. (Sriwati et al. 2021). Likewise, Chatchawan et al. (2011) found 16 genera of phytoplankton at a salinity gradient of 90-249 psu in Thai salt ponds, with the dominance of *Spirulina* spp.



A B

Figure 4. Biplot of phytoplankton (A) and zooplankton (B) abundance at three salt pond locations. Acronyms used for plankton species are the same as in Table 2

According to Hinzano et al. (2022), the phytoplankton observed in salt ponds includes the following types; Dinophyceae, Bacillariophyceae, Chlorophyceae, Euglenophyceae, Raphidophyceae, Prymnesiophyceae, Cryptophyceae, and Silicoflagellates. Hinzano et al. (2022) also state that the Bacillariophyceae and Dinophyceae groups dominate phytoplankton and live in salt ponds with salinity levels below 100 psu. Meanwhile, the Cyanophyceae and Chlorophyceae groups are spread over salinities above 100 psu. However, according to Irshad et al. (2014), many halophilic organisms can live at extreme salt levels, depending on environmental and nutritional factors for growth.

The phytoplankton species dominate in crystallization ponds and can survive at high salinity (BST, TST, and STT), including *Pleurisigma* sp., *Coscinodiscus* sp., and *Nitzschia* sp. According to some sources, this species can be found in various salinities. For example, Sriwati et al. (2021) found *Nitzschia* sp. at a salinity of 200-230 psu; Hotos (2021) observed it in Greece at a salinity of 130-160 psu; Dolapsakis et al. (2005) found this species in the salinity range of 144 psu. Meanwhile, Devi et al. (2019) observed *Nitzschia* sp., *Coscinodiscus* sp., *Navicula* sp., and *Microcystis* sp. at a salinity of 225 psu in Indian salt ponds. On the contrary, Sukmawati et al. (2013) found several phytoplankton species, including *Nitzschia* sp., *Microystus* sp., *Oscaltoria* sp., and *Pleurisigma* sp., that were able to survive at a salinity of 275 psu. Also, Rexy et al. (2022) discovered 66 phytoplankton genera in low-salinity salt ponds and 19 phytoplankton genera in high-salinity salt ponds in India. Phytoplankton adaptation can adapt to extreme salinity by actively transporting ions out of the cells or into the vacuole and regulating cellular osmolytes and EPS (extracellular polymer substance) mechanism (Steele et al. 2014). For example, the benthic diatom *Phaeodactylum tricornutum* increases EPS production by producing more uronic and sulfate acids at high salinity, potentially enabling EPS to retain more water (Rossi et al. 2016). *Nitzschia frustulum* produces more types of monosaccharide sugars, rhamnose (32 mol%) and xylose (5 mol%), at high salinity, causing the EPS gel to become thicker and limiting the diffusion of ions to the cells (Gügi et al. 2015).

According to Heinle et al. (2021), physicochemical factors such as nutrients, temperature, and light availability can influence phytoplankton abundance and predators (especially herbivorous organisms). In their study, Boas et al. (2021) observed that an increase in salinity usually leads to a decline in the zooplankton population, including cladocerans and copepods, as an increase in the population of rotifers. Furthermore, Boas et al. (2021) stated that this shift in zooplankton community structure could result in higher phytoplankton blooms and alterations in functional ecosystem parameters. These parameters may include factors such as productivity, nutrient cycling, and ecosystem metabolism that are crucial to the ecosystem's overall function and stability (Rombouts et al. 2013).

The highest abundance of zooplankton (3,339±391) was found in low salinity ponds at Brebes salt ponds (BSR), as shown in Table 2. The composition of zooplankton found in 12 genera, including 5 classes: Copepods (6 genera), Ostracoda (1 genus), Eurotatoria (2 genera), Oligotricea (1 genus), and Litostomatea (2 genera). Ciliates were the dominant zooplankton group in Brebes salt ponds, and *Didinium* sp. was the dominant genus in low-mid salinity ponds but not in high salinity (> 200 psu). Sudararaj et al. (2006) discovered *Didinium* sp. at a salinity of 187 psu in Indian salt ponds, despite its rarity. According to Soares et al. (2018), marine organisms in a salinity gradient will experience succession, gradually adapting to high salinity levels. However, various species will begin to decrease or disappear in abundance and will be replaced by halophilic bacteria. Ciliates are a diverse group of protists living in freshwater and marine environments. In addition, more than 15 species are found in moderate salinity environments (40-80). Even several species are found in hypersaline environments (80-340 psu), although they are uncommon, i.e., *Choturnia salinia* at salinity up to 120 psu (Zhuang et al. 2016); *Platynematum rossellomorai* in Spanish salt ponds at a salinity of 280 psu (Qu et al. 2020).

Moreover, Basuri et al. (2020) discovered 29 ciliate taxa from 18 genera and five classes in an Indian hypersaline lagoon at salinity 12-61 psu, with *Strombidium conicum* as the dominant species. When exposed to unfavorable environmental conditions such as food scarcity, hyper- or hypo-osmolarity, temperature, and pH extremes, ciliates can transform into a cryptobiotic form by forming cysts (Li et al. 2022). Kaur et al. (2019) stated that changes in cyst form are an adaptation strategy in response to environmental changes. The adaptive strategy against major stresses generally includes slowing down or shutting down all metabolic activities. According to Lambrecht et al. (2015), in general, Cysts have a thick protective wall, which is often double or multilayered, and it is composed of lipids, proteins (glycol), and carbohydrates such as chitin and cellulose, and some cysts can withstand drought for more than 20 years. The ability to form cysts is important in their ecology and may have contributed to their long evolutionary history and wide distribution (Verni and Rosati 2011). In addition, the diversity and variety of ciliate communities are caused by differences in geographic location and environmental heterogeneity (Zhao and Filker 2018). Furthermore, the research by Ladhar et al. (2015) and Ghannay et al. (2015) found copepod dominance in Tunisian salt ponds at salinities ranging from 60 to 90 psu, and the Litostomatea group lived in a salinity range of 81-92 psu. Furthermore, the research by Thabet et al. (2018) showed some seasonal and interannual variations in the zooplankton community in Tunisian salt ponds, with copepods, the most abundant zooplankton species. Their abundance is influenced by nutrient content variation and the level of anthropogenic pollution in each pond, but salinity changes primarily influenced their distribution. According to Corral et al. (2019), halophilic organisms are classified according to their salt requirements: halophilic organisms grow optimally at salt levels of 1-3%; moderate halophiles grow optimally at 3-5%; and extreme halophiles grow optimally at 15-30%. Some ciliates species are halotolerant, for example, *Fabrina salina* (Hotos 2019), *Cyclidium* *glaucoma*, *Euplotes* sp., and *Pseudocohnilembus persalinus* (Weinisch et al. 2018)

Table 2 illustrates the structure of the phytoplankton community had a low diversity index (H') of 0.91±0.46 at Tuban salt pond (TST); a moderate diversity showing a level of 2.05±0.20 at Sampang salt ponds (SSR); a depressed evenness index (E) ranging from 0.12-0.20; and a moderate dominance index (C) showing a level 0.14-0.34 at all three salt pond locations. That is similar to the observation in Chinese salt ponds by Deng et al. (2014), at a salinity gradient of 50-200 psu, the diversity index (H') of phytoplankton ranged from 0.13-1.57 (low), the evenness index (E) ranges from 0.01-0.58 (stressed-labile), and the dominance index (D) indicates a value above 0.5 (moderate). As the observation by Rexy et al. (2022) discovered, the diversity and evenness index decreased sharply in crystallization ponds compared to reservoir ponds in Indian salt ponds. The low diversity of phytoplankton in the salt pond environment, particularly in crystallization ponds, is due to limited nutrition and high salinity stress. Therefore, the salinity and nutrients influence phytoplankton composition (Padisák J and Flores (2021). Aside from physical and biological environmental factors, Wei et al. (2022) found that variations strongly influence the abundance and composition of phytoplankton in predatory factors. As Jales et al. (2021) stated, biological and physical factors such as environmental changes, nutrients, and the presence of herbivorous zooplankton affect the species composition and diversity of phytoplankton in the ecosystem. Furthermore, the zooplankton community had a low diversity index (H') of 0.81±0.16 at Tuban salt ponds (TSS), a moderate diversity index (H') of a level 1.64±0.04 at Brebes salt ponds (BSR), a depressed evenness index (E) that ranged from 0.18-0.22; and a low dominance index (C) that ranged from 0.03-0.30 at the three salt pond locations. Observations by Ghannay et al. (2015) in Tunisian salt ponds show that the zooplankton community diversity index decreases along with the increase of salinity, i.e., 1.54 (20 genera) at salinity 38 psu and 0.06 (4 genera) at salinity 297 psu. Ladhar et al. (2015) also found that the diversity index of copepods decreases from 1.04 (at a salinity of 42.1 psu) to 0.06 (at a salinity of 96.5 psu), indicating that salinity is a limiting factor for zooplankton abundance. The same result was found along the Tunisia salt ponds, where the diversity index decreased as salinity increased, indicating that the spatial distribution of copepods along the saltern appears to be primarily related to salinity (Thabet et al. 2018).

Correlation of environmental characteristics on plankton distribution and composition

Figure 5 shows a biplot canonical correlation analysis on phytoplankton distribution in three salt pond locations. Therefore, it can be observed that the physicochemical parameters of nitrate, nitrite, ammonia, salinity, pH, DO, total organic matter, phosphate, and temperature are strongly influenced. On the other hand, Brebes salt ponds are characterized by high concentrations of phosphate, nitrate, and total organic matter. Therefore, they have a strong effect on phytoplankton, including Bacillarophyceae (*Navicula* sp., *Pleurosigma* sp., *Rhizosolenia* sp., and *Nitzschia* sp.,), which had the highest total abundance (from low to high salinity) at 90,592 cells/L (59.80%), and also Dinophyceae (*Ceratium* sp.) with an abundance of 6,283 cells/L (4,15%). Furthermore, high nutrient concentrations in Brebes salt ponds were thought to trigger plankton growth. Meanwhile, the ponds of Sampang and Tuban were characterized by high concentrations of ammonia, pH, temperature, and salinity. Therefore, they strongly affected the abundance of Bacillarophyceae species (*Amphora* sp., *Cocconeis* sp., *Coscinodiscus* sp., *Melosira* sp., *Surirella* sp., and *Biddulphia* sp.). These species which also had the highest abundance of each salt pond in Tuban was 64,962 cells/L (53.42%) and Sampang was 77,429 (69.38%) of the total abundance of each salt pond location. Meanwhile, also the Dinophyceae (*Peridinium* sp.) had a total abundance of 20,957 cells/L (17.23%) in Tuban and 9,160 cells/L (8.20%) in Sampang salt pond. In addition, Cyanophyceae (*Trichodesmium* sp.) had an abundance of 35,683 cells/L (29.34%) in Tuban and 25,480 cells/L (22.83%) in Sampang. Bacillarophyceae were found in all three salt ponds, showing their widespread group. Most diatoms (Bacillariophyceae) are phytoplankton with high environmental tolerance and adaptability; thus, Bacillarophyceae is cosmopolitan (Van ormelingen et al. 2013). According to Padisák and Flores (2021), nutrient concentrations and salinity influence phytoplankton's abundance and composition in extreme environments.

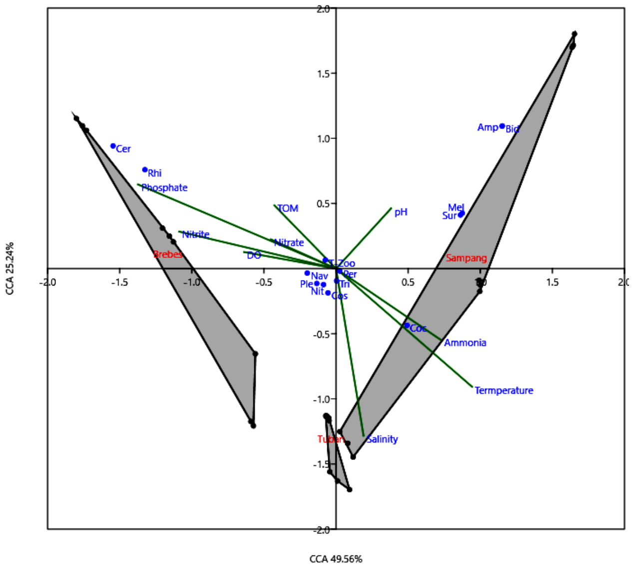


Figure 5. Canonical correspondence analysis (CCA) biplot of plankton (phytoplankton and zooplankton) species abundance against environmental variables in three salt ponds in three locations of Brebes, Tuban, and Sampang. The acronyms used for plankton species are the same as in Table 2.

Florescu et al. (2022) stated that Bacillariophyceae prefer more oxygenated, cooler, and clearer ecosystem conditions, while Cyanophyceae prefer warmer, shallower waters with low phosphate levels. As stated in Masmoudi et al. (2015), Diatom (Bacillarophyceae) require high orthophosphate (PO4-) and nitrate (NO3-) concentrations, whereas Dinophyceae require high ammonium (NH4) concentrations. Temperature, salinity, pH, nitrate, nitrite, ammonia, and silicates are all hydrochemical and physical factors influencing spatial variation and temporal phytoplankton distribution (Vajravelu et al. 2018). Nitrogen and phosphate are essential for plankton formation and distribution and the composition and biomass of phytoplankton in the ecosystem (Hoang et al. 2018). Ladhar et al. (2015) stated abiotic factors (pH, ammonium, total nitrogen, nitrite, nitrate, and total phosphorus) significantly correlate with the density and spatial variation of plankton communities in Brazilian salt pond ecosystems. According to several sources, phosphate is a limiting factor for phytoplankton growth (Arofah et al. 2021; Ballah et al. 2019). In addition, low phosphate concentrations affect the structure and distribution of the phytoplankton community in high-salinity environments (Girault et al. 2013).

Environmental conditions in ecosystems strongly influence phytoplankton distribution as primary producers, especially nutrients (Hinzano et al. 2022) and predators such as zooplankton (Ghannay et al. 2015). The canonical biplot analysis (Figure 4) shows that environmental factors (physical and chemical) had a greater influence on the abundance and composition of phytoplankton in the three salt ponds than predatory factors or zooplankton. Although Brebes and Sampang salt ponds are characterized by the dominance of *Didinium* sp., a carnivorous zooplankton type, Tuban salt ponds are characterized by *Tintinnopsis* sp., which has tendencies as an herbivore (Camarillo and Zaragoza 2021). It is widely known that zooplankton as a predator impacts plankton composition. However, in this study, the presence of total zooplankton did not have a strong influence on the abundance and composition of phytoplankton in three salt ponds. The chemical and physical factors in an ecosystem strongly influence phytoplankton composition and distribution. Bruce and Imberger (2009) reported that phytoplankton composition changes in Tunisian salt ponds did not appear to be influenced by predators (zooplankton) or nutrients but were caused by phytoplankton competition, which was more tolerant of changes in salinity in the salt pond environment.

In conclusion, the locations of salt ponds in Tuban and Sampang had similar environmental characteristics. The Brebes Salt Ponds had higher concentrations of nutrients (nitrates, nitrites, and total organic matter) compared to the other two locations, enabling a favorable plankton growth environment. The highest abundance of plankton was found in the Brebes Salt Pond at low salinity. However, along with increased salinity levels, plankton's abundance and diversity will decrease. Salinity is a limiting factor for the biological community in the salt pond environment. The biological community in the salt ponds supports the traditional salt production process. Therefore, this research is an information base for the traditional salt production process using biological systems that are environmentally friendly and promote a sustainable, high-quality production process.

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