



Evaluation of weevils (*Neochetina* spp.) existence in Rawa Pening Lake (abundance and impact on target and non-target plant)

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Abstract. Water hyacinth, *Eichhornia crassipes*, which is the most invasive species native to Amazon river, occupies more than 50% of Rawa Pening Lake's area and had an ecologically and economically negative impact. Due to the negative effects of the presence of *E. crassipes* in Rawa Pening Lake, the weevil *Neochetina* spp. was introduced in the lake in 1975 as a biocontrol agent. Although the successful biological control of water hyacinth is influenced by the abundance of agent and their ability to reduce water hyacinth population, an evaluation of weevil abundance in the lake after several years since its introduction and also the impact of weevil against *E. crassipes* and other aquatic plant species in the lake have not been reported yet. The research aimed to investigate the abundance of weevils in 4 sub-districts between September and December 2016 in the Rawa Pening Lake. It also included the evaluation of the impact of the presence of weevils on several plants, including *E. crassipes*. To evaluate the impact, we proposed ten weevils in a no choice and choice test on five plants, and we measured the *E. crassipes* foliar damage per day by weevil using BioLeaf, a portable application. The result indicated that *Neochetina bruchi* was not found, while *Neochetina eichhorniae* was established well in the lake, although it was relatively in low quantities (5-14 adult m⁻² and 2-9 larvae m⁻²). The average abundance of adult *N. eichhorniae* found at each location was similar (sig. value = 0.184, p > 0.05), but the abundance from October and November differed from December (mean difference = -3.7500*, p < 0.05; mean difference = -3.7500*, p < 0.05 respectively), and there was no significant difference in larvae abundance in the different locations and period (sig. value = 0.525, p > 0.05, sig. value = 0.254, p > 0.05 respectively). Ten *N. eichhorniae* caused foliar damage to *E. crassipes* and *Monochoria vaginalis* (1.89-8.31% and 1.33-5.23% respectively) in choice test, both of them belonging to the Pontederiaceae family. The average damage inflicted by an adult was 0.43% per *E. crassipes* leaf area per day. The low quantity of *N. eichhorniae* in the lake suppressed *E. crassipes* robustness, despite the fact that integrated control is required to facilitate the reduction in weed cover. Furthermore, the catchment area input nutrient and stream inflow regulation is essential for successful control of *E. crassipes*.

Key Words: water hyacinth, biocontrol, *Neochetina* spp., abundance, foliar damage.

Introduction. Water hyacinth, *Eichhornia crassipes* originates from South America, and it is one of the most problematic aquatic weeds, according to IUCN's list (Admas et al 2017). *E. crassipes* has a rapid growth rate and it can reproduce in a generative or vegetative manner, the seeds can survive more than 28 years and can grow again in appropriate conditions (Sullivan & Wood 2012). This plant was introduced in Indonesia in 1884 as an ornamental plant (Goltenboth et al 2006), and today exists in almost all freshwater areas in Indonesia, including Rawa Pening Lake area covered with *E. crassipes* mats was 50% of 2.670 ha (Trisakti et al 2015).

Rawa Pening is located in the Central Java Province, Indonesia. The lake borders four sub-districts, Ambarawa, Banyubiru, Bawen, and Tuntang. The approximate lake area covered with *E. crassipes* mats was 50% of 2.670 ha (Ministry of Environment

2010). Rawa Pening Lake was included among 15 national priority lakes for the 2010-2014 period in the national conference based on lake quality degradation and the presence of *E. crassipes* (Soeprbowati 2015). There were many strategies proposed to eradicate the *E. crassipes* population, such as physical, mechanical, chemical, and biological control. In fact, the use of mechanical devices to control *E. crassipes* throughout the lake was expensive. Equipment also requires maintenance, and it can change communities of aquatic organisms (Mangas-Ramirez & Elias-Gutierrez 2004). The use of chemical materials, such as glyphosate, formic and propionic acids needs a short time to kill *E. crassipes*, but it can lead to water pollution in inappropriate doses (El-Shahawy 2015). Biological control is less expensive and can establish long-term control. The grass carp (*Ctenopharyngodon idella*) was not the specific agent to control *E. crassipes* (Silva et al 2014), but it was used in Rawa Pening Lake. It failed to control *E. crassipes* because it was understocked, and the fish were caught by fishermen after they were stocked (Research and Development Agency of Marine and Fisheries 2014).

The other control agents were weevils *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae). *N. eichhorniae* and *N. bruchi* had the potential to control *E. crassipes* population in many tropical and sub-tropical countries (Yasothea & Lekeshmanaswamy 2012; Firehun et al 2015; Hamadina et al 2015; Sivaraman & Murugesan 2016). *N. eichhorniae* and *N. bruchi* were introduced in Indonesia in the years 1975 and 1994 as the biocontrol agents of *E. crassipes* (Kasno et al 2001). These *E. crassipes* weevils have also been tested and released in most of the freshwater areas, including Rawa Pening Lake, Central Java Indonesia. Monitoring and evaluation are necessary after the introduction of *E. crassipes* weevils because they are related to its existence, effectiveness, and impact on the biodiversity of aquatic plants in an ecosystem. There were recent investigations about *Neochetina* spp. existence in West Java Indonesia, such as the result of Sapdi et al (2006) which showed that *N. eichhorniae* has spread widely but in small quantities in West Java and *N. bruchi* was not found in the study sites, and his study also proved that *N. eichhorniae* feed on *Spirodela* sp., *Marsilea crenata*, *Ludwigia adscendens*, and *Ludwigia octovalvis*, even though *N. eichhorniae* cannot complete its life cycle. Kasno et al (2001) submitted that *Neochetina* spp. can actively disperse a few meters a week in all directions following the plant's existence in Situ Bagendit Lake, West Java. Weevil's density slightly increased, but the survival rate of the weevils was estimated not to be more than 5% because of many factors, such as predators. Based on references, there was lack of evaluation of weevils' existence and the implications of its presence in Rawa Pening Lake, Central Java. The objectives of this study were to determine *E. crassipes* weevils' abundance in Rawa Pening Lake, to evaluate weevil's impact on *E. crassipes* (target) and other aquatic plants (nontarget) with no-choice and choice tests in a field cage experiment, and to quantify foliar damage of *E. crassipes* caused by weevils per day. The finding result will be a valuable report to support the success of *E. crassipes* control.

Material and Method

Study areas. The research was conducted at Rawa Pening Lake, Central Java Province, Indonesia (Figure 1). The sampling stations were selected based on the purposive sampling method. Four sub-districts in the lake were chosen for estimating weevils' abundance in the presence of *E. crassipes*, related to different anthropogenic activities. These four sites are made up of different things: Tuntang (outlet, blue bridge, and paddy farming area), Bawen (floating restaurant, middle of the lake, and paddy farming area), Ambarawa (Kampung Rawa resort, paddy farming, and outlet), and Banyubiru (Bukit Cinta resort, paddy farming, and floating cage culture area).

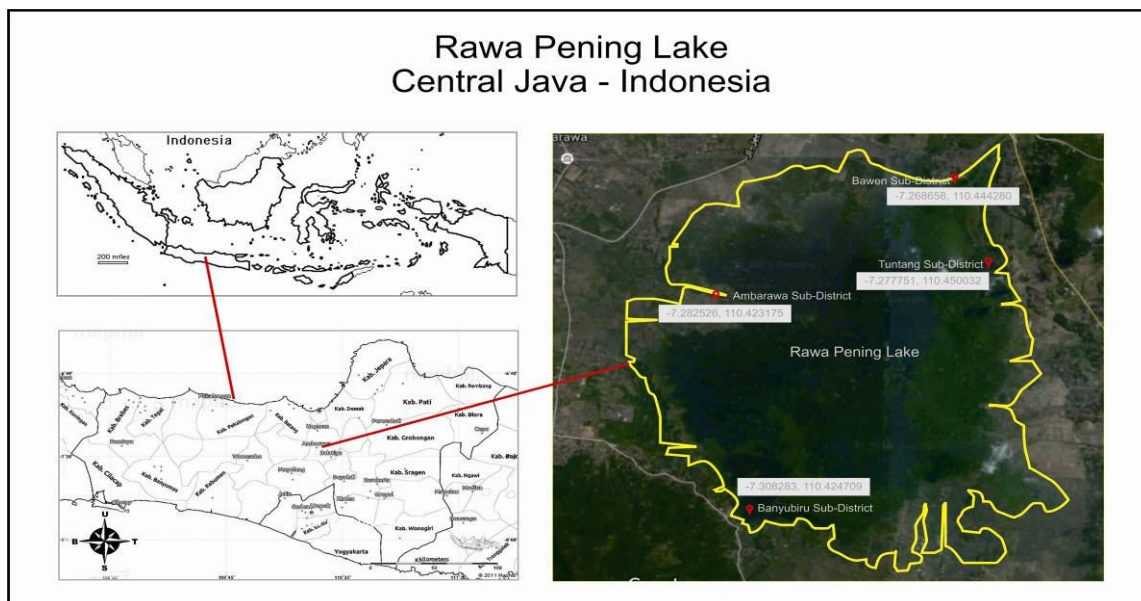


Figure 1. Map showing the position of Rawa Pening Lake in Central Java, Indonesia and the sampling points.

Sampling techniques

The abundance of weevils. To investigate the prevalence of weevils, we collected *E. crassipes* monthly, using 100 × 100 cm quadrats for each location with three sampling sites and three replications (10 m interval distance) from September to December 2016. Quadrat was one of the ecological tools for monitoring aquatic plants in a selected area and at a certain time (Johnson & Newman 2011; Madsen & Wersal 2017). During each survey, larva and adult weevils on the individual plant were sorted and counted manually.

No-choice and choice test. No choice and choice tests were conducted to predict the biological control agent's impact on the target and non-target plants (Heard 2002; Van Driesche & Reardon 2004). Plants were selected based on the level of relatedness to *E. crassipes* morphology, the same habitat, and the economically importance of species (Sheppard et al 2005). *Monochoria vaginalis*, *Pistia stratiotes*, *Limnocharis flava*, *Oryza sativa*, and *E. crassipes* were used in no choice and choice tests. The above mentioned five plants (±20-40 cm high, ±100-150-gram weight) were presented to ten adult agents in a field net covering cage experiment (20 × 20 × 45 cm) in the Banyubiru sub-district as the selected site for seven days in January 2017, with three replications.

We considered *M. vaginalis* and *E. crassipes* for the choice test based on the no-choice results that showed feeding scars on both of them. In the cage experiment, ten adult weevils were placed in the center of two plants available. The trial was repeated three times with weekly observations for one month.

Assessment of daily herbivory rate. An assessment was carried out to see the daily herbivory rate of an adult weevil per leaf area (Lupi et al 2009), by adding a piece of leaf into the net covered container (15 × 15 × 20 cm in size) with an adult inside. The five trials were conducted for 24 hours in the laboratory in January 2017.

Measuring damage level. BioLeaf-foliar analysis (version 1.0 beta), an advanced mobile application, was used to quantify leaf damage of plants caused by *E. crassipes* weevils. This freeware was promoted by a group of researchers from the Federal University of Mato Grosso do Sul - UFMS Brazil, campus of Ponta Porã - CPPP, collaborated with Dom Bosco Catholic University - UCDB researchers and the group INOVISAO as the leader.

There are four procedures to quantify foliar damage: (a) picture thresholding, (b) elimination of noise, (c) border repair using quadratic Bezier curves, and (d) foliar damage quantification. BioLeaf performs foliar damage quantification with high precision compared to what a supposed expert would do (Machado et al 2016). The foliar damage was grouped into three levels: low (< 10%), moderate (11-49%), and high (> 50%) (Zvereva & Kozlov 2014).

Data analysis. A statistical analysis with the SPSS statistics program was used in analyzing the experiment's data. The descriptive statistics described average and standard deviation, while Pearson correlation was used to learn the correlation between adult and larval quantities of *N. eichhorniae*. Two-way ANOVA and least significant difference (LSD) tests were used to see differences in quantities of adult *Neochetina* spp. and larvae between sites and sampling times at 95% confidence interval.

Results

The abundance of adults and larvae of *Neochetina* spp. In this study, it was found that *N. eichhorniae* was present in all sampling locations, but *N. bruchi* was not established. Based on the collection, it was discovered that the average number of adult *N. eichhorniae* was 5-14 individuals m^{-2} . The two-way ANOVA was used to compare the mean differences between groups of the adult and larvae (Figure 2), and the results showed that the abundance of *N. eichhorniae* adults found at four locations was similar (sig. value = 0.184, $p > 0.05$), whereas based on the time of sampling, the number of adults was different (sig. value = 0.037, $p < 0.05$). The LSD test showed that the average number of *N. eichhorniae* adults in October and November differed significantly from the average in December (mean difference = -3.7500*, $p < 0.05$; mean difference = -3.7500*, $p < 0.05$ respectively). The approximate number of larvae was 2 to 9 individual m^{-2} . The larvae of *N. eichhorniae* collected from *E. crassipes* was similar in the different locations and time (sig. value = 0.525, $p > 0.05$ and sig. value = 0.254, $p > 0.05$ respectively). Our other findings showed a very weak relationship between the adult *N. eichhorniae* quantity and larvae (Table 1) found during the research period (the correlation coefficient = 0.226**, $p < 0.05$).

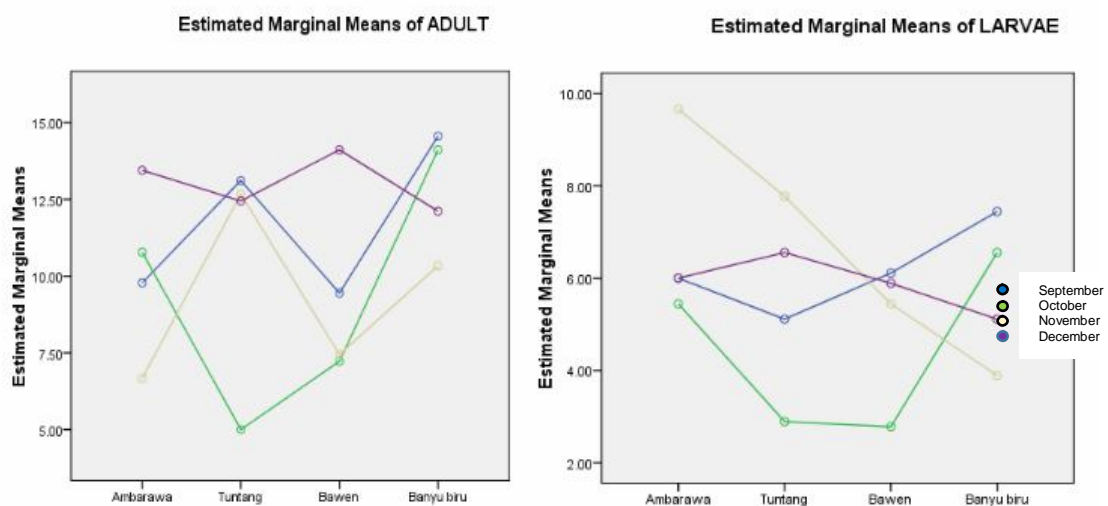


Figure 2. The marginal means of *Neochetina eichhorniae* adult and larvae found in the research location during September-December 2016.

Table 1

Correlations between adult and larvae of *Neochetina eichhorniae*

Stage	Correlations	Adult	Larvae
Adult	Pearson Correlation	1	0.226**
	Sig. (2-tailed)		0.007
	N	144	144
Larvae	Pearson Correlation	0.226**	1
	Sig. (2-tailed)	0.007	
	N	144	144

** Correlation is significant at the 0.01 level (2 tailed).

No choice and choice tests. In the no-choice test, we proved that there were no feeding scars on *P. stratiotes*, *L. flava*, and *O. sativa*, except for *E. crassipes* and *M. vaginalis* leaves (Table 2). The weevils survived for seven days test, except for the control that survived only for 2 days. The other findings, in the choice test, showed that there was low damage for *E. crassipes* and *M. vaginalis*, but the trend of foliar damage tended to increase for four weeks (Table 3, Figure 3).

Table 2

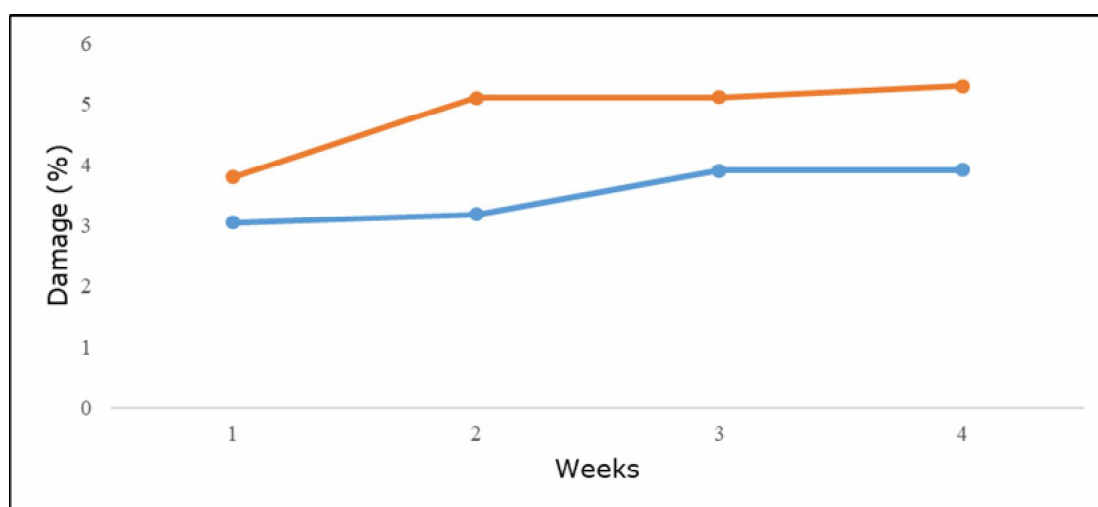
Foliar damage (%) of plants tested for *Neochetina eichhorniae* (no choice test)

Family	Species	Damage (%)			Means	Category	Day live
		X1	X2	X3			
Pontederiaceae	<i>E. crassipes</i>	3.0	2.8	5.5	3.80	Low	7
Pontederiaceae	<i>M. vaginalis</i>	1.3	1.4	4.43	2.41	Low	7
Araceae	<i>P. stratiotes</i>	0	0	0	0	No damage	7
Limnocharitaceae	<i>L. flava</i>	0	0	0	0	No damage	7
Poaceae	<i>O. sativa</i>	0	0	0	0	No damage	7
Control	No plant	-	-	-	-	Died	2

Table 3

Damage percentage of plants list for four weeks (choice test)

Species	1 week			2 weeks			3 Weeks			4 Weeks		
	X1	X2	X3	X1	X2	X3	X1	X2	X3	X1	X2	X3
<i>E. crassipes</i>	3.97	1.89	5.59	4.99	4.39	5.99	8.31	3.61	3.40	4.43	4.27	7.27
<i>M. vaginalis</i>	1.54	2.44	5.18	3.59	1.91	4.09	4.48	4.48	2.37	2.60	5.23	-

Figure 3. The average damage percentage of *Eichhornia crassipes* (orange line) and *Monochoria vaginalis* (blue line) for four weeks (choice test).

The daily herbivory rate on *E. crassipes*. Based on the laboratory experiment, the results showed that the herbivory rate of weevils was 0.43% per *E. crassipes* leaf area per day (Table 4).

Table 4
Foliar damage (%) ability of an adult of *Neochetina eichhorniae* (daily)

No	Species	Foliar damage (%)					Means
		Day I	Day II	Day III	Day IV	Day V	
1	<i>E. crassipes</i>	0.62	0.38	0.04	0.415	0.56	0.43

Discussion. The present investigation showed that *N. eichhorniae* has been established in Rawa Pening lake, whereas *N. bruchi* showed no establishment. This finding was in line with another study in West Java that found *N. eichhorniae* was widely distributed in several freshwater areas in West Java, Indonesia, but not for *N. bruchi* (Sapdi et al 2006). There were several possible factors that influenced the biocontrol agent's existence after the introduction from the origin country to new ecosystems. These included the inability to adapt to the new climate, drastic changes of seasons, competition, predation, and parasites that attack agents. According to Center & Allen (2010), the temperature was not the essential factor driving tropical agent population, and the water temperatures in Central Java were not extreme, ranging from 26 to 27°C (Sulastri et al 2016). Hence, the temperature may not interfere with the weevils' life. The other factor that caused the loss of *N. bruchi* was an inability to adapt to Rawa Pening Lake (tropical condition). These results agreed with the reports of other researchers that *N. bruchi* showed potential damage in environments with higher content of N and P because of their richer production of offspring in Australia (Heard & Winterton 2000).

Natural enemies and pathogens can also be reasons for the loss of a number of biocontrol agents. Microsporidiosis is a disease caused by microsporidia and causes the decrease in weevils' health. The symptoms of this disease are sluggishness and abnormal feeding. When it attacks, it disrupts the development of pupa and larvae by impeding their growth and development until they die (Rebelo & Center 2001; Lewis et al 2003). Another study proved that *Beauveria bassiana* (Balsmo) Vuillemin led to mortality of every life stage of *N. bruchi* (Chikwenhere & Vestergaard 2001).

Intraspecific competition did not occur between *N. bruchi* and *N. eichhorniae* according to Center et al (2005), because when a habitat is dominated by *N. bruchi*, *N. eichhorniae* has less of an effect on *E. crassipes*. The number of weevils introduced the first time also may affect the persistence of insects in a new environment (Memmott et al 1996). Therefore, in this case, there was no precise reason for the absence of *N. bruchi* in Rawa Pening Lake.

The number of *N. eichhorniae* adult and larvae were 5 to 14 and 2 to 9 individuals m⁻² respectively. This finding was higher than that of another area in Mexico that found 7 *N. bruchi* and 4 *N. eichhorniae* adults per m² four years after release (Martinez Jimenez et al 2001). The highest weevil population in India was 14.97 weevils/plant in September with high humidity, while the lowest was 2.49 weevils/plant in January (Ray & Sushilkumar 2015). Our findings showed the average number of adult and larvae weevil was not significantly different, except for adults, in the period of October, November differed from December of the year 2016. This can be attributed to the fact that more than 50% of the lake was covered by *E. crassipes* followed with successful passive weevil spreading in lakes. In general, the abundance of weevil adults has a very weak relationship with the number of larvae.

Based on the field cage experiment with the no choice test, the percent of *E. crassipes* leaves damage was 3.8% and 2.41% for *M. vaginalis*, categorized as minor damage, while no damage was recorded for the remaining three plants. *N. eichhorniae* damaged *M. vaginalis*, in addition to *E. crassipes*, the possible reason was both of them belonged to Pontederiaceae family and has similar leaf structure (Fan et al 2013). In the control cage, *N. eichhorniae* survived only two days without food and water. This is in line

with the other results which indicated that *N. eichhorniae* and *N. bruchi* can survive without food and water in humid environmental conditions up to 48 and 28 days, respectively. In the condition where the water was available, *N. eichhorniae* can survive up to 56 to 82 days, while in a place with no water and no food, it can only survive up to 3 days (Jayanth & Visalakshy 1990).

The choice test found the higher feeding scars in *E. crassipes* compared to *M. vaginalis*, and the trend of damage percentage of *E. crassipes* increased during field cage experiment period. However, there were no eggs or larvae found during the investigation. The results were in conformity with another study that explained *N. eichhorniae* would damage some aquatic plants species in the choice test (Sapdi et al 2006). However, neither eggs nor larvae were found in plants. The other investigation proved that the weevil only damaged and completed its life cycle on *E. crassipes* in host range tests with 46 plant species from 23 families (Jianging et al 2002). There was no damage on *Oxycaryum cubense*, *Thalia multiflora*, *Salvinia biloba*, *P. stratiotes*, *Ludwigia peploides*, or *Nymphaea prolifera* as plants for weevils (Martinez et al 2013). This suggests that *N. eichhorniae* has a high preference for *E. crassipes* and has no risk for other aquatic plants in the Rawa Pening Lake. For this reason, the agents were safe for controlling *E. crassipes*.

The damage caused by ten weevils in *E. crassipes* was classified as minor damage, and the daily herbivory rate of *N. eichhorniae* was only 0.43% per leaf area. The other study found that adult *N. eichhorniae* and *N. bruchi* damage the leaves up to 65.47 and 66.95 mm² per day respectively (Pichidsuwanchai 1996). The maximum scars by an adult *N. bruchi* and *N. eichhorniae* were 75 and 86 mm² per leaf per day respectively (DeLoach & Cordo 1976). The level of damage of leaves correlated positively with leaf nitrogen content (Moran 2004; Martinez et al 2013).

The leaves of *E. crassipes* with little damage caused by weevils were not proportional to the rapid growth and high biomass of *E. crassipes* in Rawa Pening Lake. This finding was similar to that of other research, which conducted an experiment to control *E. crassipes* of different sizes (small, medium, and large). The results proved that small and medium-sized specimens experienced significant damage by 20 weevils per plant, but not for large *E. crassipes* specimens (Ray et al 2009). The rapid growth of *E. crassipes* was supported by the high nutrient concentration in Rawa Pening Lake. This can be attributed to many anthropogenic activities in the catchment area, such as agriculture, floating cage culture, tourism, and restaurants. However, Rawa Pening also received run off nutrients from nine upstream rivers. Rawa Pening Lake was categorized as a hypertrophic lake with high N and P content. Based on the in-situ measurement, the high nitrogen concentration in the water was 0.081 mg L⁻¹ and 1.350 mg L⁻¹ for phosphate (Nugroho et al 2014), and the trophic state index was 57.22-68.06 (Aida & Utomo 2016). Thus, it was categorized as an eutrophic lake. Future research should investigate the integrated control method and the comprehensive approach to manage the nutrient input that came from the catchment area and the nine upstream rivers.

Conclusions. The present study showed that *N. eichhorniae* was well established in Rawa Pening Lake but not for *N. bruchi*. This study also showed that the quantities of *N. eichhorniae* adults and larvae found per m² were low in Rawa Pening Lake. In general, larvae and the adults found were not significantly distinct between locations and period. The foliar damage rate of an adult *N. eichhorniae* was 0.43% per *E. crassipes* leaf per day, and it could not control *E. crassipes* population due to the high nutrient levels in Rawa Pening Lake. Weevils damaged *E. crassipes* and *M. vaginalis* because both of them belong to the Pontederiaceae family and have uniform leaf structures. According to our investigation, *N. eichhorniae* was safe to control *E. crassipes*. However, a supplementary study should be added to examine integrated control and nutrient management between catchment area and headwater area for successful *E. crassipes* control.

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