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THE MINIMUM NUMBER OF VALVES FOR DIATOM IDENTIFICATION IN RAWAPENING LAKE, CENTRAL JAVA

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ABSTRACT

Technical challenges in using diatoms for paleolimnological work are the identification and enumeration of diatom valves. Variations exist in the minimum number of valves to identify, ranging from 100 to 700 valves of the dominant species. This task can be very time consuming, particularly when the diatom valves are not abundant. This research was conducted to determine the minimum number of valves to be identified in the diatom assemblages from Rawapening Lake, Central Java, Indonesia. Based on the 314 samples obtained from Rawapening Lake, the diatom efficiency rose above 0.85 upon the minimum count of 300 valves. The number of diatom species identified remained stable after the minimum of 300 valves. Therefore, the minimum number of diatom valves identified to represent the assemblage for paleolimnological analysis was 300.

Keywords: Diatom analysis, eutrophic, Indonesia, paleolimnology, Rawapening

INTRODUCTION

Diatoms are the common name of microalgae belong to Bacillariophyte that have wide distribution from the ocean, freshwater to humid land and part of aquatic food webs. The uniquely ornamentated silica cell wall remain undisturbed whenever fosilized. That is why diatoms are good tool for paleolimnology.

Diatom undoubtedly have potential as bioindicators for water quality changes due to their well preserved walls in sediments. Being primary producers, diatoms play significant roles in food webs. Diatoms are distributed worldwide in saline or freshwaters, have short life cycles and are responsive to environmental changes. Diatoms can live across ecological gradients, are easily sampled and can be analyzed at low cost (Dixit *et al.* 1992; Gell *et al.* 2007; Reid & Ogden 2009).

Diatoms community are responsive to environmental changes such as pH, water depth, nutrients, salinity, and also the current condition of the environment. Diatom assemblages are often specific to particular habitats, therefore diatom fossils can be used to characterize those habitats. For that reason, diatoms have been widely used to investigate the status of lakes and can explain about pollution control, water quality monitoring programs and the paleoecology of the lakes. Palaeolimnological studies offer an opportunity to understand the past environmental conditions (Bere 2014). Paleolimnological techniques can reveal long-term perturbations and transitions of lake ecosystems (Kattel *et al.* 2016).

To understand limnological change, diatom valves are often well preserved in sediments allowing the inference of water quality over time. However, Indonesia does not have standard methods for diatom analysis. Even in the temperate region countries, the protocol for identifying the number of valves differs, which may be not suitable if implemented in Indonesia.

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The analysis of diatoms consists of three steps, i.e. digestion to separate diatom valves from the sediment; sample mounting-slide preparation; and identification-enumeration. The number of valves identified in the enumeration step differs across regions. Battarbee (1986), identified 300 – 600 diatom valves in England, and many studies used Battarbee's standard. However, Bate and Newall (2002) suggested 200 valves as an adequate number to characterize the diatom assemblages. These studies are focused mainly on temperate systems. Tropical systems are very diverse which may influence diatom assemblages. Therefore, it is important to determine an adequate count size in tropical diatom assemblages to optimize the efficacy of the research effort. The lower standard number i.e. 100 valves (Round 1993) when being implemented in Indonesia, was still time consuming due to species diversity despite lower population size (Soeprobowati *et al.* 2005).

The objective of this research was to determine the minimum valves count in the identification-enumeration step of diatom analysis for paleolimnological studies.

MATERIALS AND METHODS

Rawapening Lake was chosen as a study site because the lake is small with eutrophication problems similar to other Indonesian lakes. Rawapening Lake is one of the 15 Indonesian national priority lakes in 2010-2014 (ME 2010). Rawapening Lake was chosen as a pilot project for 'Save Indonesian Lake' as it sufficiently represents the eutrophication and sedimentation problems of Indonesian lakes (ME 2011). Radical action is required to overcome lake degradation problems comprising, one clear program for action, substantial funding, and strong institutional collaboration (Soeprobowati 2015a). Paleolimnological study was among the priority programs, named Gerakan Penyelamatan Danau (Germadan) Rawapening, to save Rawapening Lake (ME 2011).

Rawapening Lake is located at 45 km south of Semarang and about 9 km east of Salatiga. Rawapening is surrounded by five volcanoes i.e. Telomojo (1,895 m asl), Butak (1,000 m asl), Balak (700 m asl), Payung (600 m asl), and Rong (600 m asl). There are four districts around the lake i.e.: Tuntang, Bawen, Ambarawa and Banyubiru. About 17 villages are situated around the lake side and their agricultural areas are frequently subjected to flooding. Rawapening Lake, situated at about 400 m asl, is about 4 km long and 2.5 km wide, with slightly sloped (7%) sides. In the 1970s its maximum capacity was 65 million m^3 and the minimum was 25 million m^3. However, there has been a clear trend of reducing volume, which has affected its capacity to generate hydropower electricity (Soeprobowati *et al.* 2012b).

Echosounding of the lake revealed extensive areas of less than 2 m depth; there were also three deeper depocentres in the lake's west, each 18 m deep (Soeprobowati 2012). Sediment cores of different lengths were collected from four research sites across Rawapening Lake. The longest sediment core (63 cm, *As*) was obtained from the Asinan site. The Panjang (*Pj*) and Tuntang (*Tg*) cores were 36.5 cm and 35.5 cm long, respectively. The Dangkel (*Dk*) core was 29 cm (Fig. 1).

Tg site was represent an outlet of Rawapening Lake, *As* site was near an inlet that come from a settlement and so represents a settlement catchment area and *Pj* site was near an inlet passing through agricultural area, thereby representing an agricultural catchment area. *Dk* site was located relatively close to the middle of the lake and so represents the lake body. Those 4 sites were determined to sufficiently represent Rawapening Lake. Diatoms were sliced every 0.5 cm based on the modified version of Battarbee *et al.* (2001). The first step of extraction is intended to separate diatom valves from organic material. Basically, depending on the type of sediment, strong acid may be used to digest sediment. This study applied 10% HCl followed by 10% H_2O_2 to digest sediment samples. In the preparation process, a mountant with refraction index of 1.7 is required. Hyrax was used in this research and the silicious striae were clearly seen under the microscope, expediting the identification process. Identification of diatom species was carried out by referencing the diatom samples to the standard texts (Kramer & Lange-Bertalot 2004a, 2004b, 2004c, 2010) and by referencing the samples to the diatom collections held at Universitas Diponegoro, Semarang, Indonesia and at the Federation University Australia, Ballarat, Australia. A total of 600 valves were counted for each sample with totals tallied at steps of 100 (i.e.

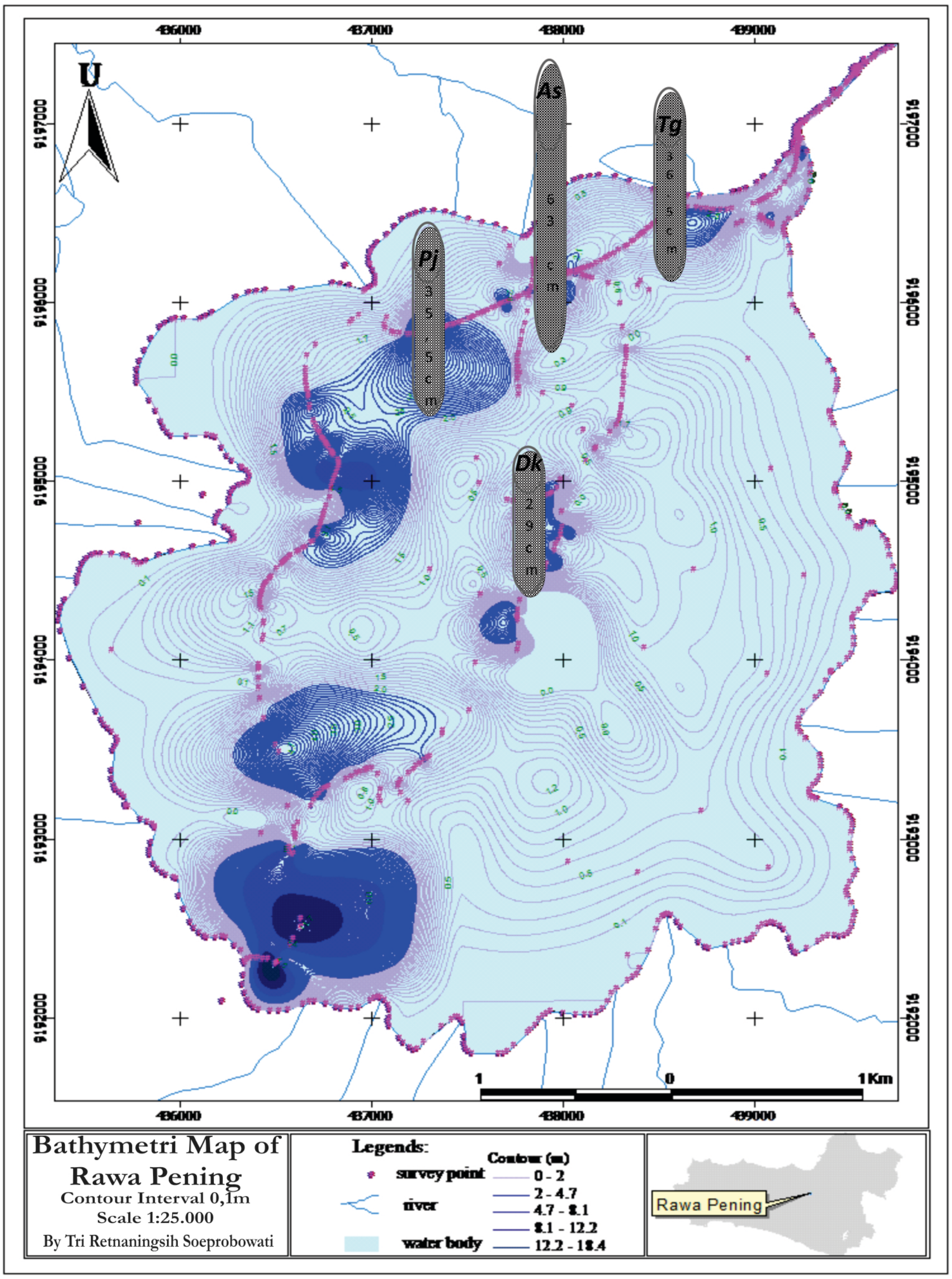

Figure 1 Study sites in Rawapening Lake for collecting sediment core samples

Notes: *As* = Asinan Site (63 cm) represents settlement catchment area, *Pj* =Panjang Site (35.5 cm) represents agricultural catchment area, *Tg*= Tuntang Site (36.5 cm) represents outlet of Rawapening, *Dk* = Dangkel Site (29 cm) represents the lake body

100, 200, 300, 400, 500, 600). Species accumulation curves were applied to identify the minimal number of valves to be counted to achieve maximum efficiency, which was calculated using the formula (Bates & Newall 2002):

$$\text{Maximum efficiency} = 1 - \frac{\text{number of species}}{\text{number of individuals}}$$

Maximum efficiency reflected the probability of new species to be found at each identification step (Pappas & Stoermer 1996; Bates & Newall 2002).

RESULTS AND DISCUSSION

Three steps commonly used in diatom analysis consist of extraction, preparation, and identification. In the identification process, the minimal number of valves identified varied among researchers. Counting the valves of

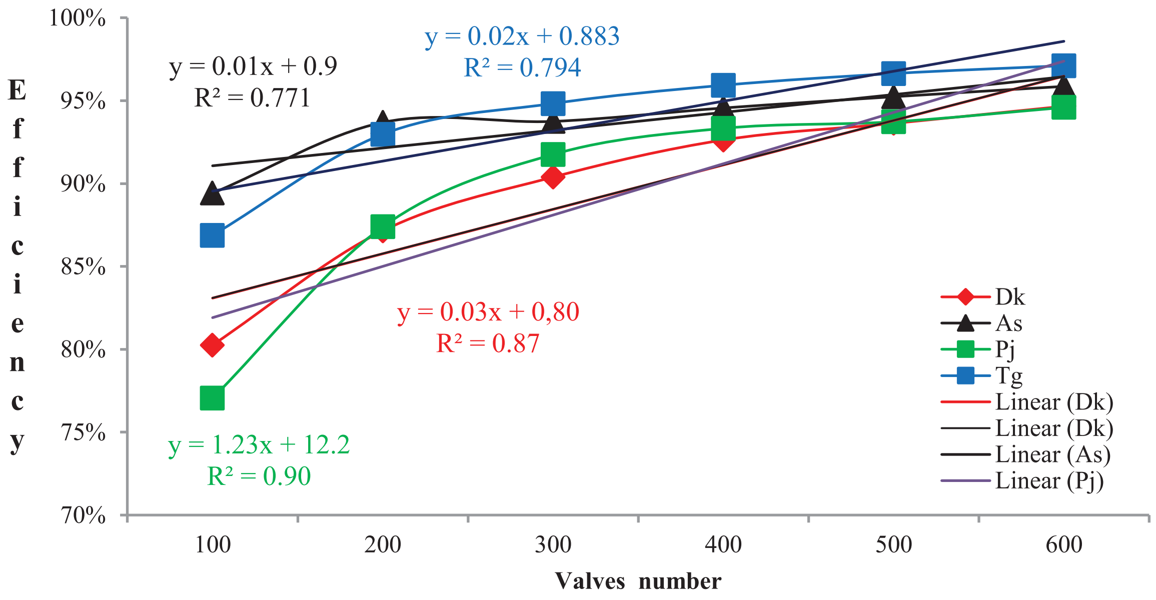

Figure 2 Maximum efficiency counts of diatoms from the upper 1 cm of sediment cores from 4 study sites

Notes: *As* = Asinan Site (63 cm) represents settlement catchment area, *Pj* = Panjang Site (35.5 cm) represents agricultural catchment area, *Tg*= Tuntang Site (36.5 cm) represents outlet of Rawapening, *Dk* = Dangkel Site (29 cm) represents the lake body

diatoms is important to provide data about the ecological condition in the past.

This study analyzed 314 samples, compared valve counts at 100 , 200, 300, 400, 500, and 600 valves, found that the uppermost (1 cm) sediment samples from each of four sites. The counting results showed maximum efficiency of 0.87 – 0.93 (Fig. 2). The number of diatom species identified remained stable after 300 valves while the maximum efficiency rose to above 0.85. New finding from this research is that 300 valves is the most efficient number of valves for paleolimnological assessment.

The number of diatom species identifed from 4 sites were different. The highest number of diatom species was found in *Dk* site having 42 species in the counts of 200 valves which remained stable in the counts of 300 through to 600 valves (Fig. 3). *Dk* was situated close to the middle of the lake, having a water depth of 2 m with sediment of peat mud. This might be correlated with numerous *Eunotia* species found only in the *Dk* site indicating its acid condition (Soeprobowati *et al.* 2012b). Typically, when the euphotic depth is shallow, the number of aquatic plants attached to the substrate decline, causing nutrients and sediments to be further released into the water column. This drives the increasing numbers of phytoplankton to continually exploit the light that is confined to the surface waters (Reid *et al.* 2007). This condition is shown in the diatom record of the Rawapening Lake sediments that are dominated by planktonic forms thriving in eutrophic, turbid and alkaline waters. The odd situation happened in *Tg* site. Thirty diatom species were found in the count of 200 – 600 valves. The R^2 of 0.42 for Tg site indicated that there was a low correlation between the number of species with the number of valves counted. In *Tg* site, large diatom species might adapt well to the moving outlet water. *As* site showed 22 diatom species in 400 valves and remained stable. *Pj* site had the lowest number of species (14 species) in the count of 200 valves and increased to 17 species in the count of 500 valves.

Statistically, comparing the results of analysis of variance, the diatom species numbers found in each sediment layer were significantly different between counts of 100, 200, 300, 400, 500, and 600. Further Least Significant Difference (LSD) analysis showed that results from the count of 500 valves were significantly different from the count of 400 and 600 valves. There was also significant difference between the diatom species number for the count of 400 and 600 valves. The increasing numbers of diatom species in the count of 600 valves were probably due to contaminant species. This was based on the fact

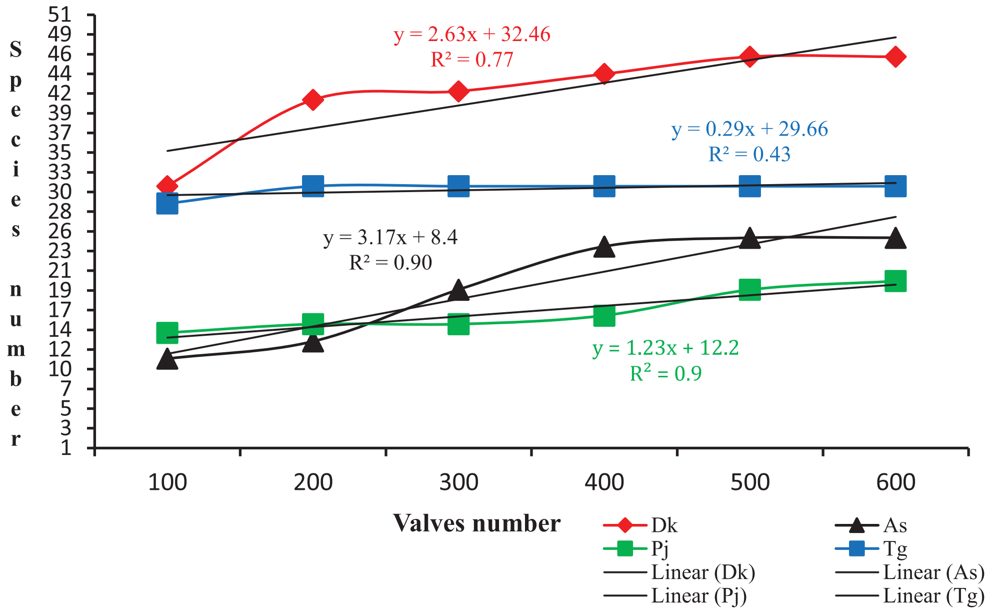

Figure 3 Number of diatom species identified from the upper 1 cm of sediment cores from 4 research sites

Notes: *As* = Asinan Site (63 cm) represents settlement catchment area, *Pj* = Panjang Site (35.5 cm) represents agricultural catchment area, *Tg*= Tuntang Site (36.5 cm) represents outlet of Rawapening, *Dk* = Dangkel Site (29 cm) represents the lake body

that there had been no or low increase in the numbers of diatom species with low populations from the 4 sites of Rawapening Lake (Fig. 3). The maximum efficiency of those 4 sitesalso indicated that the count of 400 – 600 valves had maximum efficiency above 0.9 (Fig. 2). Analysis of variance supported this result, there is no significant different. Hence, the minimum count of 300 valveshaving efficiency more than 0.85, was appropriate for the paleolimnological analysis of a tropical lake, with specific reference to the eutrophic Rawapening Lake.

In England, Battarbee *et al.* (1986; 2001) proposed counting 300 - 600 valves for routine analysis, but Round (1993) determined that 100 valves were sufficient for dominant species. In Sweden, Gothe *et al.* (2013) identified diatoms until they found at least 400 valves. In Finland, Soininen & Kononen (2004) identified 250 – 500 valves. In France, Morin *et al.* (2008) identified 300 valves. In Australia Fluin *et al.* (2010) identified diatoms until they found between 300 – 540 valves, whereas Grundell *et al.* (2012) identified 200 valves. In Canada, Koster *et al.* (2005) and Pienitz *et al.* (2006) identified as many as 500 valves. In Mexico, a minimum of 500 valves were identified (Siqueiros-Beltrones *et al.* 2005). In Uganda, Mills (2009) counted until 300 - 500 valves were found. In America, Kireta *et al.* (2012) identified 100 valves in samples with sparse diatoms. In India, it is recommended to count 400 valves (Karthick *et al.* 2010). For fossil diatoms, the count should be different, because some diatom species may have been dissolved over a period of time or diatom samples may contain diatom species from previous period of time.

In Indonesia, the minimum number of valves recommended by Round (100 valves) had been implemented, but this is less efficient and less effective, particularly given Round (1993) stipulation that 100 valves of the dominant species had to be counted (Soeprobowati *et al.* 2005, 2012a). In this study, there were significant differences between the counts of 100,and 200 and 300, while the count of 500 valves was not significantly different from 400 and 600. These results were similar to Battarbee's (1986) statement that there were marked differences between the count of 100 and 200 valves, while there was little differences between the count of 400 and 500. For this reason, he recommended that a count of 300 to 600 may be used for routine analysis purposes analysis. Based on this research, it was recommended that a count of a minimum 300 valves might be used for paleolimnological analysis in Rawapening Lake.

The counting of diatom valves is to produce a semi-quantitative approach for ecological analysis. Therefore, it is very important to determine the minimum number of valves to be counted to get a reliable approach to gauge the relative species composition at sampling sites (Karthick *et al.* 2010). The minimum total number of valves to be counted for each sample varies depending on the purpose of the assessment and the need to produce statistically sound results. Comparing the count of 200 and 600 valves, there were percentage differences of 1.89. The count of 300 and 600 valves provided 1.85 percentage differences which suggested that the count of 300 valves was sufficient for the calculation of diatom species.

Results of comparing the maximum efficiency of counting 100, 200, 300, 400, 500, and 600 valves showed that the minimum number of valves that should be identified was 300, since its maximum efficiency was more than 0.85. Maximum efficiency is considered to sufficiently represent diatom species numbers because the formula to calculate maximum efficiency includes the number of individual valves. Therefore, maximum efficiency can be used to determine the minimum valves to be identified in diatom analysis (Bates & Newall 2002).

In Australia, the minimum number of 200 valves had more than 80% efficiency, and this number was deemed suitable to be used in water quality monitoring programs (Bates & Newall 2002). There was no influence on the diatom index at counts of valves 300 or above (Prygiel *et al.* 2002).

A European Protocol for diatom enumeration, DALES (*Diatoms for Assessing Lake Ecological Status*, version 1.0 2004), determined that at least 300 valves should be identified, especially for non planktonic taxa. When the abundance of one taxon was more than one third of all individual valves, the protocol recommended to increase the sample size until a minimum of 200 valves of non planktonic diatom are found. New species found in the count above 400 valves were determined to be contaminant species. The presence of these species may cause bias for further analysis. Therefore, species found to be less than 5% of valves were considered to be rare species and were not included in the data analysis. Dominant species are considered to provide more evidence of the water quality than the rare species.

Based on the diatom efficiency and diatom species found from Rawapening Lake, a minimum count of 300 valves was sufficient for paleolimnological analysis, which is lower than the recommended valves count for temperate or polar areas. This recommendation might be related to the year round warm temperature in the tropics which increases the diatom species diversity.

A minimum count of 300 diatom valves was implemented to reconstruct the environmental condition of Rawapening Lake since the 1960s. The dominance of *Fragilaria capucina* Desm, *Luticola goeppertiana* (Bleisch) Mann, *Mayamaea atomus* (Kutzing) Lange-Bertalot, *Navicula radiosa* Kutzing, *Nitzschia palea* (Kutzing) W. Smith and in As site, *Tryblionella apiculata* Gregory,reflected eutrophic, but clear waters in 1967-1974. The presence of *Eunotia pectinalis* (Kutzing) Rabenhorst var. *undulata* (Ralfs) Rabh suggested neutral to slightly acid conditions in 1967-1974 and the appearance of *Fragilaria capucina* Desm, *Gomphonema gracilis* Ehr. and *Navicula radiosa* Kutzing suggested changes of water pH to alkaline conditions. An increase in epiphytic *Gomphonema* spp. in 1974-1983 marked an increase in aquatic macrophyte plants, perhaps in response to high nutrient levels. This change was followed promptly by the increasing numbers of acidophilous *Eunotia* spp. reflecting high organic production. A transition to a diatom community dominated by planktonic forms occurred around 1983. This community was initially dominated by more clear water, oligotrophic species such as *Discostella stelligera* (Cleve and Grunow) Houk and Klee and *Aulacoseira distans* (Ehrenberg) Simonsen, but transitions happened in 1990 to be dominated by *A. granulata* (Ehrenberg) Simonsen and ultimately *Aulacoseira ambigua* (Grunow) Simonsen. This was interpreted as a shift to a turbid water phase that contained beneficial phytoplankton, at the expense of benthic or epiphytic taxa requiring clear water. The dominance of *A. granulata* (Ehrenberg) Simonsen since the 1990s indicated that the lake experienced hypertrophic conditions with pH>9. Although a high proportion of the taxa in Rawapening Lake sediments were not represented in the European data set, Rawapening Lake experienced hypertrophic condition with pH>9 as indicated by the dominance of *Aulacoseira granulata* (Ehrenberg) Simonsen (Soeprobowati *et al.* 2012b).

However, when the identification was done using counts of less than 300 valves, some predominant species such as *Aulacoseira ambigua, Cyclotella meneghiniana, Gomphonema gracillis, Synedra ulna* were not found. The predominance of *Synedra* from 1967 to the present indicated that Rawapening Lake had been fresh and meso-eutrophic throughout. *Synedra ulna* (Nitzsch) Ehrenberg is a tolerant species, found in Indonesian rivers and lakes with high organic content with total phosphorous content of 20 - 1,000 μg/L and pH of 5 – 9. The modern sampling of pH at Rawapening Lake revealed that pH in Rawapening Lake fluctuated. Goltenboth (1994) reported that the pH of Rawapening Lake was 7.4±0.2 (dry season) and 7.3±0.1 (wet season). EPA-ERC Undip (1999) reported that the pH of Rawapening Lake was 7.96±0.42. In 2004 and 2005, the pH of the inlet and lake tended to be neutral (7.04±1.13), except in the site around the spring and floating island where the pH was 9.52 (Soeprobowati *et al.* 2005). During field work for this study (2008), pH increased up to 9.39±2.51. In recent study, those paleoreconstruction of ecological change in Rawapening proved a trend of increasing pH. Measurements of pH in June 2015 showed that pH of 14 sites in Rawapening Lake was 7.22±2.45 (Soeprobowati 2015b).

CONCLUSIONS

Diatom maximum efficiency rose to above 0.85 at the minimum count of 300 valves. The number of diatom species identified remained stable after the minimum count of 300 valves. A minimum count of 300 valves was appropriate for the paleolimnological analysis of a tropical lake, with specific reference to the eutrophic Rawapening Lake.

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