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Growth of the water fleas *Daphnia magna* (Straus, 1820) at different trophic levels of two small urban lakes in Indonesia

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Abstract: Nutrient enrichment in waters that has become a major environmental problem is related to excessive loading of nutrients into aquatic ecosystems. This nutrient enrichment, called eutrophication, favors phytoplankton growth, which can function as a natural daphnid feed. This study examined the growth performance of the water fleas *Daphnia magna* in water collected from small lakes (ponds) of different trophic levels. The water was taken from Situ Rawa Kalong, considered eutrophic from its dark green color, and the less eutrophic Situ Cibuntu with relatively clear water. Daphnids were grown in six aquaria filled with water from both ponds without artificial feeding with an initial density of five individuals/L. Samples of daphnids were taken every three to four days to observe their growth and reproduction, along with water samples to analyze the chlorophyll content and total suspended solids (TSS). The result showed that the eutrophic water of Situ Rawa Kalong favored phytoplankton growth, indicated by a consistently higher chlorophyll content in the water ranging from 35.3 to 140.7 µg/L compared to less eutrophic water of Situ Cibuntu with chlorophyll content ranging from 1.4 to 13.2 µg/L throughout the experiment. A much higher daphnid density of 151.7 individuals/L was achieved with more water chlorophyll content, meaning phytoplankton availability became a controlling factor for daphnid growth in the pond waters. This study reveals the functional relationships in the food chain between the water trophic level, the abundance of phytoplankton as the primary producer, and daphnids as the first-order predator. It also suggests that the open water trophic level can be managed to favor the daphnid growth, which can then be harvested for use as natural feed.

Keywords: *Daphnia magna*, eutrophic waters, nutrient, Situ Rawa Kalong, Situ Cibuntu

1. Introduction

The uncontrolled enrichment of nutrients in the aquatic environment, known as eutrophication, has become a common phenomenon not only in Indonesia, which is alleged to be a national problem that needs to be resolved immediately (Kementerian Lingkungan Hidup Republik Indonesia, 2011) but also throughout the world (Yang *et al.*, 2008). These nutrients are mainly derived from anthropogenic activities, including the disposal of domestic and industrial wastes, fertilizers leaching from agricultural activities, and fishery cultivation activities (Haryani, 2013; Yang *et al.*, 2008). For instance, a load of nutrient input from the upper Citarum River catchment area was around 34 tons N/day and 5.5 tons P/day (Garno, 2001). This load, together with more significant input from floating net cage fish cultivation, reaching 478 tons N/year and 68 tons P/year (Garno, 2002), leads to severe eutrophication problem in Saguling Reservoir, with N and P contents of 0.684–3.460 mg/L and 0.067–0.364 mg/L, respectively, while the chlorophyll content reached 5.364–71.126 mg/m^3 (Van der Gun, 2012; Hart *et al.*, 2003). Eutrophication problems have also occurred in Lake Limboto, with the contents of N and P recorded at 0.89–1.66 mg/L and 0.12–0.64

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mg/L respectively, and the chlorophyll content of 18.43–42,18 mg/m^3 (Chrismadha & Lukman, 2008). Likewise, the waters of Lake Maninjau are considered to be under eutrophic conditions, with the TN and TP contents of 0.37–7.43 mg/L and 0.02–0.65 mg/L, and the chlorophyll content of 0.236–0.285 mg/L (Syandri *et al.*, 2014; Lukman *et al.*, 2013). A similar problem also occurred in several lakes of West Java, such as Situ Rawa Kalong, where the average contents of chlorophyll, TN, and TP were 254.23 µg/L, 11.13 mg/L, and 0.38 mg/L, respectively (Satya *et al.*, 2018).

Eutrophication leads to phytoplankton blooms (Chrismadha & Lukman, 2008; Sulastri *et al.*, 2015). This phenomenon is a logical consequence of the phytoplankton ecological role as autotrophic organisms that utilize abundant nutrients under available solar energy for their growth (Chrismadha *et al.*, 2012; Andriani *et al.*, 2017; Meirinawati & Fitriya, 2018). According to the ecological function of the food chain, phytoplankton bloom will be an abundant source of food for first-order consumers, one of which is daphnids. There have been studies that reported the ability of Cladoceran to grow by taking advantage of phytoplankton abundance in open water (Chen *et al.*, 2009; Zhang *et al.*, 2009; Pinto-Coelho *et al.*, 2003; Pandolfini *et al.*, 2000). On a laboratory scale, daphnids have also been shown to exploit phytoplankton abundance for their growth (Chrismadha & Widoretno, 2016).

The water flea *Daphnia magna* is a planktonic animal of the lower crustacean group, belonging to the class Crustacea and the order Cladocera (Bekker *et al.*, 2018). The animal is among various natural feeds commonly used in freshwater aquaculture. Several reports indicated superior nutrition values of daphnids, including high protein content with relatively complete amino acids and unsaturated fatty acids content (El-Feky & Abo-Taleb, 2020; Fahmi *et al.*, 2019; Herawati *et al.*, 2018;). Mass cultivation of daphnids usually involves organic wastes to fertilize water to stimulate their growth (Cheban *et al.*, 2018; Darmawan, 2014).

Eutrophication in lake water can be considered the same process as water enrichment by organic wastes and can, therefore, be utilized as a medium for the mass cultivation of daphnids. A high abundance of phytoplankton in eutrophic water has been reported by Vanni and Temte (1990) and Sulastri *et al.* (2015) and has been shown to have the potential to provide natural foods to support the growth of daphnids (Chrismadha & Widoretno, 2016). Until recently, however, there was still very little information on the potential utilization of eutrophic lake water for the mass cultivation of daphnids. Therefore, this study was intended to determine the potential utilization of water from eutrophic open waters as a growth medium for the water fleas *Daphnia magna*. In this case, the enriched water is designed to be converted into phytoplankton biomass, which is then available for grazing to support the daphnids' growth.

2. Materials and Methods

2.1. Location, Research Time, and Study Biota

The research was conducted at the Research Center for Limnology LIPI in November 2018. The biota studied was the water flea *Daphnia magna* Straus, 1820. The daphnid stocks were taken from the Research Center for Limnology LIPI collection, which were maintained in the water media enriched with catfish feed pellets (Hi-Pro-Vite 781™).

This study used six aquariums measuring 40 cm × 30 cm × 30 cm equipped with an aeration system and filled with 15 L of water from Situ Cibuntu dan Situ Rawa Kalong ponds. The aquariums were placed in a room with a transparent roof and a room temperature of 25–34°C.

2.2. Eutrophic Waters

The waters at two different trophic levels were taken from ponds around Bogor Regency: Situ Cibuntu and Situ Rawa Kalong, which were subsequently used as the growth media in this experiment. Situ Cibuntu represented lightly contaminated water based on the relatively clear water and the odorless water condition, while Situ Rawa Kalong represented heavily contaminated water due to its foul smell and dark green color. The water was taken from both ponds, collected in several plastic jerry cans, and then brought to the Research Center for Limnology LIPI, where it was poured into experimental aquaria. Subsequently, each aquarium was aerated overnight before the daphnid source was inoculated.

2.3. Daphnid Culture

Large adult daphnids obtained by filtering the animals from culture stock using a net with a mesh size of 2 mm were selected for sowing daphnid cultures. After inoculation with an initial density of five individuals/L, the daphnid population was reared for 19 days without artificial feeding. This research duration was set long enough for the daphnids to reproduce in two to three generations. For this experiment, daphnids filtered by a net with a mesh size of 2 mm were considered adults, while those who passed through the net were considered juveniles.

2.4. Observation

The growth of daphnids was observed in terms of their abundance five times: on day 5, day 8, day 12, day 15, and day 19. The aquaria water was stirred slowly to homogenize the daphnid distribution before 2 L water sampling. The water was filtered using a plankton net, and daphnid samples were collected into 20 mL plankton bottles while the remaining water was returned to the aquaria. Subsequently, the population of daphnids was calculated and extrapolated into individuals/L. Each sampling was conducted in three replications.

Water chlorophyll and total suspended solids (TSS) parameters were monitored to evaluate the presence of phytoplankton, considered the primary natural feed of daphnids. These two parameters were measured at the beginning of the experiment and five times during the experiment simultaneously as sampling for daphnid abundance. A 20 mL water sample was filtered using Whatman GF/C filter paper and then frozen for storage before analysis. The chlorophyll concentration was determined by the spectroscopic method using a UV-VIS spectrophotometer (Hach DR 2800) after extraction using 90% acetone (APHA, 2017).

Meanwhile, samplings for TSS measurement were also conducted following the gravimetric method (APHA, 2017) by filtering 100 mL of water samples through Whatman GF/C filter papers previously weighed. The filter papers and filtered solids were then heated to a temperature of 55°C until the weight was constant. The TSS concentration was determined by subtracting the weight of the filter paper containing the filtered solids from the weight of the filter paper and then extrapolating it into mg/L.

Water quality parameters were also monitored during the experiment to ensure a suitable water condition for daphnid growth. They included water temperature, pH, Dissolved Oxygen (DO), conductivity, Total Dissolved Solids (TDS), and turbidity, carried out twice a week using a Multiparameter Water Quality Checker Horiba U-50.

2.5. Data Analysis

The daphnid growth performance was evaluated by calculating the specific growth rate following the formula:

$$\mathrm{SGR} = \frac{\ln \mathrm{Wt} - \ln \mathrm{Wo}}{\mathrm{T}} \times 100 \quad \text{....Eq (1)}$$

in which SGR = specific growth rate, ln Wo = natural log of daphnid abundance at day 0, ln Wt = natural log of daphnid abundance at day t, T = time (days). The above Equation 1 considered the sampling interval to determine the daphnids' growth responses to the phytoplankton development in the media.

The daphnids' responses to the water trophic status were also assessed regarding the adult-to-juvenile ratio, which assumed that the juveniles would dominate the daphnid population structure under appropriate growing conditions. It is generally known that under unfavorable conditions, the daphnids will turn their reproductive behavior from parthenogenetic to sexual mode, leaving the female adults carrying partially developed embryos that remain dormant until water conditions improve. If conditions are suitable for further development, the eggs will hatch, followed by the return of the offspring to the parthenogenetic pattern (Lawrence, 1981).

At the same time, the food availability related to the water trophic status to support daphnids' growth was evaluated in terms of water chlorophyll, TSS contents, and the chlorophyll to TSS ratio. TSS consisted of inorganic and organic materials. However, since there was no inorganic material in the experimental media, the TSS dynamics were thought to occur due only to organic materials formed by microbiotic development favored by organic contents in the waters. As Satya *et al.* (2018) reported, the eutrophic water of Situ Rawa Kalong contains nutrients and considerably high contents of total organic matter. Therefore, water can support phytoplankton growth and various microorganisms, from heterotrophic bacteria to tiny zooplankton such as rotifers (Wullur *et al*., 2019; Yang *et al*., 2008; Kritzberg *et al*., 2006). Therefore, it is crucial to consider the presence of various microbiotics that could function as food for daphnids, represented by the parameter TSS, while the share of phytoplankton in TSS was calculated using the chlorophyll to TSS ratio.

The food availability was assessed by calculating the relative number of potential foods to the abundance of daphnids, represented by the chlorophyll to daphnids ratio and the TSS to daphnids ratio in this study.

3. Results and Discussion

3.1. Dynamics of Chlorophyll and TSS

The chlorophyll and TSS contents in the water media from both ponds are shown in Figures 1a and 1b. At the beginning of the study, the chlorophyll content in the water media of Situ Rawa Kalong and Situ Cibuntu were 108.11 and 13.17 mg/m^3, while the TSS contents were 13.33 and 6.67 mg/L, respectively. Both chlorophyll and TSS contents in the water samples from Situ Rawa Kalong were much higher than in the water from Situ Cibuntu.

The chlorophyll content decreased during the first five days of the study in both pond water media. After that, the chlorophyll content tended to increase to reach 140.67 mg/m^3 on day 12 in the Situ Rawa Kalong water, while the maximum chlorophyll content in the Situ Cibuntu water was 7.34 mg/m^{3,} which occurred on day 19. The TSS contents in the Situ Rawa Kalong water increased during the study and reached a maximum value of 60 mg/L on day 19, while in the Situ Cibuntu water, the TSS contents fluctuated at low concentrations in the range of 2.3–18.0 mg/L (Figure 1b).

During the study, the proportion of chlorophyll to TSS tended to be higher in the Situ Rawa Kalong water, which ranged from 0.11% to 0.83%, compared to the Situ Cibuntu water, which ranged from 0.08% to 0.30% (Figure 1c).

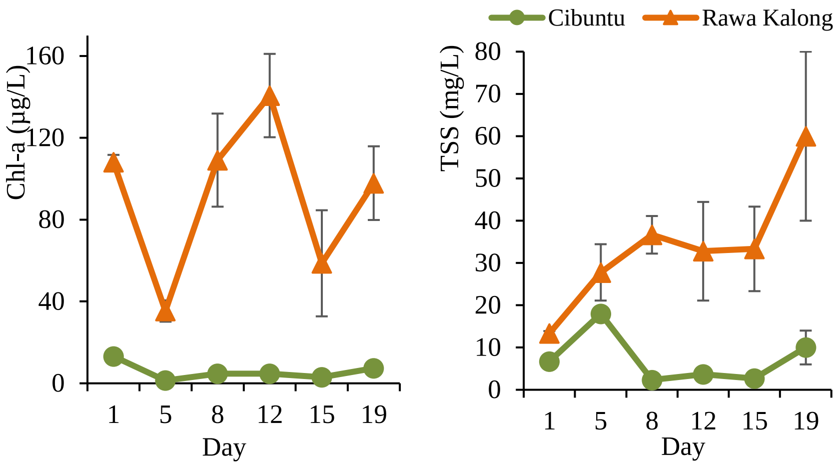
3.2. Daphnid Growth

In the first five days, the daphnid population had grown to 34–47 individuals in the Situ Cibuntu water, consisting of 27–35 juveniles and 5–12 adults, while in the Situ Rawa Kalong water, the population was 4–17 individuals consisting of 4–10 juveniles and 0–7 adults (Figure 2a). This phenomenon showed a more difficult adaptation phase in the more heavily polluted Situ Rawa Kalong water. Although both successfully reproduced immediately after stocking, the survival rates of both juveniles and adults were much better in less polluted water conditions. Figure 2c showed a better population growth rate in the Situ Cibuntu water; the rapid increase in the number of juveniles caused the proportion of adult individuals to decrease to only 20%. The decrease in the proportion of adult individuals also occurred in the water of Situ Rawa Kalong, but it was primarily due to the higher mortality rate of adult individuals.

After going through the adaptation phase, the daphnids grew better in the Situ Rawa Kalong water, and the population increased from 3–9 individuals (day 8) to 24–164 individuals (day12), 14–262 individuals (day 15), and 53–327 individuals (day 19). In the Situ Cibuntu water, the daphnids developed from 23–35 individuals (day 8) to 18–74 individuals (day 12), 15–81 individuals (day 15), and 18–85 individuals (day 19). In the Situ Cibuntu water, the daphnid growth was negative between days 5 and 8. During the study, other types of zooplankton were not found when observing the daphnid density, so it can be ascertained that the daphnids were the only consumers of phytoplankton.

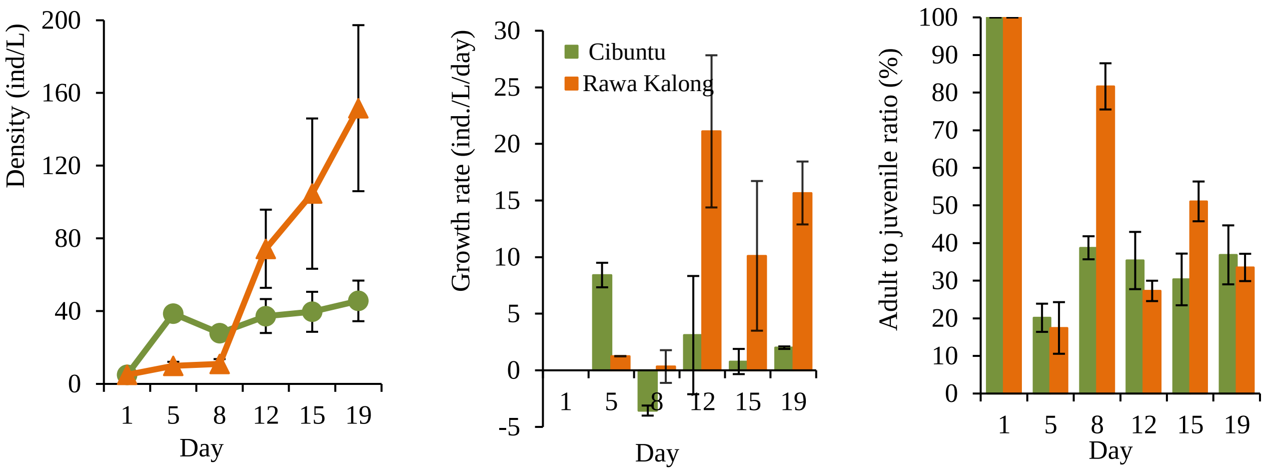
The development of the proportion of adult individuals, especially the average adult proportion of 82% on day 8 in the Situ Rawa Kalong water, indicated that the daphnid population needs adaptation to take advantage of water fertility for its growth. This adaptability varies between individuals, as seen from the survival of adult individuals on day 8 (Figure 2c), resulting in wide variations of population development in the next growth phase.

Meanwhile, the slow development of the daphnid population in the Cibuntu Situ water showed the limited capacity of the water to support daphnid growth (Figure 2a). The ratio of chlorophyll to daphnids in the Situ Cibuntu water tended to be low, indicating that the daphnids could not grow due to limited phytoplankton abundance as the feed and, at the same time, showed the preference of daphnids to feed on phytoplankton (Figure 3a). In contrast, the abundant phytoplankton in the Situ Rawa Kalong water favored the daphnid population to increase and deplete the available phytoplankton, as indicated by the ratio of chlorophyll to daphnids, which continued to decrease until it approached the same value as in the water of Situ Cibuntu (Figure 3).



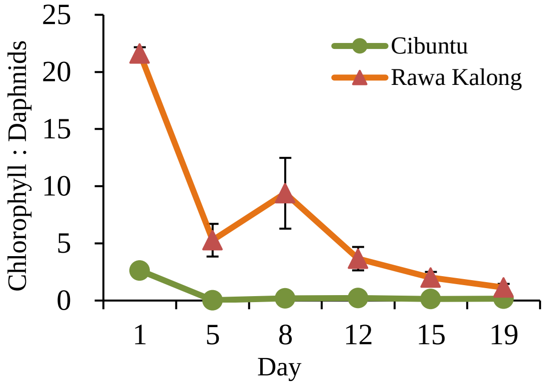
(a) (b) (c)

Figure 1. Chlorophyll content, TSS content, and Chlorophyll : TSS ratio in pond water with different trophic levels

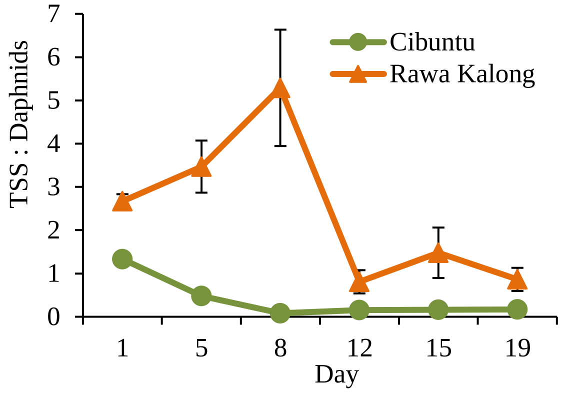


(a) (b) (c)

Figure 2. The daphnid population development in the water from both ponds: (a) Daphnid density; (b) Daphnid growth rate; and (c) Adult to juvenile ratio



(a)



(b)

Figure 3. (a) The ratio of chlorophyll to daphnids; and (b) the ratio of TSS to daphnids to indicate feed availability in both pond waters

3.3. Water Quality Condition

The water temperature during the study, measured every morning, fluctuated in the range of 24.4–27.3°C. The pH of the Situ Rawa Kalong water tended to be higher than that of the Situ Cibuntu water, 7.9–9.6 and 6.9–8.2, respectively. The dissolved oxygen (DO) concentrations also fluctuated but were still in the range of 5.7–6.7 mg/L (Table 1). The DO values tended to decrease in the Situ Rawa Kalong water, while they increased in the Situ Cibuntu water during the final phase of the study.

The conductivity values of the Situ Cibuntu water were 53–68 µS/cm, lower than that of the Situ Rawa Kalong water at 132–162 µS/cm. During the study, the conductivity values of both pond water tended to increase. At the beginning of the study, the low turbidity value of 2.79 NTU was obtained from the Situ Cibuntu water, which continued to decrease until day 19 to 0.53 NTU. At the beginning of the study, the turbidity of the Situ Rawa Kalong water was high (54.49 NTU). It decreased on day 5 to 8.25 NTU, along with the formation of microorganism flocs at the bottom of the aquarium, then rose again to reach 40.22 NTU on day 19 (Table 1). The formation of flocs of microorganisms at the bottom of the aquarium occurs in all aquariums.

Table 1. Ranges of water quality parameters during the study

Parameter	Situ Cibuntu	Situ Rawa Kalong
Temperature (°C)	24.80–27.10	25.00–27.30
pH	6.94–8.22	7.94–9.54
Dissolved Oxygen (mg/L)	5.70–6.70	5.80–6.60
Conductivity (µS/cm)	53.00–68.00	132.00–162.00
Total Dissolved Solids (mg/L)	35.00–46.00	88.00–108.00
Turbidity (NTU)	0.44–2.79	8.25–54.49

Daphnids grew better in the eutrophic water of Situ Rawa Kalong than in Situ Cibuntu water, although a more difficult adaptation process was observed at the beginning of the culture. As shown in Figure 2, less population growth occurred in Situ Kalong water in the early phase of culture; however, after going through the adaptation process, a remarkable improvement in population growth was obtained so that it exceeded that of the Situ

Cibuntu water on day 12, and at the end of the experiment, the abundance of daphnids in the Situ Rawa Kalong water was higher by more than threefold. This superior growth can also be expressed in the specific growth rate, which reached 10–20% per day in the Situ Rawa Kalong water after day 8, while in the Situ Cibuntu water, it was only 2–5% per day (Figure 2). The growth of daphnids in the Situ Rawa Kalong water from this experiment was comparable to that reported by Chrismadha & Widoretno (2016) for daphnids grown in water enriched with fish pellet feed, where the maximum population size was 192 individuals/L. However, this was much lower than the total population of daphnids grown in the water medium enriched with various organic matters, where the peak population size was reported to be up to 10,000 individuals/L (El-Feky & Abo-Taleb, 2020; Darmawan, 2014; Izzah *et al.*, 2014). These findings could indicate that although eutrophic open water can support daphnid growth, it cannot yet be used for mass production.

A higher ratio of adults to juvenile daphnids in response to heavier water contamination was observed during the initial phase of culture, especially on day 8 in the Situ Rawa Kalong water compared to that in the Situ Cibuntu water (Figure 2). The lower proportion of juveniles in the Situ Rawa Kalong water during this phase can be attributed to the inability of juveniles to survive in the higher pollution conditions of the Situ Rawa Kalong water. After passing through the eight-day adaptation period, the juvenile proportion tended to increase.

This experiment also showed the population response to food shortage conditions, which was observed in daphnids reared in the Situ Cibuntu water, where juvenile blooms occurred immediately after inoculation until day 5. However, after that, the population experienced depletion due to the high mortality of juveniles, which could be particularly associated with low chlorophyll content in the water. Consistent with this observation, Ranta *et al.* (1993) and Nogueira *et al.* (2004) reported that the growth of daphnids was highly dependent on feed availability.

The initial chlorophyll concentration in Situ Rawa Kalong waters was 108.11 µg/L, while in Situ Cibuntu, it was 13.17 µg/L (Figure 1a). These values indicated that the trophic level of Situ Rawa Kalong was more than eight times higher than that of Situ Cibuntu, which was also supported by the higher content of TSS, conductivity, and turbidity values (Table 1). As pH measurements were conducted during the daytime, a higher pH was also observed in the Situ Rawa Kalong waters, which could be associated with the higher chlorophyll content. All water quality parameters (Table 1), including temperature and DO, were within the conditions suitable for daphnids' growth (El-Deeb Ghazy *et al.*, 2011; Ebert, 2005).

Chlorophyll content has been widely used to represent the abundance of phytoplankton in open water and is considered in assessing the trophic status of waters (Carlson & Simpson, 1996; Chapman, 1996). Sulastri *et al.* (2015) reported the dynamics of phytoplankton composition in Lake Maninjau from observations with an interval of four years from 2001 to 2014, mainly related to seasonal nutrient availability. In the waters of Situ Rawa Kalong, the high nutrient contents of 11.13 mg/L of TN and 0.38 mg/L of TP were reported by Satya et al. (2018). Meanwhile, considerably lower nutrient contents in Situ Cibuntu water, which ranged from 0.068 to 3.623 mg/L of N-NO_3 and 0.0007–0.101 mg/L of P-PO_4 was reported (Meutia, 2000), and an increase in the phytoplankton abundance with phosphorous enrichment was demonstrated (Chrismadha & Maulana, 2012). In addition, Figure 1 also showed a higher chlorophyll to TSS ratio in Situ Rawa Kalong water, which means a higher phytoplankton share in the TSS content in the Situ Rawa Kalong compared to that in the Situ Cibuntu.

The indications of a relatively stable chlorophyll to TSS ratio in the final stages of daphnid culture (Figure 1c) and the chlorophyll to daphnid ratio in the Situ Cibuntu water (Figure 3a) revealed the preference for daphnid feeding on phytoplankton. There was no population growth in this condition. In contrast, the higher phytoplankton abundance in the Situ Rawa Kalong water allowed the daphnid population to increase sustainably and to deplete the availability of phytoplankton as indicated by the ratio of chlorophyll to daphnids, which continued to decrease down to the values almost the same as in the Situ Cibuntu water

(Figure 3a). Although generally, daphnids are known as filter feeders with no preference feeds (Jensen *et al.*, 2001), some pieces of evidence have been reported to emphasize the dependence of the animals on phytoplankton to fulfill the nutritional requirement, particularly essential lipids (Cheban *et al.*, 2017; Martin-Creuzburg *et al.*, 2011). Yin *et al.* (2010) have also demonstrated the ability of *Daphnia magna* to select more nutritious algal cells for feed, while Lotocka (2001) and Mohamed (2001) reported the ability of the animal to reject toxic blue-green alga during feed filtration.

This experiment points out that the abundance of phytoplankton is highly dependent on the trophic status of water and becomes a controlling factor for daphnid growth in pond waters. It also shows that eutrophic open waters are not fertile enough to support a mass culture of daphnids, unlike in cultivation activities where measured organic fertilization is provided to enhance phytoplankton and other natural feed development. Further experiments are still required to elaborate the possibility of employing eutrophic waters to produce daphnid biomass, among others, by implementing a continuous culture system, in which eutrophic water is constantly supplied to the culture vessel to maintain adequate levels of nutrients over time, subsequently to support the growth of phytoplankton and daphnids.

4. Conclusion

This study reveals that trophic level determines the capacity of open waters for growing daphnids, and phytoplankton development is demonstrated to be the controlling factor. It is considered that open eutrophic water is insufficient to be used for mass production purposes of daphnids. It is suggested that controlling the water trophic level at an adequate level, such as by providing a continuous flow into the culture system, can overcome the insufficiency of water trophic level and make the eutrophic open water usable for growing daphnids. Further studies regarding this topic are still needed.

Data availability statement

All data included and used in this study is not confidential and available upon request.

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Conflict of interests

The authors state that we do not have any conflicts of interest to declare.

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Authors contribution

TC developed the experimental concept, provided the daphnid stock, analyzed the data, and prepared the manuscript. **LRT** collected the open water for experimental culture media, conducted the experiment, analyzed the data, and prepared the manuscript. **EN** carried out the water quality monitoring and chlorophyll analysis.

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