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The Biodiversity of Freshwater Copepods in Five Provinces of Northern Thailand

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Abstract

The biodiversity and richness of copepod species in five provinces of northern Thailand, including Tak, Kamphaeng Phet, Sukhothai, Phetchabun, and Phitsanulok was studied. Eighty-nine samples were collected from 11 caves and 78 freshwater bodies from July 2021 to May 2022. A total of 22 species of calanoids, 12 cyclopoids, and 4 harpacticoids were found in the samples. Among the 89 sampling localities, 29 localities, comprising 4 caves and 25 freshwater habitats, were studied in both the pre-monsoon and post-monsoon seasons. A paired sample t-test showed that there was no significant difference in the environmental characteristics between the pre-monsoon and post-monsoon seasons. The comparison of the species richness between the pre-monsoon and post-monsoon seasons was carried out, and the diversity of copepods from the cave localities in northern Thailand was also shown in this study.

KEYWORDS: Freshwater copepod, Harpacticoida, Calanoida, Cyclopoida, northern Thailand

1 INTRODUCTION

Copepods are one of the most successful animals on earth, with over 14,000 known species. They are categorized under Phylum Arthropoda von Siebold, 1848; Subphylum Crustacea Brünnich, 1772; and Subclass Copepoda Milne Edwards, 1840. Copepods have a wide range of habitats, from pools of glacial meltwater to hot springs, and from hypersaline lakes to phytotelmata, and they comprise mostly planktonic, benthic, and groundwater communities (Boxshall and Defaye 2008). Copepods from the orders Calanoida, Cyclopoida, and Harpacticoida are found free-living in freshwater habitats.

In Thailand, the freshwater copepod diversity has been studied since 1981, and 109 species of freshwater copepods have been found, including 26 new species from temporary and permanent water bodies, including cave habitats (Janpong, and Sanoamuang, 2020). The diversity of copepod in Thailand was studied from the northern region (Proongkiat and Sanoamuang, 2008; Brancelj et al., 2010; Watiroyram et al., 2015), the north-eastern region (Sanoamuang, 1999; Reddy et al., 2000; Sanoamuang, 2001; Sanoamuang and Yindee, 2001; Sanoamuang and Athibai, 2002; Sanoamuang, 2004; Watiroyram et al., 2015), the southern region (Watiroyram et al., 2017; Boonyanusith et al., 2020), and central Thailand (Chittapun et al., 2009; Chullasorn et al., 2008; Boonyanusith et al.; 2013). The past studies about copepods in Thailand have focused mostly on the diversity and abundance of copepods in limnetic waters. This paper focuses on the diversity of copepod species in caves and freshwater bodies in the northern part of Thailand and their differences between the pre-monsoon and post-monsoon seasons.

2 RESEARCH OBJECTIVES

This study focuses on the diversity of copepods in the caves and freshwater bodies in Tak, Kamphaeng Phet, Sukhothai, Phetchabun, and Phitsanulok provinces in northern Thailand. The species richness of copepods from the freshwater bodies is compared between the pre-monsoon (July 2021) and the post-monsoon (February 2022).

3 LITERATURE REVIEW

Copepods are one of the most abundant metazoans on the planet because of their high densities in the worlds oceans (Reid and Williamson 2010). They belong to the subclass Copepoda, which is the largest entomostracean subclass with over 14,000 known species, and among them, 2,814 species have been reported from freshwater habitats (Boxshall and Defaye, 2008). Copepods can be found in a wide variety of habitats, including subterranean habitats such as karstic caves. About 1,000 copepod species have been recorded in subterranean habitats out of more than 2,800 species found in freshwater habitats around the world (Galassi, 2001; Galassi et al., 2009). The majority of these species belong to the Harpacticoida and Cyclopoida families (Boxshall and Defaye 2008). Cyclopoid copepods have been known as predators for mosquito larvae (Hutlburt, 1938; Lindberg, 1949; Bonnet and Mukaida, 1957; Fryer, 1957). Cyclopoids are used for large-scale biological control as most species are easy and inexpensive to mass culture (Støttrup and Norsker, 1997). Certain copepod species have the potential to be used as biological indicators for eutrophication on large scales as they are sensitive to different environmental variables (Matsumura-Tundisi and Tundisi, 2005). They also serve as an important link between phytoplankton primary production and higher-trophic-level consumers like fish, crustaceans, and whales (Kwok et al., 2015).

Cave habitats provide an especially rich and unique fauna belonging to the subterranean environment (Culver et al., 2004). Subterranean habitats usually host a higher proportion of geographically isolated taxa than surface environments (Gibert and Deharveng, 2002). The study on the diversity of freshwater copepods in Thailand and neighboring Southeast Asian countries primarily originated from the sampling of various freshwater resources such as ricefields, reservoirs, lakes, rivers, and temporary water bodies (Bricker et al., 1978; Boonsom, 1984; Lim and Fernando, 1985; Sanoamuang, 1999; Alekseev and Sanoamuang, 2006; Chaichareon and Sanoamuang, 2011; Sanoamuang and Dabseepai, 2021). Due to intensive sampling, the study of copepods from subterranean regions has increased. The study of copepod diversity in Thailand has been ongoing since 1981, and 109 species of freshwater copepods have been found, including 26 new species from temporary and permanent water bodies, including cave habitats (Janpong and Sanoamuang, 2020). These include 42 calanoid species (Dumont et al., 1996; Reddy and Dumont, 1998; Sanoamuang, 2001a; Sanoamuang, 2001b; Sanoamuang and Yindee, 2001; Sanoamuang and Athibai, 2002; Sanoamuang 2004; Sanoamuang and Teeramaethee, 2006; Proongkiat and Sanoamuang, 2008; Watiroyram and Sanoamuang, 2017; Sanoamuang and Watiroyram, 2018; Sanoamuang and Watiroyram, 2020), 8 cyclopoid species (Alekseev and Sanoamuang, 2006; Watiroyram et al., 2012; Watiroyram et al;., 2015a; Karanovic, Koomput and Sanoamuang, 2017; Boonyanusith, Sanoamuang and Brancelj, 2018; Watiroyram, 2018), and 6 harpacticoid species (Brancelj et al., 2010; Watiroyram

et al., 2015b; Watiroyram and Brancelj, 2016; Watiroyram et al., 2017).

4 RESEARCH METHODOLOGY

4.1 Study Area

The study area of this research includes Tak, Kamphaeng Phet, Sukhothai, Phetchabun, and Phitsanulok provinces in the northern part of Thailand (Figure 1). The samples were taken from 10 types of freshwater habitats (Figure 2), comprising temporary ponds, reservoirs, permanent ponds, rivers, lakes, caves, canals, rice fields, roadside canals and irrigation canals.

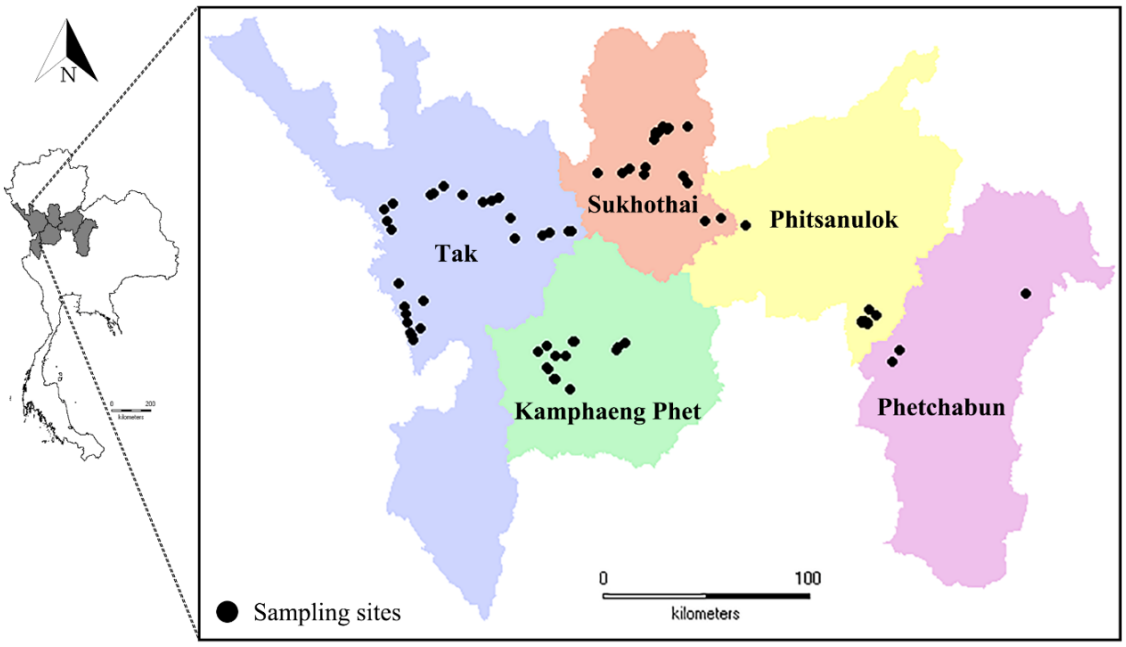

Figure 1: *The 89 sampling sites in five provinces in northern Thailand.*

4.2 Study Period

Copepods were collected quantitatively during the rainy season (July, 2021), winter (February, 2022), and dry season (May, 2022) from 89 localities in Tak, Kanphaeng Phet, Sukhothai, Phetchabun, and Phitsanulok provinces.

4.3 Environmental variables

Physical characteristics and chemical water parameters were measured immediately at each sampling site, including altitude, water temperature, total dissolved solids (TDS), pH value, and electrical conductivity (EC), with a multi-parameter probe (HANNA HI98129 Multi-Parameter Pen Water Quality Analyzer).

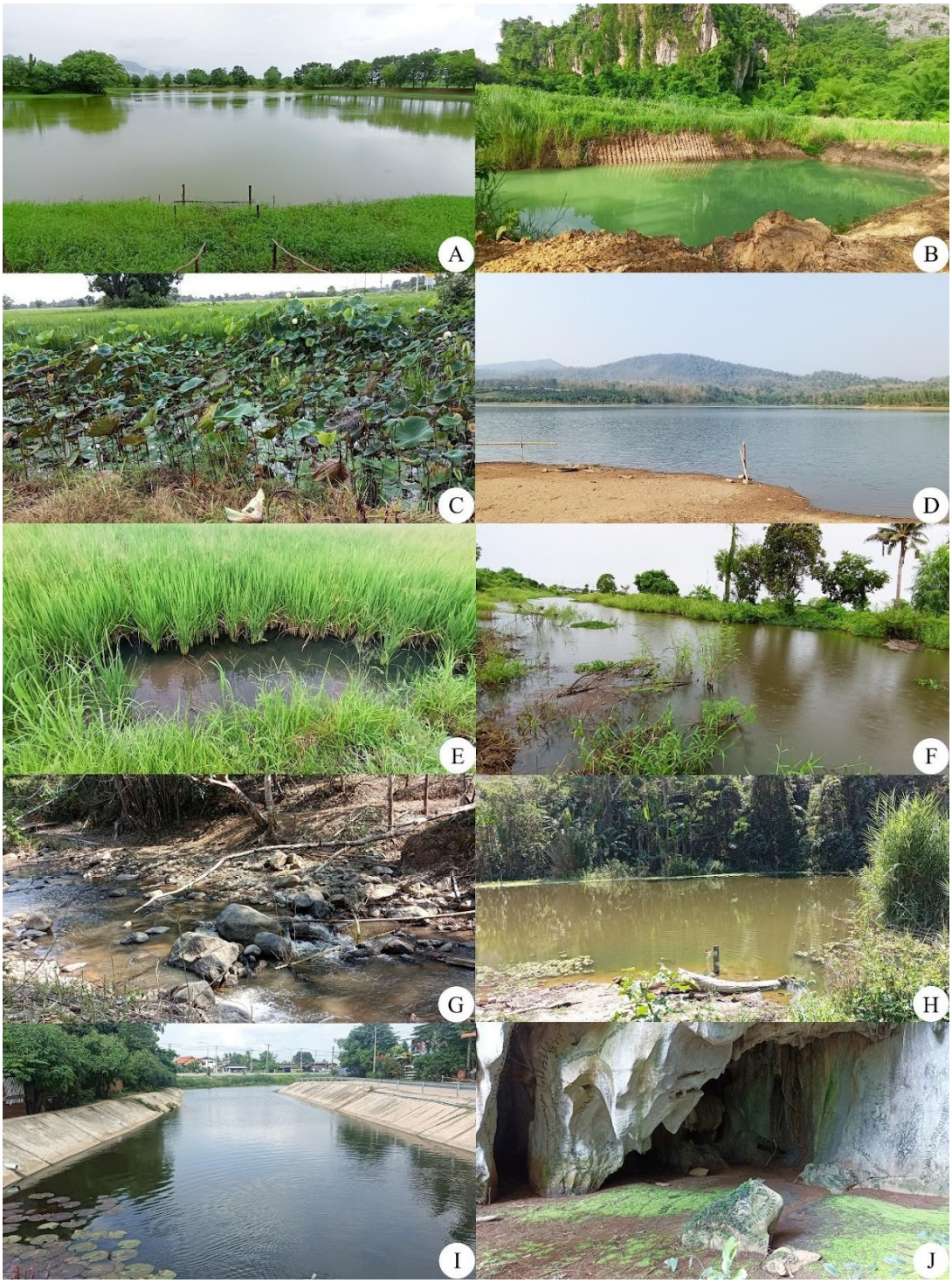

Figure 2: *Photographs of different sampling sites: A: river, B: pond, C: roadside canal, D: lake, E: rice field, F: temporary pond, G: river, H: permanent pond, I: irrigation canal, and J: cave.*

4.4 Sampling and Identification

The study areas were distributed throughout Tak, Kamphaeng Phet, Sukhothai, Phetchabun, and Phitsanulok provinces. Samples were collected from 11 caves and 78 freshwater bodies (Table 1). The samples were collected by water filtration through a plankton net with a 60 ţm mesh size in an oblique direction. The collected samples were transferred into plastic bottles and fixed with a 4% formaldehyde solution. The samples were examined under a stereomicroscope for sorting. Specimen identification was made under an Olympus CH30 compound microscope at 10-100x magnification and using current taxonomic keys (Ueda and Reid, 2003; Sanoamuang, 2002; Sanoamuang and Dabseepai, 2021; Alekseev and Sanoamuang, 2006).

4.5 Data Analysis

The paired sample t-test was applied to compare the difference between the species richness and water parameters between the pre-monsoon and post-monsoon seasons. The relative occurrence of each copepod species was calculated based on the number of sampling localities where the species were found. The Sørensen-Dice index (Cs) (Magurran, 2004) was applied to calculate the similarity between the species richness of the pre-monsoon and post-monsoon seasons.

$$C_c = 2_a 2_{s+b+c}$$

a = the total number of species that are shared by the assemblages 1 and 2.
b = the number of species that are found only in the assemblage 1.
c = the number of species that are found only in the assemblage 2.

5 RESULTS

5.1 Species diversity

One hundred and seven samples were collected from 11 caves and 78 freshwater bodies from July 2021 to May 2022. A total of 22 species, comprising 6 calanoids from 5 genera of the family Diaptomidae, 12 cyclopoids from 4 genera of the family Cyclopoidae, and 4 harpacticoids from 2 genera of the family Canthocamptidae, were identified from the samples. The list of copepod species recorded during this study is shown in Table 1.

According to the calculated relative occurrence, the most common species was Mesocyclops ogunnus Onabamiro, 1957 (22.68%), followed by Mongolodiaptomus botulifer (Kiefer, 1974) (19.59%), and Mesocyclops thermocyclopoides Harada, 1931 (18.56%). Neodiaptomus schmackeri (Poppe & Richard, 1893), Bryocyclops maewaensis Watiroyram, Brancelj & Sanoamuang, 2012, Thermocyclops sp., Attheyella (Canthosella) thailandica (Borutzky, 1967), and Attheyella (Canthosella) vietnamica (Borutzky, 1967) occurred in 1.03% of the collected samples. In total, 22 species were found: 11 species were found in both the pre-monsoon and post-monsoon seasons, while 8 species were found only in the pre-monsoon season and 3 species in the post-monsoon season. The Sørensen-Dice index (Cs) of the two seasons was

Table 1: *List of copepod species recorded from the freshwater localities with their habitat types and distribution in the freshwater habitats from five provinces of Northern Thailand. Habitat types are represented as Tp = temporary pond, Re = reservoir, Pp = permanent pond, Ri = river, L = lake, Ca = cave, C = canal, Rf = rice field, Rc = roadside canal and Ir = irrigation canal. Species marked with an asterisk (*) are species recorded from the same sites in both pre-monsoon and post-monsoon seasons.*

No	Species list	Hapitat Types										Pre-monsoon	Post-monsoon	Number of Sites Recorded	Relative occurrence (%)
		Tp	Re	Pp	Ri	L	Ca	C	Rf	Rc	Ir				
	Order Calanoida														
1	Eodiaptomus sanoamuangae (Reddy & Dumont, 1998)					+						-	+	1	1.03
2	Mongolodiaptomus botulifer (Kiefer, 1974)	+	+	+	+		+	+	+	+		+	+	19	19.59
3	Mongolodiaptomus calcarus (Kikuchi, 1936)		+	+	+				+	+		+	+	10	10.31
4	Neodiaptomus schmackeri (Poppe & Richard, 1893)									+		+	-	1	1.03
5	Phyllodiaptomus (Ctenodiaptomus) praedictus praedictus Dumont& Ranga Reddy, 1994	+		+			+		+	+		+	+	15	15.46
6	Vietodiaptomus blachei (Brehm, 1951)			+	+				+	+		-	+	4	4.12
	Order Cyclopoida														
7	Bryocyclops maewaensis Watiroyram, Brancelj & Sanoamuang, 2012						+					-	+	1	1.03
8	Mesocyclops affinis van de Velde, 1987	+		+	+	+			+	+	+	+	+	11	11.34
9	Mesocyclops aspericornis (Daday, 1906)			+		+			+	+		+	+	8	8.25
10	Mesocyclops ogunnus Onabamiro, 1957	+		+	+	+	+		+	+		+	+	22	22.68
11	Mesocyclops thermocyclopoides Harada, 1931		+	+		+			+	+		+	+	18	18.56
12	Microcyclops. varicans varicans (Sars G.O., 1863)	+							+			+	+	3	3.09
13	Paracyclops sp.											+	+	2	2.06
14	Thermocyclops decipiens (Kiefer, 1929)	+	+	+	+		+			+	+	+	+	19	19.59
15	Thermocyclops operculifer Kiefer, 1930	+			+					+	+	+	+	10	10.31
16	Thermocyclops vermifer vermifer Lindberg, 1935	+			+			+			+	+	-	8	8.25
17	Thermocyclops sp.											+	-	1	1.03
18	Tropocyclops sp.											+	-	3	3.09
19	Attheyella (Canthosella) thailandica (Borutzky, 1967)							+				+	-	1	1.03
20	Attheyella (Canthosella) vietnamica (Borutzky, 1967)							+				+	-	1	1.03
21	Elaphoidella bidens decorata (Daday, 1901)							+				+	-	2	2.06
22	Elaphoidella sp.														

0.67, which means that there is 67% similarity between the species of the pre-monsoon and post-monsoon seasons.

5.2 Seasonal occurrence

Environmental variables including water temperature $(°C)$, pH, total dissolved solids (TDS) (mgL^{-1}), and electrical conductivity $(\mu Scm^{-1}$) were measured at each sampling site and presented in Table 3. The pair-sample t-test indicated that there was no significant difference $(p > 0.05)$ in water parameters between the pre-monsoon and post-monsoon seasons.

Table 2: *Environmental variables and their means, standard deviations, and range were measured for both pre-monsoon and post-monsoon seasons.*

Water Parameters	Pre-Monsoon	Post-Monsoon
Water Temperature (C)	27.21 ś 2.21	28.38 ś 2.76
	(23.1-31.4)	(22.3-32.7)
pH	8.72 ś 1.62	7.75 ś 0.64
	(7.3-11.1)	(6.88-9.3)
Total dissolved solids TDS (mg L-1)	124.35 ś 67.12	168.28ś 81.73
	(61-242)	(49-295)
Electrical conductivity (ţS cm-1)	241.35 ś 131.22	337.14 ś 181.70
	(120-476)	(9-594)

A total of 29 sites, comprising 25 freshwater localities and 4 caves, were collected in both the pre-monsoon and post-monsoon seasons. Among them, 12 sampling localities could not be sampled in the post-monsoon season as the water had dried out. The number of copepod species found in each type of habitat between the pre-monsoon and post-monsoon seasons was also compared in Figure 2. The habitat type of rice fields showed the highest species richness with 8 species in the pre-monsoon season, while the habitat of permanent ponds followed with 7 species in the pre-monsoon season and the habitat of roadside canals had the same number of species. The habitats, reservoir, and river had no species in the post-monsoon season, and the lake habitat showed no species in the pre-monsoon season.

5.3 The diversity of copepod species from the cave habitats in Northern Thailand.

Total four species of Order Harpacticoida, namely *Elaphoidella bidens decorata, Elaphoidella* sp., *Attheyella (Canthosella) thailandica* and *Attheyella (Canthosella) vietnamica* were found from the cave habitats. Some of the cave habitats have plenty of water, although some caves only have dripping water from the cave ceilings. The common calanoid copepod species, such as *Mongolodiaptomus botulifer* and *Phyllodiaptomus preadictus*, were also found in some cave localities. Cyclopoid copepod species such as *Thermocyclpos* sp. (Tham Pha Kong Cave), *Tropocyclops* sp. (Phra kay Kaen Cave and Mae La Ong Nam Thip Cave), and *Paracyclops* sp. (Tham Yai Cave) were also recorded. But because of the insufficient amounts of zooplankton samples and characteristics, the samples were unable to identify into species name.

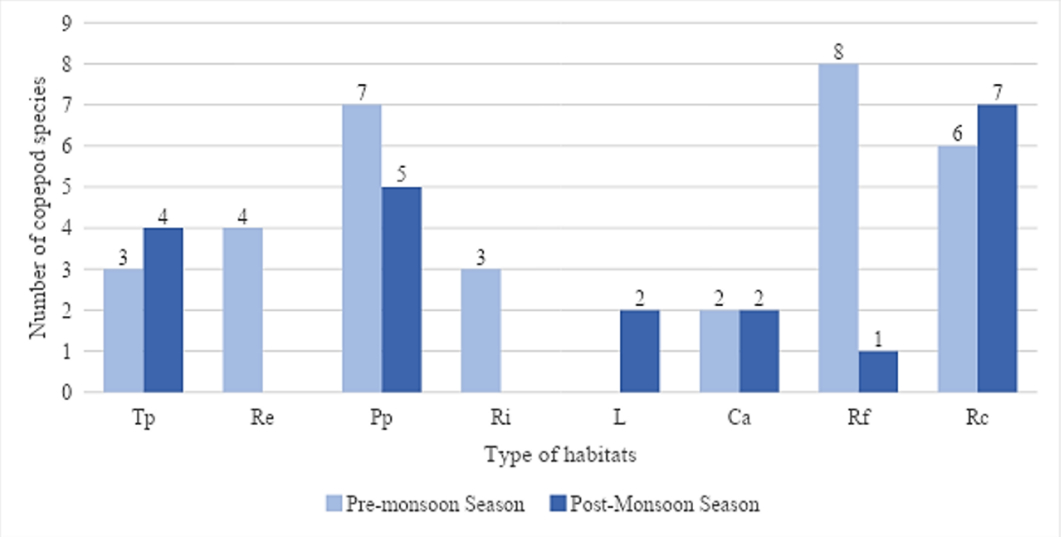

Figure 3: *Comparison of species richness in each type of habitat between the pre-monsoon and post-monsoon seasons.*

6 DISCUSSION AND CONCLUSION

This study described the diversity of freshwater copepods in freshwater bodies and cave habitats of northern Thailand. A total of eighty-nine samples were collected from both the caves and freshwater habitats in Tak, Kamphaeng Phet, Sukhotahi, Phetchabun, and Phitsanulok provinces. According to the relative occurrence of copepod species in different types of habitats, *Mesocyclops ogunnus* is the most common species of copepod recorded during this study, followed by *Mongolodiaptomus botulifer* and *Mesocyclops thermocyclopoides*. Although the study of freshwater diaptomid copepods in Thailand has been documented since 1981 (Lai and Fernando, 1980, 1981; Reddy et al., 1998; Sanoamuang, 1999), Eodiaptomus species were only recently recorded. The diaptomid copepod, *Eodiaptomus sanoamuangae*, was first recorded from a roadside canal in Khon Kaen Province, northeastern Thailand (Reddy and Dumont, 1998). In previous studies, this species was recorded not only from the canals but also from lakes and swamp localities in north and northeast Thailand (Sanoamuang, 2001). During this study, *Eodiaptomus sanoamuangae* was found in a lake in Tak Province in the post-monsoon season.

The differences in species richness between the pre-monsoon season and post-monsoon season were also investigated. According to the calculated Sørensen-Dice index (Cs) (=0.67), the similarity of species richness between the two periods was 67% similar, with 11 shared species out of 22 species in both seasons. Among the 89 sampling localities, 29 localities, including 4 caves and 25 freshwater localities, were studied in both the pre-monsoon and post-monsoon seasons. The environmental characteristics of these localities, including water temperature, pH value, total dissolved solids, and electrical conductivities, were measured and compared for both the pre- and post-monsoon seasons. A pair-sample t-test was applied to compare the water parameters and found that there was no significant difference between the two periods ($p > 0.05$).

Table 3: *List of copepod species found in the cave habitats of northern Thailand.*

No	Species List	Code of the Cave
Order Calanoida		
1	Mongolodiaptomus botulifer (Kiefer, 1974)	CPK, CML
2	Phyllodiaptomus praedictus (Poppe & Richard, 1893)	CTT
Order Cyclopoida		
3	Bryocyclops maewanensis Watiroyram, Brancelj & Sanoamuang, 2012	CPK
4	Paracyclops sp.	CSP, CML, CTY
5	Thermocyclops sp.	CTP
6	Tropocyclops sp.	CPK, CTL, CML
Order Harpacticoida		
7	Attheyella (Canthosella) thailandica (Borutzky, 1967)	CPD
8	Attheyella (Canthosella) vietnamica (Borutzky, 1967)	CTL
9	E. biden dercorata (Daday, 1901)	CTP, CTL
10	Elaphoidella sp.	CML

CPK= Phra kay Kaen Cave, CSP= Sang Phet Cave, CTP= Tham Pha Kong Cave, CML= Mae La Ong Nam Thip Cave, CTY= Tham Yai (Nakharat) Cave, CPD= Cave Padaeng, CTL= Thamlom Thamwong Cave

Copepods are one of the most successful metazoans in subterranean habitats, where they are found in saturated as well as unsaturated zones in karstic aquifers (Boonyanusith et al., 2018). In this study, a total of 10 species of copepods from three orders, Order Calanoida, Cyclopoida, and Harpacticoida were recorded from cave localities. The diaptomid copepods, *Mongolodiaptomus botulifer* and *Phyllodiaptomus praedictus*, which are the common calanoid copepods, are usually found in surface waterbodies. These species might be washed down to the caves during the rainy season. Four species of copepods from three genera of the Order Harpacticoida were found in the caves in the present study. The genus *Attheyella* has a wide range of habitats and is also found in groundwater (Chang and Kim, 1992). The species *Attheyella (Canthosella) thailandica* is widely distributed in Thailand and is the second member of the genus to be found in this country, after *Attheyella (Canthosella) vietnamica*, which has similar characteristics to it. During this study, the canthocamptid copepod *Attheyella (Canthosella) thailandica* was found at the site CPD, and the species Attheyella (Canthosella) vietnamica was found at the site CTL in the pre-monsoon season. This species was first described from a cave water reservoir in Vietnam, but it was also found in Thailand. The species was collected from the pools filled by percolating water in Tham Bot Wangna cave (Phitsanulok Province) (Watiroyram et al., 2015a). In addition, the species *Ellaphoidella biden ecorate* was found in CTP and CTL in the pre-monsoon season. The genus *Elaphoidella* is distributed worldwide, and its representatives are commonly found in the karstic environments of Thailand (Watiroyram et al., 2015). Copepods from the order Cyclopoida were found in caves more frequently than harpacticoid copepods in this study. Copepods from the genera *Thermocyclops*, *Tropocyclops*, and *Paracyclops* were discovered in caves, but the copepods could not be identified into exact species names due to an insufficient sample size and a lack of distinguishing characteristics.

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