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### Comparative evaluation of phytoplankton-based indices for water quality assessment in tropical lentic ecosystems of Thailand

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### ABSTRACT

Phytoplankton are widely acknowledged as sensitive and effective bioindicators for water quality assessment due to their rapid and quantifiable responses to environmental changes. This study assessed the performance of two phytoplankton-based indices: Palmer's algal pollution index (API) and the applied algal research laboratory - phytoplankton score (AARL-PP score). Water samples were collected from 264 sites across 50 lentic water bodies in four regions of Thailand during April and May 2024, and analyzed for chlorophyll-a ( $\mu$ g/L), total nitrogen (TN,  $\mu$ g/L), and total phosphorus (TP,  $\mu$ g/L). The results show that the mean API scores (± SD) across trophic categories were oligotrophic = 27, mesotrophic =  $16 \pm 7.11$ , eutrophic =  $18 \pm 6.26$ , and hypereutrophic =  $16 \pm 6.51$ . Corresponding AARL-PP scores were oligotrophic = 7.67, mesotrophic =  $6.00 \pm 1.23$ , eutrophic =  $7.37 \pm 1.01$ , and hypereutrophic =  $7.40 \pm 0.84$ . Although API values showed no significant differences among trophic groups (Tukey's HSD test), suggesting limited sensitivity to nutrient gradients, AARL-PP scores demonstrated significant distinctions between mesotrophic and both eutrophic and hypereutrophic systems. Furthermore, AARL-PP score exhibited a stronger correlation with the trophic state index (TSI) ( $R^2 = 0.0946$ ) than API ( $R^2 = 0.045$ ). As one of the first large-scale, regionally diverse comparisons of phytoplankton indices and nutrient-based indicators in tropical lentic ecosystems, this study addresses a critical research gap by integrating chemical and biological assessments and applying a locally developed index. The findings highlight the ecological relevance and practical utility of AARL-PP score and provide valuable guidance for selecting bioassessment tools tailored to tropical freshwater environments, supporting future water resource management and ecological monitoring in Thailand and similar regions.

**Keywords:** applied algal research laboratory – phytoplankton score, Palmer's algal pollution index, eutrophication, phytoplankton, trophic state index, water quality.

### INTRODUCTION

Phytoplankton are microscopic, photosynthetic organisms that inhabit both freshwater and marine ecosystems worldwide. They play a fundamental role as primary producers at the base of aquatic food webs by converting solar energy into chemical energy through photosynthesis, thereby supporting higher trophic levels including zooplankton, fish, and larger aquatic animals (Jahan et al., 2023). The diversity and structure of phytoplankton communities are highly responsive to environmental changes, particularly in relation to nutrient concentrations, organic pollutants, and various physicochemical parameters such as pH, temperature, dissolved oxygen, and water transparency (Zhang et al., 2021). Due to their sensitivity and rapid response to environmental fluctuations, phytoplankton are widely utilized as biological indicators for assessing water quality and pollution levels (Ugya et al., 2025). Analyzing phytoplankton community composition provides valuable insights into the long-term impacts of pollution, which are often undetectable through single-time-point measurements of physical or chemical parameters. This is because phytoplankton communities undergo dynamic changes in response to sustained environmental pressures [Essa et al. 2024]. For instance, a clear example of this sensitivity can be observed in tropical oxidation pond systems like those in Laem Phak Bia, where unialgal cyanobacteria blooms (e.g., *Microcystis* spp.) are frequently reported. This case illustrates the direct response of phytoplankton communities to nutrient enrichment and environmental conditions (Chaichana and Dampin, 2016).

In recent decades, a variety of bioindices have been developed to evaluate water quality based on phytoplankton assemblages. One of the earliest and most widely recognized is the Palmer's API, developed in 1959 by Charles M. Palmer (Palmer, 1969). This index evaluates the abundance of phytoplankton genera that are tolerant of high levels of organic pollution, with cumulative scores indicating degrees of water pollution. The simplicity of the Palmer index allows for rapid field-based assessments. Although the Palmer's API was originally developed using phytoplankton data from temperate freshwater systems in the United States (Palmer, 1969), its applicability has extended beyond its initial context. The index has been successfully utilized in various tropical and subtropical environments to assess organic pollution, demonstrating its flexibility and potential for broader ecological relevance. For example, Noel and Rajan (Noel and Rajan, 2015) used the API in conjunction with physicochemical analysis to assess the water quality of the Vaigai River in India, finding that the index reliably indicated elevated organic pollution levels. These studies underscore the adaptability of the API across different geographic contexts but also highlight the importance of regional calibration to improve ecological relevance in tropical regions like Thailand.

In contrast, the applied algal research laboratory – phytoplankton score (AARL-PP score) was developed in the context of Thailand's tropical freshwater environments (Peerapormpisal et al., 2007). This index groups phytoplankton according to their associations with nutrient levels and assigns scores reflecting trophic conditions and water quality status. The AARL-PP score has demonstrated strong correlations with chemical indices such as chlorophyll-a concentration and the levels of key nutrients (nitrogen and phosphorus), achieving an accuracy of up to 95% in classifying trophic states (Chaipiputnakhajorn and Gunbua, 2023). As such, it is considered highly suitable for assessing water quality in tropical environments characterized by high biodiversity and unique climatic conditions. A study conducted by Yossan and Moonsin (Yosaan and Moonsin, 2015) at Huay Samran in Sisaket Province, Thailand employed dominant phytoplankton genera to assess water quality and found that Oscillatoria and Closterium were strongly associated with elevated biochemical oxygen demand (BOD), an indicator of organic pollution. This case highlights the ecological validity of using tropical-specific genera in water quality assessments and illustrates the practical relevance of phytoplankton-based tools like AARL-PP score within Thai freshwater ecosystems.

Nevertheless, while both the Palmer pollution index and the AARL-PP score have proven useful for phytoplankton-based water quality assessment, further comparative studies are needed to determine which index more accurately reflects actual water conditions in Thailand. This consideration becomes particularly important when compared against chemical-based indices such as the trophic state index (TSI), which is derived from direct measurements of parameters including chlorophyll-a, total nitrogen, and total phosphorus (Paulic et al., 1996). Therefore, the objective of this study is to evaluate the potential of phytoplankton as bioindicators of lentic water quality in Thailand by comparing the outputs of two phytoplankton-based bioindices, Palmer's algal pollution index and the AARL-PP score, with the trophic state index. To date, few studies have systematically compared multiple phytoplanktonbased indices with nutrient-based indicators in tropical lentic ecosystems. This study addresses this research gap by conducting the first largescale, regionally diverse evaluation of these indices across 50 freshwater bodies representing varied trophic states and ecological conditions in Thailand. By integrating chemical measurements with biological assessments and incorporating a locally developed scoring system (AARL-PP score), this study offers novel insights into the ecological relevance and practical utility of phytoplankton-based indices in tropical contexts. The findings aim to guide the selection and calibration of reliable bioassessment tools tailored to tropical freshwater ecosystems, supporting future water resource management and ecological monitoring efforts in Thailand and beyond.

#### **METHODS**

#### Selection of sampling area

This study was conducted across 50 lentic water bodies located in the Central, Eastern, Northeastern, and Northern regions of Thailand, as illustrated in Figure 1. These water bodies vary in size, ranging from small ponds to large reservoirs, and serve different purposes such as irrigation and agricultural use. They also exhibit a wide range of nutrient levels and eutrophication status. This diversity was intentionally included to ensure that the study covers the full spectrum of lentic water types found in Thailand. To classify the nutrient status and productivity of each site, the water bodies were categorized based on their TSI, following the method of Paulic et al. [Paulic et al. 1996]. During the sampling period in April-May 2024, average temperatures recorded were 31.32 °C in the Central region, 31.66 °C in the Northeastern region, 30.04 °C in the Eastern region, and

31.36 °C in the Northern region. The corresponding monthly rainfall levels were 4.11 mm, 3.84 mm, 5.39 mm, and 2.83 mm, respectively.

#### Water and phytoplankton sampling

Water and phytoplankton samples were collected from 50 lentic water bodies representing a range of nutrient levels. The sampling campaign, conducted at 264 points, included detailed stratification based on the size of the water bodies: 9 large-sized sites (9 samples each, totaling 81), 15 medium-sized sites (7 samples each, totaling 105), and 26 small-sized sites (3 samples each, totaling 78). This systematic approach ensured one sample per point during April and May 2024. Water sampling procedures and nutrient analyses (chlorophyll-a ( $\mu$ g/L), TP ( $\mu$ g/L), and TN ( $\mu$ g/L)) followed the methods described in Phonmat et al., (2025), where full methodological details are provided. The TSI was calculated



Figure 1. Locations of lentic water bodies across 4 regions of Thailand

following the method described by Paulic et al., (1996). The formulas used were as follows: TSI  $(chl-a) = 16.8 + [14.4 \times ln(chl-a)], TSI (TP) =$  $18.6 \times [\ln (TP \times 1000)] - 18.4$ , and TSI (TN) =  $56 + [19.8 \times \ln (TN)]$ . The overall TSI was then computed using the formula: TSI = [(TSI (chl-a))]+ (TSI (TN) + TSI (TP)) / 2) ] / 2. Based on the resulting TSI values, water bodies were classified into four categories: hypereutrophic (TSI > 70), eutrophic (TSI between 51–70), mesotrophic (TSI between 40-50), and oligotrophic (TSI < 40). Chl-a refers to chlorophyll-a, TN to total nitrogen, and TP to total phosphorus. The TSI values and the results of water body classification based on TSI were specified in (Phonmat et al., 2025).

In this study, we focus on the phytoplankton component. Phytoplankton samples were obtained by filtering surface water (20 liters) through 22  $\mu$ m plankton nets. The samples were preserved in 4% formaldehyde and stored at 4 °C. Phytoplankton identification up to species level and enumeration were carried out using a Sedgwick-Rafter counting chamber under a microscope, following the taxonomic reference by Ladda (Wongrat et al., 1987). The calculation of phytoplankton density followed the method described by Ladda and Sophon (Wongrat and Boonyapiwat,

Table 1. Pollution index scale

Pollution index	Status of pollution
0–10	Lack of organic pollution
10–15	Moderate organic pollution
15–20	Probable high organic pollution
> 20	Confirms high organic pollution

Note: Palmer (1969).

Table 2. Palmer's algal pollution index value

2003). Phytoplankton density (units per liter) was calculated using the formula: phytoplankton density (units per liter) =  $(N \times V2) / V1$ , where N is average number of phytoplankton counted per 1 milliliter (units), V1 is volume of water filtered through the plankton net (liters) and V2 is volume of water in the sample bottle (milliliters).

#### Palmer's algal pollution index (API)

The study categorized lake water samples as either high or low polluted with organic matter, based on algal populations using the Palmer's API (Palmer, 1969). Palmer identified a list of 20 algal genera that exhibit high tolerance to organic pollution, each assigned a specific pollution index score, and developed a pollution index scale (Table 1) and the Palmer's algal pollution index value (Table 2) as detailed below.

# Applied algal research laboratory – phytoplankton score (AARL-PP score)

The assessment of water quality using the AARL-PP score is a method for assigning scores to various phytoplankton groups based on their trophic levels in the water. This index was developed by Peerapornpisan et al. (2007). Each water body is assigned a score calculated by comparing it with reference standards of water quality. This method is a tool for assessing water quality by analyzing phytoplankton communities in conjunction with physical and chemical water quality parameters. The use of the AARL-PP score has been validated with 95% accuracy when compared to physical and chemical water quality indices. In Part 1, the water quality score is divided

Genus	Index value	Genus	Index value
Anacystis	1	Micractinium	1
Ankistrodesmus	2	Navicula	3
Chlamydomonas	4	Nitzschia	3
Chlorella	3	Oscillatoria	5
Closterium	1	Pandorina	1
Cyclotella	1	Phacus	2
Euglena	5	Phormidium	1
Gomphonema	1	Scenedesmus	4
Lepocinclis	1	Stigeoclonium	2
Melosira	1	Synedra	2

Note: Palmer (1969).

into six levels as follows: oligotrophic status, oligotrophic-mesotrophic status, mesotrophic status, mesotrophic-eutrophic status, eutrophic status, hypereutrophic status. The scores range from 1 to 10 (as shown in Table 3).

In Part 2, the assessment of water quality using phytoplankton-based indices involves a systematic scoring procedure. Initially, the dominant phytoplankton genus, determined by either the highest biovolume or visual prominence, is identified along with the second and third most dominant genera. To ensure ecological relevance, each selected genus must constitute more than 30% of the total phytoplankton biovolume. Subsequently, water quality scores are assigned to each genus based on trophic-level classifications as outlined in the phytoplankton score reference table (Table 4). The scores from the three selected genera are then averaged to yield a composite phytoplankton score. This average is compared against the established water quality classification thresholds (Table 3), thereby enabling the determination of the trophic status and overall water quality condition of the sampled site.

#### **Statistical analysis**

The relationships between the TSI, Palmer's algal pollution index, and AARL-PP score with various phytoplankton metrics were examined using correlation analysis in SPSS version 29.0.2.0 (licensed user). A correlation coefficient nearing +1 or -1 was interpreted as a strong positive or negative association, respectively. This study also employed regression analysis and the coefficient of determination to examine the relationship between biological index values and TSI values. To further assess differences among waterbody groups classified by TSI, data distribution was initially tested using the test of homogeneity of variance and welch's robust test of equality of means. Subsequently, a one-way ANOVA was

conducted, followed by Tukey's HSD post hoc test for multiple variable comparisons. Statistical significance was determined at a threshold of p < 0.05, indicating meaningful associations or differences. Conversely, a p-value  $\geq 0.05$  suggested no significant relationship or difference (SPSS Inc).

### RESULTS

# Water quality assessment using Palmer's algal pollution index

Assessment of water quality using the Palmer's API revealed that oligotrophic water bodies (O14M) exhibited the highest score of 27, indicating a confirmed high organic pollution. In contrast, the average scores for mesotrophic, eutrophic, and hypereutrophic water bodies were  $16 \pm 7.11$ ,  $18 \pm 6.26$ , and  $16 \pm 6.51$ , respectively, suggesting a probable high organic pollution (as shown in Table 5). The elevated Palmer pollution index score in the oligotrophic site suggests a discrepancy between trophic classification based on TSI and actual water quality conditions as detected by biological assessment.

# Water quality assessment using applied algal research laboratory – phytoplankton score

Water quality assessment using the AARL-PP score revealed that the oligotrophic site, classified by TSI, exhibited a surprisingly high score of 7.7, indicating poor water quality. Please note that only one oligotrophic water body was included in the study, therefore, no standard deviation (SD) is reported for this category. This score was even higher than the average AARL-PP scores observed in the mesotrophic, eutrophic, and hypereutrophic groups, which were  $6.00 \pm 1.23$ ,  $7.37 \pm 1.01$ , and  $7.40 \pm 0.84$ , respectively. These scores

Table 3. Standard water quality scores based on trophic levels for water quality assessment using the AARL-PP score

Score	Water quality based on trophic levels	Water quality
1.0–2.0	Low nutrient levels (Oligotrophic status)	Good
2.1–3.5	Low to moderate nutrient levels (Oligo-mesotrophic status)	Good to moderate
3.6–5.5	Moderate nutrient levels (Mesotrophic status)	Moderate
5.6–7.5	Moderate to high nutrient levels (Meso-eutrophic status)	Moderately poor
7.6–9.0	High nutrient levels (Eutrophic status)	Poor
9.1–10.0	Very high nutrient levels (Hypereutrophic status)	Very poor

Note: Peerapornpisan et al. (2007).

Genus	Score	Genus	Score	Genus	Score
Actinastrum	5	Dimorphococcus	7	Oscillatoria	9
Acanthoceras	5	Dinobryon	1	Pandorina	6
Amphora	6	Encyonema	6	Pediastrum	7
Anabaena	8	Epithemia	6	Peridiniopsis	6
Ankistrodesmus	7	Euastrum	3	Peridinium	6
Aphanocapsa	5	Eudorina	6	Phacus	8
Aphanothece	5	Euglena	10	Phormidium	9
Aulacoseira	6	Eunotia	2	Pinnularia	5
Bacillaria	7	Fragilaria	5	Planktolyngbya	7
Botryococcus	4	Golenkinia	5	Pseudanabaena	7
Centritractus	4	Gomphonema	6	Rhizosolenia	6
Ceratium	4	Gonium	6	Rhodomonas	8
Chlamydomonas	6	Gymnodinium	6	Rhopalodia	5
Chlorella	6	Gyrosigma	7	Scenedesmus	8
Chroococcus	6	Isthmochloron	5	Staurastrum	3
Closterium	6	Kirchneriella	5	Staurodesmus	3
Cocconeis	6	Melosiera	5	Stauroneis	5
Coelastrum	7	Merismopedia	9	Strombomonas	8
Cosmarium	2	Micractinium	7	Surirella	6
Crucigenia	7	Micrasterias	2	Synedra	6
Crucigeniella	7	Microcystis	8	Synura	8
Cryptomonas	8	Monoraphidium	7	Tetraedron	6
Cyclotella	2	Navicula	5	Trachelomonas	8
Cylindrospermopsis	7	Nephrocytium	5	Volvox	6
Cymbella	5	Nitzschia	9		
Dictyosphaerium	7	Oocystis	6		

Table 4. Scores of phytoplankton genera indicating water quality

Note: Peerapornpisan et al. (2007).

generally reflect moderately poor water quality across the three trophic categories. Notably, this finding mirrors the results obtained from the API, which similarly showed inconsistencies with TSI-based trophic classifications. Such discrepancies highlight the potential limitations of relying solely on nutrient-based indices like TSI.

To support the calculation of AARL-PP scores, the three most dominant phytoplankton genera in each trophic state were identified based on their relative abundance in each sampling site. As outlined in Part 2, each selected genus contributed more than 30% of the total phytoplankton volume. The dominant genera used for score assignment in each site are presented in Table 6. The scores derived from these selected genera were then averaged to obtain the AARL-PP score discussed above.

# Comparative analysis of biological indices in relation to trophic state index

The results of the comparative analysis using regression analysis between biological indices and the TSI for the Palmer's API and AARL-PP score are presented in Figure 2. The analysis indicates that the AARL-PP score demonstrates a stronger correlation with TSI compared to the API. Specifically, the AARL-PP score demonstrated a higher coefficient of determination (R<sup>2</sup> = 0.0946) compared to the API (R<sup>2</sup> = 0.045), suggesting that AARL-PP score accounts for a greater proportion of variance in TSI scores and shows a positive correlation. In contrast, the API was found to have a negative correlation with TSI, implying a weaker or potentially inverse relationship with trophic status. These findings underscore that while neither index perfectly mirrors the nutrient-based TSI classification, the

Genus	Ande	0100	Cuala	<b>-</b>	Comm	1	Minu	Mari	A /:4-	Onei	Dama	Dha	0.000	0	Total
Water bodies		Clos	Cyclo	Eug	Gomp	Lepo	MIC	Navi	INITZ	Osci	Pana	Pna	Scen	Syn	score
E1L	+	+	+	+		+		+	+	+		+	+	+	29
E2L		+		+		+		+		+	+	+	+		22
E3L		+	+				+	+	+	+	+	+	+		21
H4L		+	+			+		+	+	+	+	+	+		23
H5L				+		+		+	+	+	+		+	+	24
E6L	+					+		+		+			+	+	17
E7L		+		+		+				+		+	+		18
H8L										+	+				6
H9L							ĺ			+		+			7
E10M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	32
E11M				+						+	+	+			13
H12M		+				+	+			+		+	+	+	16
M13M	+	+		+	+			+		+	+	+	+		24
O14M	+	+	+	+		+		+		+	+	+	+	+	27
H15M	+	+				+				+			+	+	15
E16M								+		+	+		+		13
E17M	+	+			+	+		+					+	+	14
E18M				+		+				+	+	+	+		17
E19M		+	+	+		+			+	+	+	+	+		23
H20M		+	+	+		+			+	+	+	+	+		23
H21M	+	+		+	+	+	+		+	+	+	+	+	+	28
H22M		+		+		+		+		+			+		19
H23M				+		+		+		+		+	+		20
H24M										+	+				6
F25S	+	+		+	+	+		+				+	+	+	21
H26S				+		+		+		+	+	+	+		21
F27S										+	+	+			8
H28S				+		+				-	-	+	+		12
M29S	+		+			+				+		-	-		9
H30S		+		+								+			8
F31S										+	+		+		10
H32S		+		+	+	+				+		+		+	17
H33S		+				+				+		+	+		13
H34S										+			+		۱۵ ۵
H35S										+	+				6
Н365					+				+	+	+	+	+	+	22
M37S										· +	· ·				6
L1285	-			-		-						-			15
M200	- T			- T		- T					- T	- T			10
MADE		+	+	+		т Т		+		т	+	- T	+	-	10
		- T	- T	- T				- T			- T		- T		10
E400				+		+				+	+	+	+		20
E428	+			+						+	+	+	+		20
H435	+		+	+		+				+		+	+		19
H445						+		+		+	+	+	+		16
H45S			+	+		+		+		+	+	+	+		22
M46S			+	+		+	+	+	1	+	+	+	+		23

Table 5. Distribution of API indicator gene	a of phytoplankton and	l pollution scores in	n 50 water bodies
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E47S		+				+		+	+		+	14
H48S		+		+		+		+	+	+	+	17
H49S		+	+	+		+	+	+	+	+	+	26
E50S			+	+	+			+	+	+	+	19

**Note:** Ank: *Ankistrodesmus*; Clos: *Closterium*; Cyclo: *Cyclotella*; Eug: *Euglena*; Gomp: *Gomphonema*; Lepo: *Lepocinclis*; Micr: *Micractinium*; Navi: *Navicula*; Nitz: *Nitzschia*; Osci: *Oscillatoria*; Pand: *Pandorina*; Pha: *Phacus*; Scen: *Scenedesmus*; Syn: *Synedra*. + represents the presence of phytoplankton, O represents an oligotrophic water body, M represents a mesotrophic water body, E represents a eutrophic water body, and H represents a hypereutrophic water body. S denotes a small water body, M denotes a medium-sized water body, and L denotes a large water body. The numbers in the figure correspond to the water bodies studied, numbered from 1 to 50.

1.		1		
Water bodies	AARL-PP scores (Mean ± SD)	Most abundant genus	2 <sup>nd</sup> most abundant genus	3 <sup>rd</sup> most abundant genus
Oligotrophic	7.7	Oscillatoria	Microcystis	Peridinium
Mesotrophic	6.00 ± 1.23	Staurastrum	Peridinium	Oscillatoria
Eutrophic	7.37 ± 1.01	Oscillatoria	Microcystis	Peridinium
Hypereutrophic	7.40 ± 0.84	Oscillatoria	Microcystis	Strombomonas

Table 6. Dominant phytoplankton genera by trophic state used in AARL-PP score calculation

AARL-PP score offers a more reliable approximation and may thus serve as a more suitable tool for biological water quality assessment within this context.

### Relationship between trophic state index, Palmer's algal pollution index and AARL-PP score

The correlation coefficients illustrating the relationships between the TSI, Palmer's API, AARL-PP score, and key phytoplankton community metrics are shown in Table 7. TSI exhibited a strong positive association with chlorophyll-a (Chl-a) (r = 0.768, p < 0.01), total nitrogen (TN) (r = 0.698, p < 0.01), and total phosphorus (TP) (r = 0.681, p < 0.01), suggesting that increased nutrient levels are strongly linked to higher trophic status and enhanced algal biomass. In contrast, the associations of TSI with phytoplankton density (r = 0.097) and species richness (r = -0.159) were weak and not statistically significant, implying that eutrophication may alter community composition without necessarily increasing abundance or diversity. The API showed a negative correlation with Chl-a (r = -0.335, p < 0.05) and a strong positive correlation with species richness (r =0.804, p < 0.05). Although a weak negative trend was observed between API and TSI (r = -0.212), this relationship was not statistically significant, suggesting that trophic status may not directly predict pollution-tolerant algal assemblages as

measured by the API. No significant relationships were observed between API and TN or TP. Meanwhile, AARL-PP score showed a moderate positive correlation with TSI (r = 0.301, p < 0.05), but exhibited no significant association with Chl-a, TN, TP, phytoplankton density, or species richness. These findings highlight the complex and potentially nonlinear interactions among nutrient enrichment, trophic status, pollution indicators, and phytoplankton community structure in freshwater ecosystems.

## Comparison of water quality indices across trophic states

A comparison of the TSI, Palmer's API, and AARL-PP score across water bodies classified by trophic status - namely Oligotrophic, Mesotrophic, Eutrophic, and Hypereutrophic - is presented in Figure 3. The results show that TSI values differ significantly among all trophic groups (as indicated by different letters a, b, c based on Tukey's HSD test), demonstrating the effectiveness of TSI in distinguishing trophic status of aquatic ecosystems. In contrast, API values did not show significant differences among the trophic groups, highlighting a limitation of API as an indicator of nutrient status. On the other hand, AARL-PP scores differed significantly between Mesotrophic and both Eutrophic and Hypereutrophic groups, suggesting a stronger alignment with TSI. Based on these findings, AARL-PP score



Figure 2. Regression analysis between TSI and (a) Palmer's API and (b) AARL-PP score. The scatterplots illustrate the linear regression and coefficient of determination (R<sup>2</sup>) for each index, highlighting the direction and strength of the relationships

 Table 7. Correlation coefficients (r) between TSI, Palmer pollution index, and AARL-PP score with phytoplankton metrics

Parameter	TSI	API	AARL-PP	TN	TP	Chl-a	Phytoplankton density	Species richness
TSI	1	-0.212	0.301*	0.698**	0.681*	0.768**	0.097	-0.159
API	-0.212	1	-0.262	-0.089	-0.230	-0.335*	0.131	0.804*
AARL-PP	0.301*	-0.262	1	0.155	0.238	0.257	0.208	-0.262

**Note:** Chl-a – chlorophyll-a; TN – total nitrogen; TP – total phosphorus; TSI – trophic state index; API – Palmer's algal pollution index; AARL-PP score – applied algal research laboratory-phytoplankton score, \* significantly different at p < 0.05, and \*\* significantly different at p < 0.01.

appears to be a more appropriate index for assessing the trophic status of tropical lentic water bodies in Thailand, particularly when using TSI as a reference framework.

### DISCUSSION

# Comparison of phytoplankton genera and scoring systems in API and AARL-PP score

The comparison of phytoplankton genera and their assigned scores in Palmer's API and the AARL-PP scoring system indicates a consistent ecological signal of water quality degradation. Although the two indices differ in design, API emphasizes tolerance to organic pollution, while AARL-PP score reflects nutrient enrichment levels, the presence of certain key genera such as *Oscillatoria, Euglena, Phacus,* and *Scenedesmus* contributes to similarly high scores in both systems. These genera are well-documented indicators of environmental stress and are commonly associated with organically polluted or eutrophic water bodies (Dodds and Whiles, 2010). For instance, *Oscillatoria* and *Euglena* receive maximum or near-maximum scores in both indices



**Figure 3.** Comparison of mean index values (±SE) of TSI, Palmer's API, and AARL-PP score across four trophic states. (Oligotrophic, Mesotrophic, Eutrophic and Hypereutrophic). Letters (a, b, c) above bars indicate statistically significant differences among groups (Tukey's HSD, p < 0.05).

(API: 5; AARL-PP: 9-10), demonstrating their strong association with poor water quality conditions. The high frequency and dominance of these genera in eutrophic and hypereutrophic sites resulted in elevated index values that align well between the two systems. This consistency suggests that despite the differences in scoring methodology and interpretation framework, both indices are effective in identifying biological responses to water quality degradation. Furthermore, the overlap in indicator genera implies that both indices can be used complementarily for ecological assessments in tropical lentic ecosystems. The API offers a rapid assessment of organic pollution using presencebased scoring, while AARL-PP score provides a more nuanced evaluation based on the dominance and trophic association of phytoplankton communities. Therefore, integrating both approaches can enhance the reliability of biological monitoring and support a more comprehensive understanding of aquatic ecosystem health in Thailand. Moreover, while API treats each indicator genus independently and aggregates their scores, AARL-PP score relies on the average score of the three

dominant genera, weighted by their relative abundance. This methodological difference likely contributes to the observed variance in sensitivity. For example, genera like *Chlamydomonas* and *Closterium* receive low API scores (4 and 1, respectively), yet are assigned moderate to high scores in AARL-PP (6), reflecting differences in their ecological interpretation. These discrepancies highlight the limitations of applying indices developed in one biogeographic region to another without calibration. Overall, AARL-PP score appears to offer a more ecologically coherent framework for tropical lentic systems, although its application in oligotrophic contexts remains constrained by the lack of diverse reference data.

#### Statistical assessment of API and AARL-PP score

The comparative analysis of the Palmer's API and the AARL-PP score highlights key differences in their effectiveness for assessing water quality in tropical lentic systems. The API exhibited a negative correlation with the TSI (r = -0.212), indicating a weak alignment with

nutrient-based trophic conditions in tropical environments. This may be due to the inclusion of algal genera that are not representative of tropical phytoplankton communities, such as Cyclotella, which is commonly found in temperate or cold-water environments, and Synedra, often associated with cold water or temperate regions (Kilham et al., 1996). Moreover, the API showed high pollution scores in oligotrophic waters, suggesting that it may misclassify systems with naturally diverse but low-biomass communities as polluted due to the presence of certain tolerant genera (Palmer, 1969). In contrast, the AARL-PP score, developed specifically for tropical freshwater systems, demonstrated a stronger positive correlation with TSI (r = 0.301, p < 0.05) and a higher coefficient of determination  $(R^2 = 0.0946)$  than API. Although this correlation remains modest, it suggests that AARL-PP score better captures trophic variation, especially in eutrophic and hypereutrophic waters. Its reliance on dominant phytoplankton genera more commonly found in tropical waters likely improves its ecological relevance (Peerapormpisal et al., 2007; Chaipiputnakhajorn and Gunbua, 2023). Phytoplankton communities in such regions are influenced by a wider array of environmental gradients including hydrology and temperature regimes that are not fully accounted for by trophic-level models (Reynolds, 2006). Additionally, the relatively low R<sup>2</sup> values highlight the multifactorial nature of phytoplankton community structure and suggest that singlemetric indices may not adequately capture the ecological nuances of lentic water bodies.

Furthermore, the statistical comparison using Tukey's HSD test revealed that API values did not significantly differ across trophic categories, reinforcing concerns about its limited sensitivity to nutrient gradients in tropical systems. In contrast, AARL-PP scores showed significant differences between mesotrophic and both eutrophic and hypereutrophic groups. This suggests that beyond its moderate correlation with TSI, AARL-PP may offer improved discriminatory power in detecting trophic shifts, particularly in nutrient-rich environments (Pinmongkhonkul et al., 2002). These results further support the ecological validity of AARL-PP score for tropical lentic systems, especially when used as part of a tiered or integrated water quality assessment framework. Similarly, Kadam et al., (2020) reported that most water bodies in the Doon

valley of India, an area with climatic conditions comparable to Thailand, were classified by the API as highly organically polluted, reinforcing concerns about its applicability in tropical and subtropical regions. These findings highlight the need for refining and calibrating biological indices to match the ecological characteristics of the regions where they are applied. Integrated assessment frameworks that combine biological indices like AARL-PP score with physicochemical parameters (e.g., nutrient concentrations, chlorophyll-a) and other biological indicators (e.g., zooplankton, benthic macroinvertebrates) may offer more comprehensive and accurate evaluations of water quality (Paulic et al., 1996). This integrated approach has also been demonstrated in urban tropical environments such as the Mak Khaeng Canal in Udon Thani Province, Thailand where the combination of chemicalbased water quality index (WQI) and biological assessments enhanced the resolution and ecological relevance of water quality classification (Wongaree, 2019).

To build upon the current findings, future studies should incorporate multi-seasonal data collection. This study focused on the hot-dry season (April-May), as it is typically the period when problematic phytoplankton blooms most frequently occur in tropical freshwater systems. During this time, high temperatures, intense solar radiation, and stable water columns often promote bloom formation and shifts in community structure. However, tropical systems undergo pronounced seasonal variation. The rainy season usually brings increased nutrient input via surface runoff, elevated turbidity, and reduced light penetration, which can suppress phytoplankton growth. In contrast, the dry season following the monsoon is associated with improved light conditions, lower turbidity, and enhanced vertical mixing, which can favor eutrophic taxa and alter community dynamics (Kondowe et al., 2022; Wang et al., 2022). These seasonal fluctuations can influence index scores and trophic state interpretations. Therefore, to improve the temporal robustness and ecological generalizability of phytoplankton-based bioindicators, future research should include data from multiple seasons across the annual climatic cycle.

# Challenges in applying phytoplankton-based indices to tropical systems

A key limitation of this study is the underrepresentation of oligotrophic water bodies, with only one site (O14M) classified in this category based on the TSI. This limited sample size weakens the robustness of comparative analyses and introduces uncertainty regarding the ecological validity of both Palmer's API and the AARL-PP score in nutrient-poor systems. Notably, the oligotrophic site yielded a Palmer's index score of 27, indicative of confirmed high organic pollution, despite having a low TSI value of 33.43. A similarly elevated AARL-PP score of 7.7, corresponding to poor water quality, also contradicts its trophic classification. These inconsistencies raise concerns about the applicability of both indices in tropical oligotrophic settings, where phytoplankton communities may not align with assumptions embedded in indices developed for temperate regions. The dominant genera observed at this site, Oscillatoria, Microcystis, and Peridinium, are typically associated with high nutrient availability and algal blooms, further complicating classification (Swann et al., 2024; Phonmat et al., 2025). This observation is consistent with findings from a study in Eastern Thailand, which also frequently reported Oscillatoria and Microcystis in reservoirs with AARL-PP scores ranging from 7 to 8, aligning with the results of our study (Wongaree, 2019).

Moreover, the API showed high pollution scores in oligotrophic waters, suggesting that it may misclassify systems with naturally diverse but low-biomass communities as polluted due to the presence of certain tolerant genera (Palmer, 1969). To enhance the ecological validity of bioassessment tools (API and AARL-PP score), especially under low-nutrient conditions, continuous monitoring of oligotrophic water bodies such as site O14M is strongly recommended. For this site, increasing sampling frequency to every two months and expanding the number of sampling points within the lake would improve the resolution of phytoplankton community composition and reduce uncertainties in index interpretation. Additionally, it is essential to broaden the scope of sampling to include more oligotrophic lakes across diverse geographic and ecological settings. This would help achieve a more balanced representation of trophic states and strengthen the reliability of inter-category comparisons.

Incorporating seasonal dynamics and key environmental drivers such as watershed land use, thermal stratification, and hydrological connectivity could also provide valuable insights into the mechanisms shaping phytoplankton assemblages and improve the robustness of phytoplanktonbased indices in tropical freshwater systems.

### CONCLUSIONS

This study highlights the vital role of phytoplankton-based indices in the ecological assessment of lentic water bodies, particularly in tropical environments where conventional nutrient-based metrics alone may be insufficient. Combining these indices with measurements of nutrient concentrations in the water provides a more comprehensive understanding of the factors influencing phytoplankton communities and thus offers a fuller picture of the ecosystem status in lentic systems. Among the indices evaluated, the AARL-PP score demonstrated superior performance over Palmer's API, exhibiting a stronger correlation with the TSI and thereby offering a more accurate reflection of nutrient-driven trophic conditions. Notably, AARL-PP score exhibited greater sensitivity in distinguishing mesotrophic from eutrophic and hypereutrophic systems, reinforcing its potential as a reliable indicator for detecting subtle trophic transitions in nutrient-enriched environments. However, the discrepancies observed in the single oligotrophic site highlight the limitations of applying generalized indices without ecosystem-specific calibration. This is partly due to the low representation of oligotrophic systems and sampling restricted to the hot-dry season, which may not fully capture temporal variability in phytoplankton communities. The AARL-PP score may require refinement as more comprehensive data from oligotrophic phytoplankton communities become available. Future assessments should prioritize multi-seasonal datasets and broaden sampling coverage to include a wider range of oligotrophic and geographically diverse sites. This approach will enhance the ecological resolution of phytoplankton-based assessments, support the reassessment of genus-level thresholds, and improve the index's sensitivity and accuracy for tropical environments.

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