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Diversity of Zooplankton in the Rice Fields in Suphan Buri Province, Thailand, with a New Record of Cyclopoid Copepod

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Abstract: Rice fields are dynamic ecosystems with complex niche structures for organisms, and they have been hypothesised to have high biodiversity. Accordingly, in this study, the diversity of zooplankton in rice fields in Suphan Buri Province, a large area of rice plantations in the central region of Thailand, was examined. A total of 100 species, including 52 rotifers, 18 cladocerans, and 30 copepods, were recorded, including *Mesocyclops kayi*, which was a new record in Thailand. A high Simpson's diversity index (0.63) and a low Pielou's species evenness index (0.02) confirmed various potential niches for zooplankton in this ecosystem, leading to a low Jaccard similarity index both among the current rice fields and those in other regions. Moreover, the species richness estimators suggested that more species are expected to be discovered in the rice fields. Rotifer and copepod communities are influenced by local environmental variables, including dissolved oxygen, salinity, conductivity, and chlorophyll a. The results of this study fill a gap in the knowledge regarding the diversity and ecology of zooplankton in rice fields. However, further research is needed to fully comprehend the function of this ecosystem and the actual diversity in Thailand.

Keywords: temporary pond; agroecosystem; *Mesocyclops kayi*

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1. Introduction

Rice fields represent large and complex temporary ecosystems. Their long existence, the vast extent of land they occupy in humid tropical regions, the array of ecological habitats they encompass, and the different phases they pass through during a cultivation cycle make rice fields unique ecosystems [1,2]. The hydroregime in rice fields is an important feature of transient aquatic ecosystems that not only affects species diversity and community structures but also the life histories of microscopic animals. Moreover, rice fields are dynamic and undergo rapid physical, chemical, and biological changes. The heterogeneity of rice fields provides various niches for microscopic animals and contributes to their richness and varied biodiversity [3]. Rice fields contain both common species and species that have specific needs. Therefore, rice fields may be considered as an agronomically managed temporary wetland ecosystem that provides a home for a range of microscopic organisms. Moreover, in the rice growth stages, including the seedling, tillering, and flowering stages, different environmental conditions, such as the levels of water and dissolved oxygen, result in high heterogeneity of this habitat. These conditions offer suitable niches for a wide range of animals. Indeed, it has been reported that different organisms thrive in each stage of rice planting [4]. For example, in the early stage of the crop cycle, rice fields are mostly covered with water, which favours planktonic species. Meanwhile, in the late stage of the crop cycle, rice fields are mostly covered with vegetation, resulting in a high proportion of littoral species [5], and it is more likely that activities like watering, fertilising, pesticide use, and micro-plastic contamination will have an effect on the rice field ecosystem [1].

Thailand is a region in Asia characterised by the intensive cultivation of rice fields with diverse features. Consequently, the diversity of animals inhabiting this habitat is high, especially zooplankton [6,7]. Such diversity may increase the fertility of this environment and improve the efficiency of nutrient circulation and waste disposal in the ecosystem. It may also increase agroecosystem productivity, and with more diversity, ecosystems are more resilient to perturbations. Further, diversification can help to maintain and increase soil fertility and mitigate the impacts of pests and diseases [8,9].

Although few studies have examined the zooplankton in rice fields, the results indicate that there is a high diversity of zooplankton [4–6,10,11]. In particular, a recent study identified 121 species of zooplankton in rice fields in Nakhon Ratchasima Province, which is located in north-eastern Thailand [11]. Moreover, a recent report of a new species, *Tropodiaptomus megahyaline* [12], in rice fields in eastern Thailand indicates that rice fields have the potential to be habitats for diverse organisms. Therefore, it is likely that there are still more hidden species in this freshwater ecosystem. Suphan Buri Province is a large area, about 80% of which is used for rice cultivation. In addition, there are various rice cultivation methods, including paddy fields, off-season rice plantations, organic rice fields, and chemical rice fields. We hypothesised that these diverse habitats would contain a diverse range of organisms. Therefore, this study examined the diversity of zooplankton in rice fields in Suphan Buri Province, central Thailand, which has one of the largest concentrations of rice plantations in Thailand.

2. Materials and Methods

2.1. Study Area

Sampling was carried out in five rice fields located in Suphan Buri Province, which has the most area dedicated to rice plantations in central Thailand (Figure 1). These sites were chosen because they are representative of the different characteristics of the rice fields in this area. The characteristics, sampling periods, and environmental factors related to each rice field are presented in Table 1.

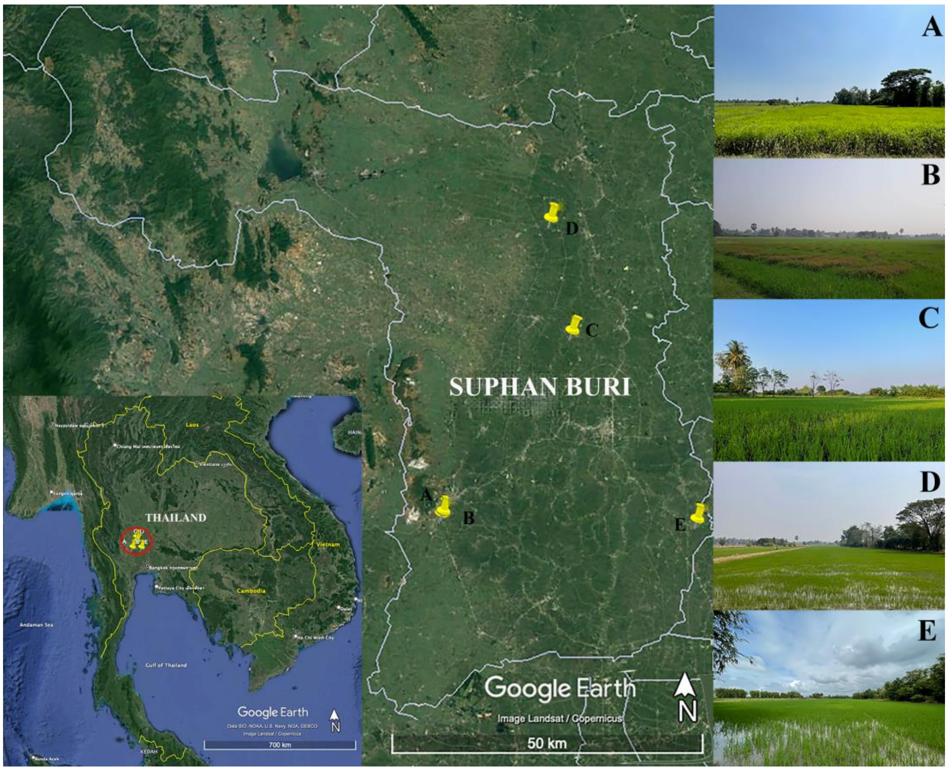

Figure 1. Map of study sites in Suphan Buri Province, Thailand. (**A**–**E**) = rice field A–E. (Google Earth Pro 7.3.6.9345).

Table 1. Characters and environmental parameters in each rice field.

Rice Field	Sampling Period	General Characteristics	Environmental Factors							
			Temp. (°C)	Cond. (µS cm^{-1})	TDS (mg L^{-1})	Sal. (psu)	DO (mg L^{-1})	Chl a (µg L^{-1})	Phyco-Cyanin (µg L^{-1})	pH
A	February–March 2023	Organic rice fields, irrigation water twice during rice plantations, and weeds were present.	28.5–31.1	403.2–406.2	235–247	0.17–0.18	2.55–17.22	5.16–79.33	0.11–3.45	7.5–7.6
B	March–April 2023	Organic rice field, irrigation water, managed weeds, and no drying period during rice cultivation	28.5–33.2	283.8–440	173–253	0.13–0.18	2.63–12.07	3.17–5.12	0.12–0.81	3.9–7.5
C	November 2022–February 2023	Chemical rice field, irrigation water, managed weeds, water level was 30–40 cm, no dried period during rice cultivation	22.2–28.7	200.3–345.7	129–237	0.09–0.17	2.25–7.48	3.74–15.72	0.07–0.8	7.6–7.8
D	November 2022	Chemical rice field, irrigation water, long drying period, water level of about 20 cm, switch to growing *Crotalaria juncea* during the off-season.	28.26	465.5	285	0.21	2.17	21.3	0.76	7.3
E	March–April 2023	Chemical rice field, roadside, irrigation water, water level was 20–30 cm, 3 months of rice cultivation whereas others are 4 months, flooding before rice plantation period every year.	31.8–36.1	1176.5–1449.9	77–676	0.21–0.58	7.54–7.54	5.31–17.39	0.17–17.39	7.0–7.3

Note: Code of each rice field referred from Figure 1.

2.2. Sample Collection, Species Identification, and Count

A 5 L bucket was used to randomly collect the 50 L of water covering each rice field plot. Rotifers, cladocerans, and copepods were then quantitatively sampled by filtering the 50 L of water through a 20 μm mesh-size plankton net. Samples were collected 1–3 times from each rice field (depending on the amount of water available) during a crop cycle between October 2022 and April 2023. Environmental parameters, including temperature (temp.), conductivity (cond.), total dissolved solid (TDS), salinity (sal.), dissolved oxygen (DO), chlorophyll a (Chl a), phycocyanin, and pH, were measured using a YSI EXO1 Multiparametre Sonde and YSI EXO Handheld Display 599150.

In the laboratory, rotifers, cladocerans, and copepods were sorted, identified, and counted using a stereo microscope (Olympus SZ51) and compound light microscope (Olympus CH2). Identification based on morphological characters was made at the species level when possible following keys and up-to-date references [13–26].

2.3. Data Analyses

Density was calculated and expressed as individuals per litre (ind. L^{-1}) with a standard error. Relative abundance was calculated by counting the number of individuals in each sample. Simpson's diversity index, Pielou's species evenness index [27], and the Jaccard similarity index were calculated with Microsoft Excel for Mac version 16.77.1. Jaccard's similarity index was used to compare the similarity of species composition between the present rice fields and to compare the similarity of species composition with two previous studies on rice fields from central [6] and northeastern Thailand [11] that studied all three groups of zooplankton with similar sampling design. Species richness estimators, including Chao 1, Chao 2, Jacknife 1, and Jacknife 2, were analysed based on a species accumulation curve using the EstimateS programme.

The relationship between the species distributions and the local environmental variables of all the rice field samples was determined by canonical correspondence analysis (CCA) using PC-ORD software version 7.0 [28]. Rare species were deleted from the data set, on the basis that rare species contribute little to the community analysis but add noise to the statistical solution [29]. The 10% rule was used; species that occurred in less than 10% of the samples were omitted from the analysis so that variance was decreased.

3. Results

3.1. Diversity and Distribution of Zooplankton

One hundred zooplankton species were recorded, including 52 rotifers, 18 cladocerans, and 30 copepod species. Of these, one cyclopoid copepod, *Mesocyclops kayi*, was recorded for the first time in Thailand, and 19 other species were newly recorded in rice field habitats in Thailand (19% of all records) (Table 2). The pictures of representatives of the zooplankton found in the present study are shown in the Supplementary File. The total number of species found in each rice field ranged from 28 to 89 species. Copepods were found in 7–21 species per sample. Moreover, the results showed a high value for the Simpson's diversity index (0.63) but a low value for the Pielou's species evenness index (0.02). The results of three estimators (Chao2, Jacknife1, and Jacknife2) for the species accumulation curve of zooplankton species richness were greater than the number of observed species. The Jacknife2 estimator yielded the greatest maximum value of about 137 species, while Chao2 method resulted in the closest estimate (122 species) (Figure 2).

Table 2. Zooplankton found in the present study.

Species	
Rotifers	**Cladocerans**
1. *Anuraeopsis fissa* Gosse, 1851	1. *Anthalona harti* Van Damme, Sinev & Dumont, 2011
2. *Anuraeopsis* sp.	2. *Bosminopsis deitersi* Richard, 1895
3. *Ascomorpha* sp.	3. *Ceriodaphnia cornuta* Sars, 1885

Table 2. *Cont.*

Species	
4. *Asplanchna sieboldii* (Leydig, 1854)	4. *Chydorus eurynotus* Sars, 1901
5. *Asplanchna* sp.	5. *Coronatella monacantha* (Sars, 1901)
6. *Brachionus angularis* Gosse, 1851	6. *Coronatella rectangula* (Sars, 1861)
7. *Brachionus budapestinensis* Daday, 1885 **	7. *Diaphanosoma excisum* Sars, 1885
8. *Brachionus calyciflorus* s.l.	8. *Guernella raphaelis* Richard, 1892
9. *Brachionus caudatus* Barrois and Daday, 1894	9. *Kurzia longirostris* (Daday, 1898)
10. *Brachionus falcatus* Zacharias, 1898	10. *Leydigia acanthocercoides* (Fischer, 1854)
11. *Brachionus lyratus* Shephard, 1911 **	11. *Macrothrix spinosa* King, 1853
12. *Brachionus quadridentatus* Hermann, 1783	12. *Moina micrura* Kurz, 1875
13. *Colurella* sp.	13. *Moinodaphnia macleayi* King, 1853
14. *Conochilus* sp.	14. *Ovalona cambouei* (Guerne & Richard, 1983)
15. *Dipleuchlanis propatula* (Gosse, 1886)	15. *Ovalona pulchella* (King, 1853)
16. *Euchlanis dilatata* Ehrenberg, 1832	16. *Pseudosida bidentata* Herrick, 1884
17. *Filinia longiseta* (Ehrenberg, 1834)	17. *Scapholeberis kingi* Sars, 1888
18. *Filinia opoliensis* (Zacharias, 1898)	18. *Simocephalus heilongigensis* Shi & Shi, 1994
19. *Filinia terminalis* (Plate, 1886) **	**Copepods**
20. *Keratella cochlearis* (Gosse, 1851)	1. *Mongolodiaptomus botulifer* (Kiefer, 1974)
21. *Keratella tropica* (Apstein, 1907)	2. *Phyllodiaptomus christineae* Dumont, Ranga Reddy & Sanoamuang, 1996 **
22. *Lecane aculeata* (Jakubski, 1912)	3. *Phyllodiaptomus praedictus* Dumont & Ranga Reddy, 1994
23. *Lecane bulla* (Gosse, 1851)	4. *Pseudodiaptomus* sp.
24. *Lecane chinesensis* Zhuge & Koste 1996 **	5. *Cryptocyclops* sp.
25. *Lecane closterocerca* (Schmarda, 1859)	6. *Ectocyclops* sp.
26. *Lecane curvicornis* (Murray, 1913)	7. *Eucyclops serrulatus* (Fischer, 1851) **
27. *Lecane hornemanni* (Ehrenberg, 1834) **	8. *Eucyclops* sp.
28. *Lecane hamata* (Stokes, 1896)	9. *Mesocyclops affinis* van de Velde, 1987
29. *Lecane lateralis* Sharma, 1978	10. *Mesocyclops aspericornis* (Daday, 1906)
30. *Lecane luna* (Müller, 1776)	11. *Mesocyclops ogunnus* Onabamiro, 1957
31. *Lecane papuana* (Murray, 1913)	12. *Mesocyclops thermocyclopoides* Harada, 1931
32. *Lecane quadridentata* (Ehrenberg, 1830)	13. *Mesocyclops kayi* Holynska & Brown, 2003 *,**
33. *Lecane signifera* (Jennings, 1896)	14. *Mesocyclops* sp.
34. *Lecane stenroosi* (Meissner, 1908)	15. *Microcyclops* sp. 1
35. *Lecane unguitata* (Fadeev, 1926)	16. *Microcyclops* sp. 2
36. *Lecane ungulata* (Gosse, 1887)	17. *Microcyclops* sp. 3
37. *Lepadella dactyliseta* (Stenroos, 1898) **	18. *Microcyclops* sp. 4
38. *Lepadella* sp.	19. *Paracyclops affinis* (Sars G.O., 1863) **
39. *Manfredium* sp.	20. *Thermocyclops crassus* (Fischer, 1853) **
40. *Mytilina ventralis* (Ehrenberg, 1830)	21. *Thermocyclops decipiens* Kiefer, 1929
41. *Platyias quadricornis* (Ehrenberg, 1832)	22. *Thermocyclops operculifer* Kiefer, 1930 **
42. *Plationus patulus* (Müller, 1786)	23. *Thermocyclops taihokuensis* Harada, 1931 **
43. *Polyarthra* sp.	24. *Thermocyclops rylovi* (Smirnov, 1928) **
44. *Scaridium longicauda* (Müller, 1786) **	25. *Thermocyclops vermifer* Lindberg, 1935 **
45. *Testudinella incisa* (Ternetz, 1892)	26. *Thermocyclops woltereckі* Kiefer, 1938 **
46. *Testudinella patina* (Hermann, 1783)	27. *Thermocyclops* sp. 1
47. *Trichocerca similis* (Wierzejski, 1893)	28. *Thermocyclops* sp. 2
48. *Trichocerca* sp. 1	29. *Elaphoidella* sp.
49. *Trichocerca* sp. 2	30. *Onychocamptus vitiospinulosa* (Shen & Tai, 1963) **
50. *Trichocerca* sp. 3	
51. *Trichotria tetractis* (Ehrenberg, 1830) **	
52. *Trochosphaera aequatorialis* Semper, 1872	

* New record in Thailand ** New record in rice field in Thailand.

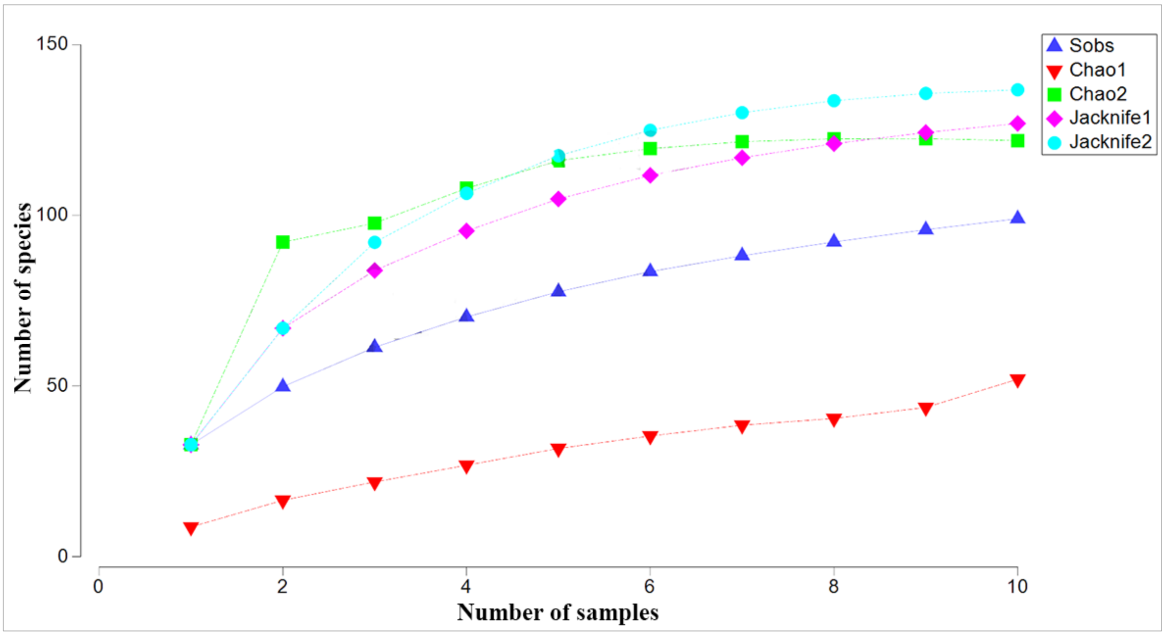

Figure 2. Species accumulation curve with the estimation curves of four estimators.

The most diverse genus of rotifer was *Lecane* (fifteen species), followed by *Brachionus* (seven species), and *Trichocerca* (five species). The most diverse genus of copepods was *Thermocyclops* (nine species), followed by *Mesocyclops* (six species), and *Microcyclops* (four species). However, most cladoceran genera were present with a single species except for *Coronatella*, which comprised two species. In addition, rotifers were found in 1–13 samples (5–65% of all the samples). *Lecane papuana* was the most frequently found species (65% of all the samples), followed by *L. bulla* (60% of all the samples), and *Plationus patulus* (50% of all the samples). Cladocerans were found in 1–11 samples (5–55% of all the samples). *Ceriodaphnia cornuta* and *Moinodaphnia macleayi* were the two most frequently found species (55% of all the samples), followed by *Diaphanosoma excisum* (50% of all the samples). Copepods were found in 1–17 samples (5–85% of all the samples), with *Mesocyclops aspericornis*, *Microcyclops* sp. 2, and *Cryptocyclops* sp. being the three most frequently found species (85% of all the species).

3.2. New Record of Mesocyclops kayi Holynska & Brown, 2003 in Thailand

This is the second report of the species *Mesocyclops kayi*, which was first described in 2003 in Burma [23]. In this study, about 1–3 individuals L^{-1} were distributed in 10 samples (50% of all the samples), ranking it among the three most dominant copepod species in rice field A. Most of the morphological characteristics were consistent with the original paper from Burma, although there were some differences, as noted in the remarks.

3.2.1. Short Description

Female: Total body length (Figure 3A), measured from anterior margin of the rostrum to the posterior margin of the caudal rami; length about 1076–1457 µm (mean = 1200 µm; n = 10). Pediger 5 with dorsally and laterally pilose. Genital double-somite (Figure 3B,C); length about 1.0–1.1 times as long as wide (mean = 1.0; n = 10). Genital double-somite and succeeding two urosomites with transverse ridges on the dorsal and ventral surfaces (Figure 3B–E). Caudal ramus length about 3.2–4.0 times as long as wide (mean = 3.6; n = 10), with a longitudinal row of hair on the inner margin, and with numerous minute spinules on the dorsal and ventral surfaces (Figure 3D,E). Spinules were present at the implantation of the outermost terminal seta, but spinules were absent at the implantation of the lateral seta.

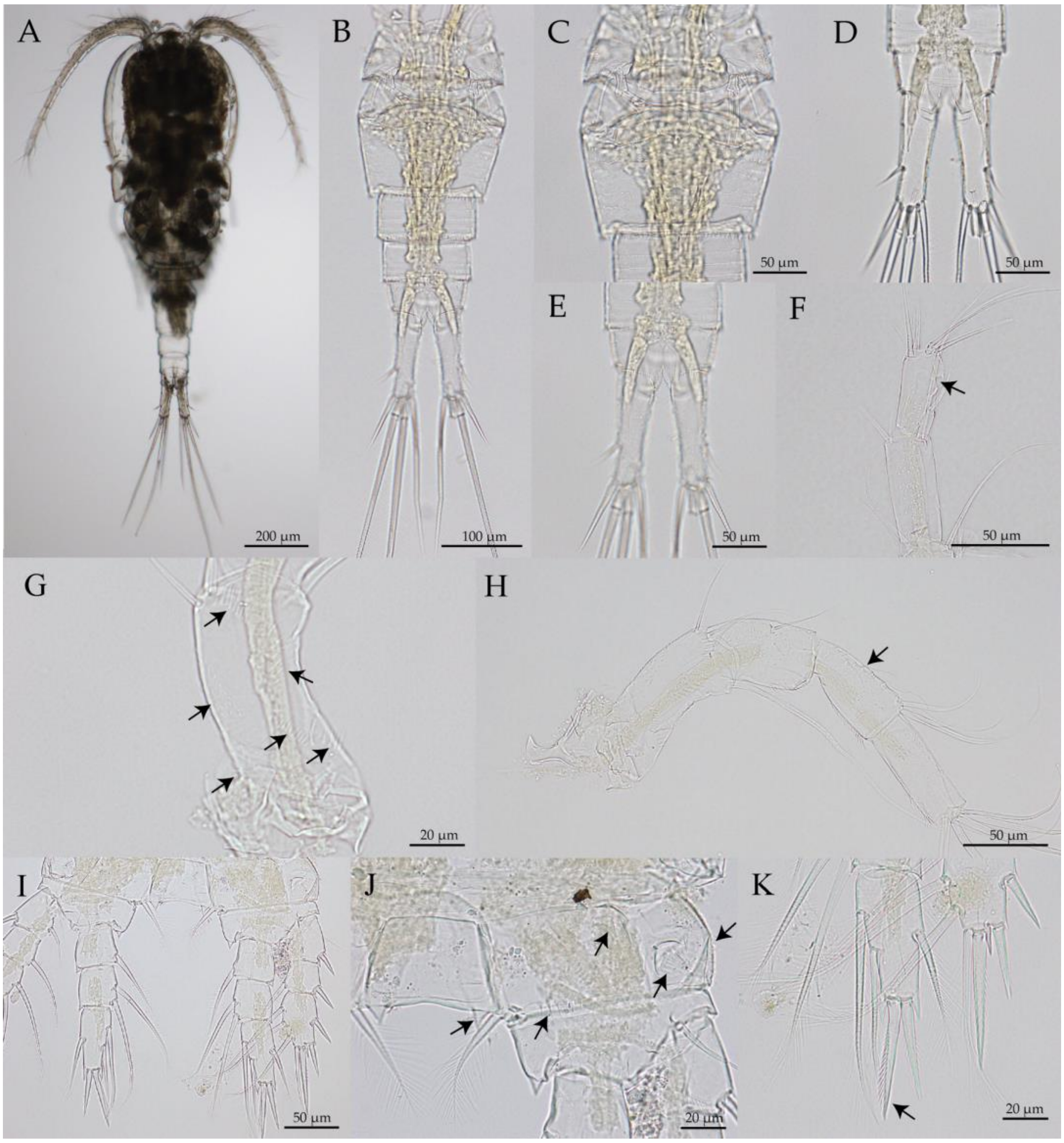

Figure 3. *Mesocyclops kayi* Holynska & Brown, 2003. Adult female (**A**) habitus, dorsal view; (**B**) urosome, ventral view; (**C**) pediger 5, genital double-somite and leg 5, ventral view; (**D**) anal somite and caudal ramus, dorsal view; (**E**) anal somite and caudal ramus, ventral view; (**F**) last segment of antennules; (**G**) basis of antenna, caudal view; (**H**) antenna; (**I**) leg 4; (**J**) intercoxal sclerite, coxa, and basis of leg 4; (**K**) apical spine of third endopod segment of leg 4. An explanation of the arrows is given in the text.

The antennule were 17-segmented, with a serrate hyaline membrane and with one notch on the last segment of the antennules (Figure 3F, arrow). The basis segment of the antenna had three setae, and the caudal surface of the basis was ornamented with the following: (i) long spinules on the lateral margin near the base, (ii) oblique row of large spinules next to the former group, (iii) longitudinal row of long spinules along the lateral margin, (iv) row of large spinules near the implantation of the mediodistal setae, (v) oblique row of minute spinules on the middle of the medial margin, and (vi) spinule on the distal of the segment (Figure 3H, arrow). The second endopodal segment of the antenna was ornamented with nine setae (Figure 3G, arrows).

The fourth swimming leg was as follows: the intercoxal sclerite was smooth on the caudal and frontal surfaces, and with two large and acute projections on the distal margin;

the caudal surface of the coxa were ornamented with the following: (i) 3–6 spinules near the distal margin, (ii) 2–5 spinules near the middle proximal margin, (iii) one oblique row of spinules on the laterodistal margin, and (iv) hairs on the lateral margin (Figure 3I,J, arrows); the basis had a row of hair on the inner distal margin and outer margin; the distal endopodal segment length was about 2.2–2.5 time as long as wide (mean = 2.3; n = 10), the inner apical spine of the distal endopodal segment length was about 1.0–1.4 times as long as the outer apical spine (mean = 1.3; n = 10), the lateral edge of the inner apical spine was smooth or with 1–4 teeth (Figure 3K, arrow).The fifth swimming leg was 2-segmented; the proximal segment had one lateral smooth seta; the distal segment had two apical setae: the spiniform inner apical seta was 0.9–1.2 times as long as the outer apical seta (mean = 1.2; n = 10) (Figure 3B,C).

3.2.2. Remark

Most morphological characteristics were consistent with the original description, but there were some that were slightly different. There were spinules at the implantation of the outermost terminal seta of the caudal ramus in our specimens, but they were absent in the original description. The ratio of the caudal ramus also differed, as the length was about 3.2–4.0 times as long in our specimens compared to 3.6–3.8 times in the original description. Despite the fact that there were few differences, the use of effective tools like molecular systematics and morphological examinations would aid in the confirmation of species status.

3.2.3. Ecological Distribution

This species was originally described in a copper mine and old fishpond in Burma. In the present study, 1–4 individuals L^{-1} were found in three rice fields, which consisted of both organic and chemical rice fields. The field measurements revealed the following environmental characteristics: water temperature was 25.6–33.2 °C, conductivity was 200.3–440 µs cm^{-1}, salinity was 0.09–0.18 ppt, total dissolved solids was 147–253 mg L^{-1}, dissolved oxygen was 2.55–17.22 mg L^{-1}, pH was 6.96–7.57, the water depth was 0.15–0.30 m, chlorophyll a was 3.17–79.33 µg L^{-1}, and phycocyanin was 0.11–3.45 µg L^{-1}.

3.3. Density, Relative Abundance, and Dominant Species

The results revealed different proportions of rotifers, cladocerans, and copepods in different rice fields. Notably, cladoceran had a higher relative abundance compared to the rotifers and copepods in all the rice fields, except rice field C, in which the copepods and rotifers were more prevalent (Figure 4). The highest cladoceran density was recorded in rice field E (5452 $\pm$ 6715 ind. L^{-1}) followed by rice field A (870 $\pm$ 1228 ind. L^{-1}) and rice field B (57 $\pm$ 74 ind. L^{-1}). There was a high proportion of rotifers in rice field C, with a density of 9.26 $\pm$ 74 ind. L^{-1}.

In addition, the results showed that each rice field shared some dominant species. Rotifers, including *Plationus patulus*, *Lecane papuana*, *L. bulla*, *Polyarthra* sp., and *Mytilina ventralis*, were dominant in most of the rice fields, except rice field A, which was dominated by the genus *Brachionus*, including *B. calyciflorus* s.l., *B. angularis*, and *B. quadridentatus* (Figure 5). Regarding cladocerans, *Ovalona cambouei*, *Diaphanosoma excisum*, *Ceriodaphnia cornuta*, *Moina micrura*, and *Moinodaphnia macleayi* were dominant in all the rice fields. Regarding copepods, cyclopoid copepods, including *Mesocyclops aspericornis*, *M. kayi*, *M. affinis*, *Microcyclops* sp. 1 and *Microcyclops* sp. 2, *Thermocyclops decipiens*, *T. rylovi*, and *Cryptocyclops* sp., were dominant in all the rice fields, whereas only the calanoid copepod, *Mongolodiaptomus botulifer*, was dominant in rice field E.

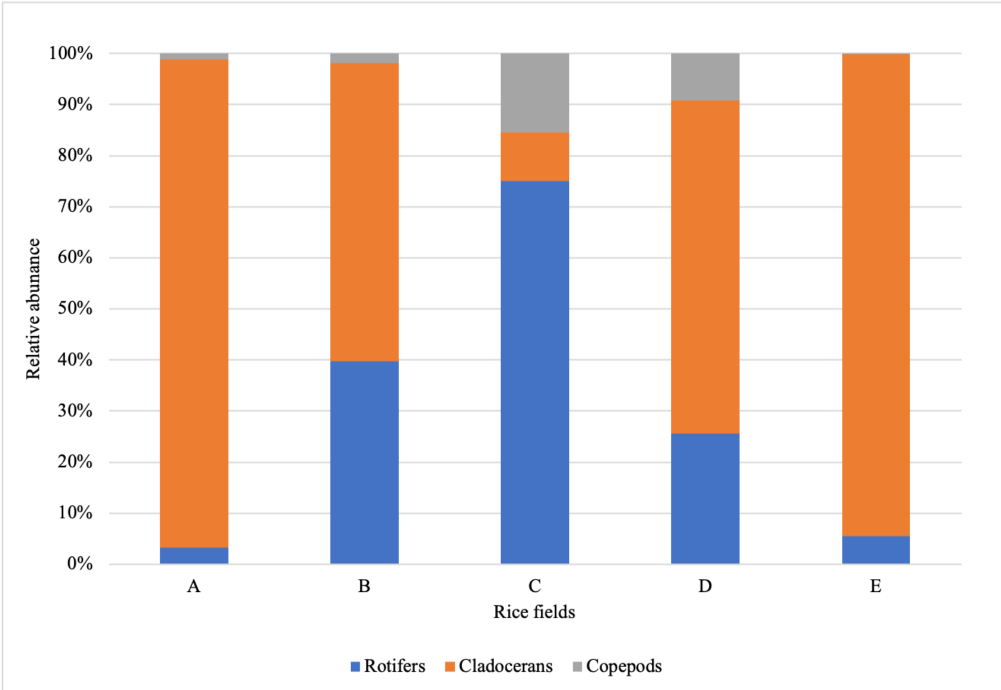

Figure 4. Relative abundance of each zooplankton group in rice fields A–E.

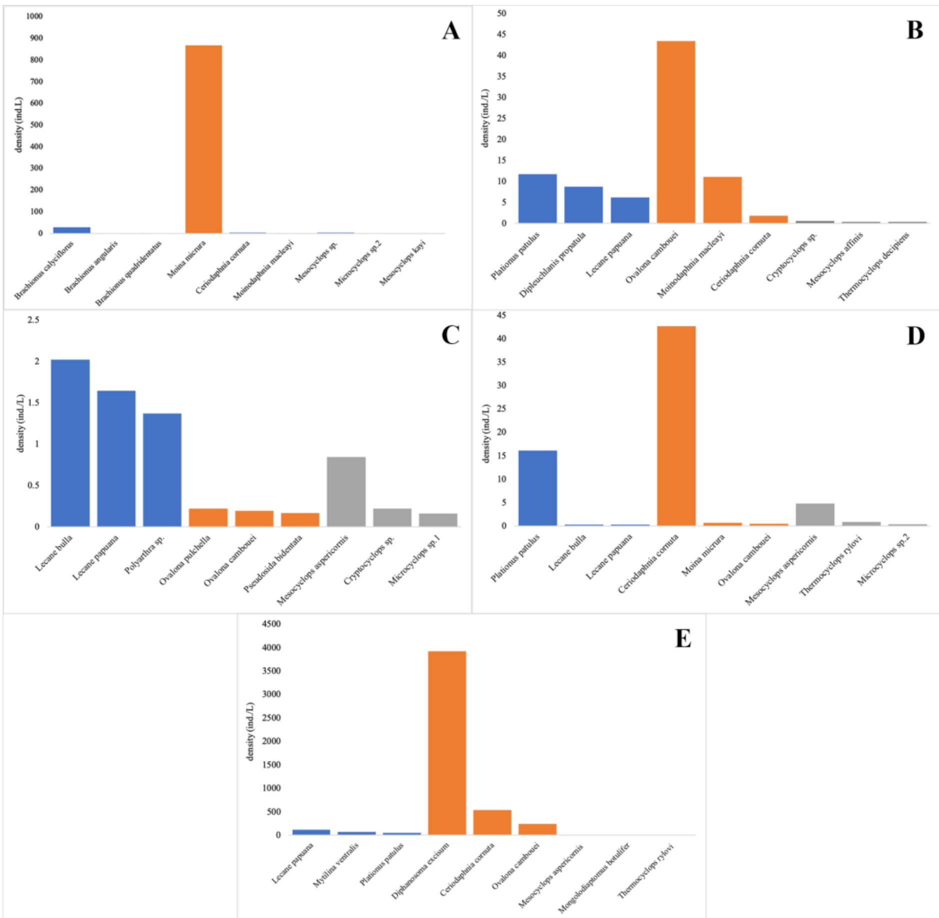

Figure 5. The three most dominant species of each zooplankton group in the rice fields (**A**–**E**). Blue = rotifers; orange = cladocerans; grey = copepods.

3.4. Similarity of Species Composition between the Present Rice Fields and Those in Previous Studies in Thailand

The Jaccard similarity index for the rice fields examined in this study was 0.31–0.85, mostly with less than 70% similarity (Table 3). In addition, the results showed fewer similarities between the species composition in the present rice fields and those from two previous studies (0.20–0.36) (Table 4).

Table 3. Jaccard similarity index among present studied rice fields.

Rice Fields	B	C	D	E
A	0.7	0.38	0.75	0.61
B	-	0.52	0.72	0.85
C	-	-	0.31	0.49
D	-	-	-	0.62
E	-	-	-	-

Table 4. Jaccard similarity index among previous and present zooplankton species composition.

	Central [5]	Northeast [11]
Central (present study)	0.36	0.20
Central	-	0.35
Northeast	-	-

3.5. Species–Environment Associations

CCA did not reveal statistically significantly variations in all species based on environmental variables. However, when analysing each zooplankton group separately, the results showed statistically significant differences in the rotifer (p = 0.01) and copepod (p = 0.03) but not in the cladoceran community (p = 0.26). For rotifers, the first two axes explained 86.4% of the total variance. Dissolved oxygen, chlorophyll a, salinity, and conductivity were important environmental variables associated with the first axis. Of these, dissolved oxygen and chlorophyll a showed a higher positive correlation with rice field A, which was dominated by *Brachionus calyciflorus* s.l. In addition, salinity and conductivity showed a higher positive correlation with rice field E, and *B. falcatus*, *Testudinella incisa*, *Mytilina ventralis*, *Lecane papuana*, *Dipleuchlanis propatula*, and *Plationus patulus* were dominant in this rice field (Figure 6). For copepods, the first two axes explained 84.4% of the total variance. Salinity and conductivity also showed a higher positive correlation with rice field E. Most copepods, including *Thermocyclops* sp. 1, *Thermocyclops operculifer*, *Phyllodiaptomus praedictus*, and *Mongolodiaptomus botulifer* were dominant in this rice field. In addition, chlorophyll a showed a higher positive correlation with rice field A. Some copepods, such as *Mesocyclops thermocyclopoides*, *Mesocyclops* sp., *M. kayi*, *Elaphoidella* sp., and *Microcyclops* sp. were highly distributed in this rice field (Figure 7).

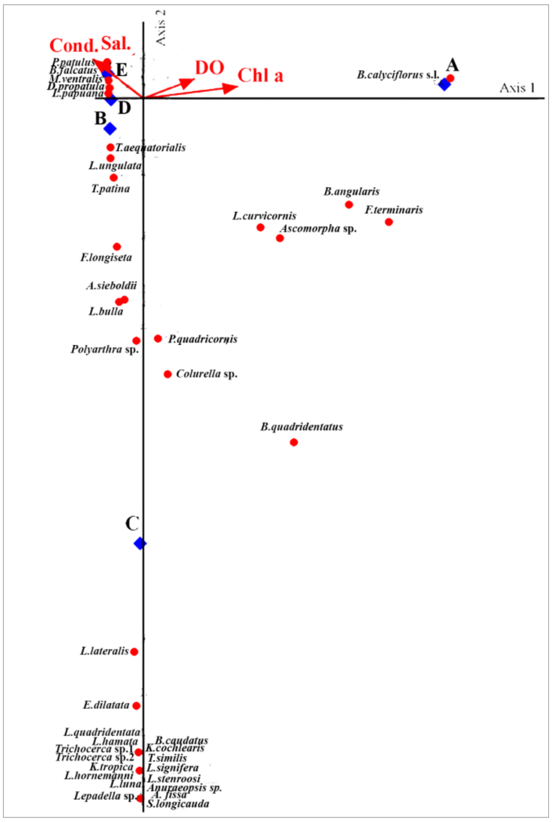

Figure 6. First two axes from the canonical correspondence analysis of rotifer species, rice field, and environmental variables. The analysis deals with 38 rotifer species from 10 samples related to 8 factors (Table 1). Vectors indicate the significant environmental variables ($p < 0.05$), (A–E) represent rice field A–E, red circles represent rotifer species.

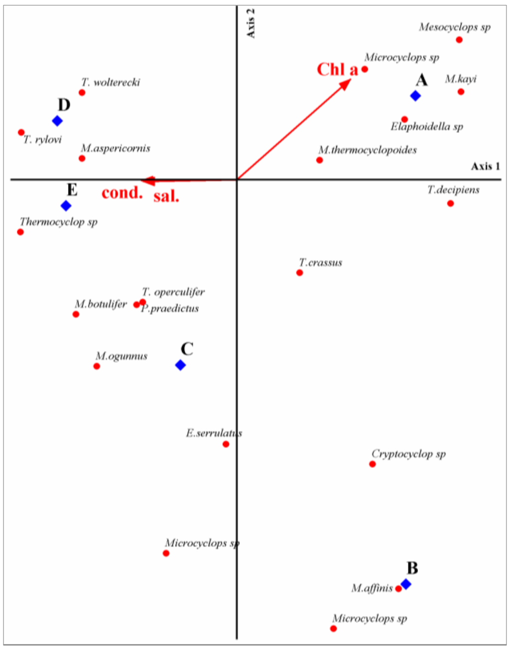

Figure 7. First two axes from the canonical correspondence analysis of copepod species, rice field and environmental variables. The analysis deals with 20 copepod species from 10 samples related to 8 factors (Table 1). Vectors indicate the significant environmental variables ($p < 0.05$), (A–E) represent rice field A–E, red circles represent rotifer species.

4. Discussion

One hundred zooplankton species were found in the rice fields in the present study. One of these, *Mesocyclops kayi*, increased the total number of copepods recorded in Thailand to 173 [30–32]. This is the first time this species has been found outside of its type locality [19]. Based on its current ecological distribution in copper mines, fishponds, and rice fields, it seems that this species prefers to live in certain habitats, so it would be interesting to learn more about its ecology. Moreover, the morphological characters of some other species, such as *Mesocyclops* sp. and *Thermocyclops* sp. 1, are inconsistent with any species in the genus, and they are possibly new species to science. Therefore, further studies including a combination of morphological characteristics and DNA taxonomy would help to confirm their taxonomic status. According to the Chao 2, Jacknife 1, and Jacknife 2 estimators, the number of species recorded was still far from the actual species richness. This suggests that many species have yet to be discovered in the rice fields, and more are expected to be found in these ecosystems.

Further, the number of species recorded in the present study was higher than that in a previous study on rice fields in Pathum Thani province, central Thailand which recorded a total of 88 species [6]. However, it was less than a recent report on rice fields in northeast Thailand, which recorded a total of 121 species [11]. It has been found that rice cultivation techniques, such as organic and chemical fields, the application of pesticides and/or herbicides, geographical conditions, and local environmental factors could affect the diversity of animals in rice fields, including zooplankton [10,11,33]. The results of this study support such findings, as the similarity of species composition was low among rice fields from different areas. Although the species richness among the five studied rice fields could not be compared in the present study due to inconsistencies in sampling efforts, the analysis of these fields, which are representative of rice fields in this area, showed a high diversity index and low evenness of zooplankton. The results indicated that, while there is a wide range of microhabitats for many species, the local environmental factors were more suitable for some species, such as *Diaphanosoma excisum*, *Moina micrura*, and *Ceriodaphnia cornuta*, which were found in high densities in most of the rice fields.

In addition, the presence of different microhabitats in different stages of the cultivation cycle, such as when there is more or less water or when the rice fields are covered with algae and vegetation or are barren, could also explain the specificity of the occurrence of zooplankton species [5]. Notably, 81% of all the species records were found in less than 50% of all the samples, and there were only a few species of copepods, *Mesocyclops aspericornis*, *Cryptocyclops* sp., and *Microcyclops* sp. 2, that were found in up to 85% of all the samples. *Lecane papuana*, *L. bulla*, and *Plationus patulus* were the predominant rotifer species in this study, confirming prior findings indicating that these species are widely distributed and can be found in diverse habitats [34,35]. Moreover, from a zoogeographical point of view, they are cosmopolitan species [36].

In addition, the high occurrence of *Ceriodaphnia cornuta*, *Moinodaphnia macleayi*, and *Diaphanosoma excisum* is consistent with previous studies that studied zooplankton in rice fields in south [4,5], central [6], and northeast Thailand [10,11]. These species were not only widely distributed in the samples but also showed relatively high densities, which is consistent with their short life cycle and their ability to produce a large number of offspring [37,38]. Moreover, these large-sized cladocerans are often good at escaping from predators like mysids and insects [36]. Among crustacean plankton, cladocerans have been found to be a powerful suppressor of rotifer densities and have greater clearance rates than rotifers, whereas copepods play a minor role [6,39,40]. Therefore, high cladoceran density results in the suppression of rotifer abundance [39]. Additionally, rotifers have a narrower food niche and range of food than cladocerans, so rotifer populations can be limited by cladocerans [39]. Interestingly, there was a rich diversity of cyclopoid copepods in the present study, with 26 species being investigated, and 11 of these species being recorded for the first time in rice fields in Thailand. In addition, up to 21 copepod species were found per sample (mostly 13–17 species), whereas 1–18 species per sample have been typically found in other types of habitats in Thailand (e.g., ponds, swamps, and lakes) [41–44]. The richest habitat that was previously reported was Thale Noi Lake in southern Thailand, consisting of 18 copepod species per sample [41–44]. However, Thale Noi is large and contains a variety of algae and aquatic plants that are suitable for many zooplankton and other fauna [44,45]. Therefore, the rice field ecosystem, a small temporary habitat characterised by its relatively high richness, is exceptionally interesting. Cyclopoid copepods, which are capable of storing sperm and undergoing rapid individual development, exhibit a unique survival strategy during drought periods, when they reside in the sediment of temporary habitats. As a result, the initial and early colonisation by cyclopoids is likely to have a profound effect on the propagule bank and the composition of future plankton communities as the habitat refills [46]. This leads to swift dispersal and the eventual dominance of this group. Another explanation is the abundance of food choices within rice fields, ranging from bacteria and phytoplankton to smaller zooplankton and mosquito larvae, including *Anopheles* [47].

Each zooplankton group was found in a similar proportion based on its relative abundance in rice field C, whereas either rotifers or cladocerans were dominant in the other rice fields. This was probably because there was a large amount of water in this rice field throughout the entire rice cultivation period (Table 1), which is long enough for many species of rotifers, cladocerans, and copepods to complete their life cycles [48–50]. Moreover, while *Brachionus calyciflorus* s.l., which might be more than one species in this study, was found in all the rice fields, its density was relatively high in rice field C due to the higher levels of dissolved oxygen and chlorophyll a. This finding corresponds with previous reports showing that this species is widely spread in a range of environments [51] and reaches high densities when dissolved oxygen levels exceed 5.0 mgL^{-1} [52]. In addition, it has been reported that an increase in saline results in a decrease in the species richness and diversity of rotifers [53,54]. Some species, such as those in the genera *Brachionus* and *Testudinella*, are halophilic, meaning that they are able to distribute in saline water [55]. The presence of *Brachionus falcatus* and *Testudinella incisa* in rice field E, which had higher salinity (up to 0.58 psu) and conductivity (up to 1449.9 $\mu S\ cm^{-1}$) levels, confirms previous findings.

In addition, although rice fields are large agroecosystems distributed throughout every part of Thailand, there are few studies on diversity in this type of freshwater habitat, although there are many studies concerning other types of freshwater habitats throughout Thailand [31,56,57]. In the last 15 years, there have only been five papers published on four sampling sites from the central, south, and northeast regions [4–6,10,11] and only two studied all rotifers, cladocerans, and copepods [6,11]. The similarities of species composition vary considerably among rice fields, as each field has its own characteristics, including environmental characteristics and rice cultivation procedures. Some areas have a short period of maintaining water levels, while other areas have a long period. Moreover, a recent study found that rice fields without pesticide applications exhibit a higher diversity of zooplankton [6,7]. In addition, besides the water column, the muddy bottom of flooded rice fields also harbours a variety of zooplankton [58].

5. Conclusions

The findings of the present study support those of previous reports showing that, for various types of niches, rice field ecosystems support a high diversity of organisms. However, it is expected that many species have yet to be found in rice fields as the number of current records is far from the actual species richness and more species will be discovered. Thus, more research is needed to reveal hidden species in rice field ecosystems. In addition, some zooplankton species showed a relationship with certain environmental variables. Therefore, they can be considered a bioindicator for monitoring changing environmental conditions and the effects of contamination, for example, by pesticides and microplastics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15101054/s1, Figure S1: Representatives of the rotifers found in the present study. Figure S2: Representatives of the cladocerans found in the present study. Figure S3: Representatives of the copepods found in the present study.

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