PHILIPP AGRIC SCIENTIST
Vol. 108 No. 1, 7 – 12
March 2025

ISSN 0031-7454
https://doi.org/10.62550/CK21018024

Influence of Nitrogen and Phosphorus Rates on the Growth of Water Hyacinth (*Pontederia crassipes* Mart.)

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Received: March 21, 2024/ Revised: February 04, 2025/ Accepted: February 17, 2025

Research has been conducted globally to investigate the growth and colonization patterns of *Pontederia crassipes* Mart. This research aimed to examine the impact of varying rates of nitrogen (N) and phosphorus (P) on the growth of *P. crassipes* and determine the nutrient levels that would result in the greatest growth response. *P. crassipes* plants were placed in plastic containers filled with 5 L of distilled water. Nitrogen and phosphorus were applied separately at varying concentrations. Specifically, N was added at 0, 10, 20, 30, 40, or 50 mg L^{-1} and P at 0, 1, 3, 5, 7, or 9 mg L^{-1}. *P. crassipes* demonstrated high nutrient uptake efficiency, absorbing 57% to 87% of applied N and 96% to 99% of the applied P. Total weekly biomass increased with N application to an optimal concentration of 25 to 30 mg N L^{-1}, after which growth declined. Leaf production was highest at 30 mg N L^{-1}, while N and P both accelerated offshoot production, with optimal reproductive growth and flowering observed at 20 mg N L^{-1}. No significant effects were observed with varying P rates on biomass or leaf production, suggesting that P may not be a limiting factor under the conditions evaluated. Overall, these findings indicate that *P. crassipes* efficiently utilizes N and P, contributing to its widespread abundance. In areas with high concentrations of these nutrients, *P. crassipes* is likely to thrive and continue to proliferate, underscoring the need for effective management strategies to mitigate its ecological impact.

Keywords: nitrogen and phosphorus response, optimal nutrient level, *Pontederia crassipes*, water hyacinth

Introduction

Pontederia crassipes Mart., commonly known as water hyacinth, is a perennial free-floating and flowering aquatic plant that originated from the Amazon Basin, South America. It has spread across freshwater systems in more than 50 countries on 5 continents, with significant invasions in Southeast Asia, the southeastern United States, Central and Western Africa, and Central America (Bartodziej and Weymouth 1995; Brendonck et al. 2003; Lu et al. 2007; Martínez Jiménez and Gómez Balandra 2007). It first appeared in the Philippines in 1912 and later spread across the Pasig River and the Laguna de Bay (Francisco 2020). Today, *P. crassipes* has infested most of the Philippine waters due to its rapid proliferation rate. In Laguna Lake alone, approximately 800 ha are covered with water hyacinth (Lemuel N Apusaga, Department of Science and Technology, personal communication, 2022 Aug 18). Moreover, a report by the Technical Working Group (TWG) on Task Force Water Hyacinth in Regions III and IV-A stated that an average of 1 M metric tons of *P. crassipes* are collected monthly from bodies of water surrounding these regions (Regions III and IV-A Task Force Water Hyacinth TWG 2022, unpublished data).

P. crassipes can reproduce both sexually and asexually. Through sexual reproduction, a single flower can produce up to 300 seeds, which can remain viable for up to 20 yr (Barrett 1980). *P. crassipes* can also spread through asexual reproduction, where a single plant can produce over 3,000 daughter plants in 2 mo. This results in the production of dense mats of black, feathery roots and spongy, inflated stalks, which can double in size in 6 to 18 d. A cluster size of half a hectare can weigh up to 200 tons (Osmond and Petroeschevsky 2013). Thus, *P. crassipes* has the potential to destroy the ecological system if left to proliferate, as the formation of thick mats in the water can drastically reduce the oxygen content and destroy aquatic biodiversity. Additionally, it tends to block waterways, making it difficult to do boating, sailing, or fishing activities (Francisco 2020).

Several nutrient-rich aquatic ecosystems such as lakes, ponds, rivers, wetlands, and marshes support the growth of *P. crassipes*. Nitrogen- (N) and phosphorus- (P) containing substances are essential for biological growth, and the oxidized forms of these nutrients, such as nitrates and phosphates, can hasten the eutrophication process and alter the quality of water (Yang et al. 2008). Excess N and P from agricultural runoff and urban waste enter water systems, leading to nutrient loading and disrupting ecosystem processes and functions (Metson et al. 2020). These macronutrients, when present in excessive amounts, can accelerate and promote the growth of large aquatic plants. Significant increases of these nutrients in water can negatively affect habitats, food sources, water quality, and oxygen availability for fish and other aquatic life. Large aquatic plant growths also have the potential to drastically reduce or completely remove oxygen from the water, which can cause fish diseases and mass fish mortality. When large aquatic plant growths produce excessive toxins and harmful bacteria, they can negatively impact humans.

There have been several studies on the growth and colonization of *P. crassipes* around the world (Jafari 2010); however, little is known about how different nutrients affect the proliferation of this species, especially in the Philippines. Understanding the varying N and P levels on the growth dynamics of *P. crassipes* is essential in elucidating how these nutrients drive the species' proliferation across different aquatic ecosystems in the Philippines.

Materials and Methods

Pontederia crassipes Collection

P. crassipes plants were collected within the vicinity of Laguna de Bay, Los Banos, Laguna (14°10′56.9″N, 121°13′24.3″E). For uniform size and age, collected plants were initially grown in a plastic container with tap water for 2 wk to allow them to produce daughter plants. After 2 wk, daughter plants weighing between 14.5 to 17.5 g per plant, each with 4 to 5 leaves, were separated from the mother plant and transferred individually into 28 × 22 × 22 cm plastic containers filled with distilled water. Distilled water was used in the study to ensure that no N or P was present initially. Daughter plants were allowed to acclimate inside the greenhouse for 3 d before treatment application.

Experimental Setup

P. crassipes plants were placed singly in plastic containers filled with 5 L of distilled water (Fig. 1). Nitrogen as urea (NH_2CONH_2) was added at rates of 0, 10, 20, 30, 40, or 50 mg L^{-1} and P as Duofos ($CaH_4O_8P_2$) at 0, 1, 3, 5, 7, or 9 mg L^{-1}. The 20 and 3 mg L^{-1} are the N and P rates for the optimum growth of *P. crassipes*, respectively (Gaikwad and Gavande 2017). Nitrogen-assigned plants had 3 mg P L^{-1} added while P-assigned plants had 20 mg N L^{-1} added. All plants received 53 mg L^{-1} of P (muriate of potash, KCl). Water in the containers was mixed constantly to avoid stratification. Initial weight, number of leaves, and initial height were determined before treatment application. Prior to the conduct of the experiment, the water pH was also measured to be 6.83.

To provide sufficient water for the plants, approximately 1.7 and 2.2 L of distilled water was added per container 2 and 1 wk after establishment (WAE) for Run 1 and Run 2, respectively. The total precipitation during the first run of the experiment (2023 Jan 30 – Feb 27) was 11 mm with 7 d of precipitation. The average temperature during this period ranged from 23.0 to 29.8 °C. For Run 2 (2023 Mar 23 – Apr 20), the total precipitation was 35 mm with 12 d of precipitation. During this period, a tropical depression occurred and produced heavy rains (Gile et al. 2023).

Fresh biomass, number of offshoots, number of leaves, days to flowering, days to seed production, and days to offshoot production were measured at weekly intervals for 1 mo. At 4 WAE, plants were harvested. The final fresh weight, number of leaves, final height, and number of offshoots were recorded. Plants were air-dried for 3 d then oven-dried at 70 °C for 5 d and dry weights were recorded. Water samples from each treatment were submitted to the Analytical Service Laboratory of the Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños for N and P content analysis.

Experimental Design and Statistical Analysis

The experiments were arranged in a randomized complete block design. Treatments were replicated 3 times, and the study was repeated. All data were subjected to ANOVA and treatment means were separated using Fisher's Protected LSD at $\alpha = 0.05$. Where appropriate, regression analyses were conducted using Sigma Plot 12 procedures to evaluate the relationship between *P. crassipes* growth parameters and nutrient rate.

Fig. 1 ***Pontederia crassipes*** **as affected by nitrogen and phosphorus rates, 3 wk after stablishment**

Results and Discussion

Nutrient removal by *Pontederia crassipes*

To assess the amount of N and P removed by *P. crassipes*, water samples were collected at the termination of the experiment. Depending on the rate, *P. crassipes* removed 57% to 87% of the applied N (Table 1). This result is similar to that of Fox et al. (2008) who reported that *P. crassipes* accounted for 60% to 85% of the N removed from the solution. The highest N removal was observed at 30 (85.21%) and 40 mg L^{-1} (86.68%). Lower rates (10 and 20 mg L^{-1}) also showed a high removal rate but efficiency declined at the highest rate of 50 mg L^{-1} (55.93%), possibly due to saturation or reduced uptake capacity (Fox et al. 2008).

In contrast to N removal, *P. crassipes* removed 96% to 99% of the applied P regardless of rate applied. This value is higher than what was reported by Xu et al. (2010) where P removal in *P. crassipes* accounted for 83%. On the other hand, Jayaweera and Kasturiarachchi (2004) reported 100% removal of total P by *P. crassipes* from wastewater at the end of the ninth week of the experimental setups.

Reddy and DeBusk (1984) reported that *P. crassipes* can absorb N and P from the water through its efficient nutrient uptake mechanisms and rapid growth characteristics. Its extensive root system facilitated the absorption of N and P compounds from the surrounding water. They further explained that *P. crassipes* has a significant affinity for N and P and possesses specialized transport proteins in its roots that aid in the uptake of N compounds such as ammonium (NH_4^+) and nitrate (NO_3^-) and P compounds such as phospholipids and nucleic acids.

Table 1 Percent nutrient removal of *Pontederia crassipes* as affected by different rates of nitrogen and phosphorus

Nutrient	Rate	Initial amount	Final amount*	Removal
		mg L^{-1}		%
Nitrogen	0	0.00	0.00	0.00
	10	21.70	5.42 ± 0.06	75.02 b
	20	43.50	9.64 ± 0.06	77.84 b
	30	65.20	9.64 ± 0.10	85.21 a
	40	87.10	11.60 ± 0.50	86.68 a
	50	108.70	47.20 ± 0.20	55.93 c
Phosphorus	0	0.00	0.00	0.00
	1	10.40	0.32 ± 0.00	96.92
	3	31.18	0.21 ± 0.02	99.33
	5	52.01	0.21 ± 0.01	99.60
	7	72.81	0.47 ± 0.03	99.35
	9	93.61	0.41 ± 0.01	99.56

*Limit of detection: 0.1 mg N L^{-1} and 0.01 P L^{-1}. Means ± standard error. Means within each column for the same nutrient, followed by the same letter are not significantly different based on Fisher's Protected LSD ($\alpha \geq 0.05$).

Total weekly biomass of *Pontederia crassipes*

Overall, weekly biomass increased with N rate but only up to a certain level, beyond which it started to decline, indicating that there was an optimal N rate for biomass accumulation of *P. crassipes* (Fig. 2). At 1 WAE, biomass was lowest and showed a relatively flat response compared to other weeks, possibly due to early establishment and acclimation to nutrient conditions. In contrast, at 2 WAE, biomass increased more sharply with N rate but started to decline beyond 30 mg L^{-1}. A similar trend was observed at 3 and 4 WAE, although the rate of biomass increase was less pronounced than 2 WAE.

The results suggest that the optimal rate for biomass accumulation ranged from 25 to 30 mg N L^{-1}, beyond which biomass tended to decline. The quadratic nature of the curves highlights that excessive N levels (greater than 30 mg L^{-1}) may negatively impact plant growth. This reduction in growth may be attributed to a disrupted nutrient balance, which interfered with nutrient uptake and may have led to toxicity, ultimately reducing biomass accumulation (Chatzistathis and Therios 2013).

Regression analyses of the weekly biomass of *P. crassipes* in response to P rates revealed no significant differences among treatments, indicating that P application did not have a clear effect on biomass accumulation (data not shown). This suggests that P may not be a limiting or influential factor for biomass accumulation at these stages of the weed. Further investigation into other factors such as environmental conditions may be necessary to identify critical influences on biomass accumulation during these stages of *P. crassipes* development.

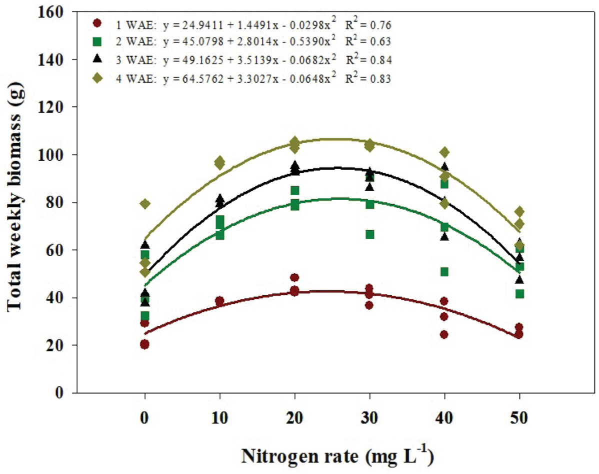

Fig. 2 Total weekly biomass of *Pontederia crassipes* in response to different rates of nitrogen grown in plastic container conditions (WAE - weeks after establishment)

Weekly leaf production of *Pontederia crassipes*

There were no differences in the weekly leaf production of *P. crassipes* among P treatments; thus, only data for N was presented. The most leaves were consistently produced at N rate of 30 mg L^{-1}, with leaf production of about 5.5 per week (Table 2). Other N rates produced about 1 to 3 leaves per week. Nitrogen is crucial for plant growth as sufficient N rate promotes production and expansion of *P. crassipes* leaves through increased photosynthesis, carbohydrate production, protein synthesis, and enzyme activity (Xie et al. 2004). In contrast, excessive amounts of N can negatively affect plant physiology by disrupting nutrient ratios and interfering with metabolic processes, resulting in suboptimal leaf production (Perchlik and Tegeder 2018). Thus, further studies should be conducted to verify the optimum rate of N that will lead to maximum leaf production.

Table 2 Weekly leaf production of *Pontederia crassipes* in response to different rates of nitrogen grown in plastic contrainer conditions

Nutrient	Rate	1 WAE*	2 WAE	3 WAE	4 WAE
	mg L^{-1}	number of new leaves			
Nitrogen	0	0.67 ^{b}	1.00 b	0.83 b	1.17 b
	10	1.67 b	1.07 b	1.40 b	2.17 b
	20	1.70 b	1.83 b	1.67 b	1.50 b
	30	5.50 a	5.50 a	5.67 b	5.40 a
	40	1.83 b	2.50 ab	2.83 ab	2.25 b
	50	2.00 b	2.83 ab	3.17 ab	2.67 b

*WAE - weeks after establishment. Means within a column with the same letter are not significantly different based on Fisher's Protected LSD ($\alpha \geq 0.05$).

Days to offshoot and flower production of *Pontederia crassipes*

Days to offshoot production of *P. crassipes* was significantly influenced by varying N and P rates (Table 3). All plants that received N produced offshoots earlier than those without N application. Specifically, plants that received 20 mg N L^{-1} produced offshoots as early as 7 d after establishment (DAE), while the other N-applied plants developed offshoots by 12 DAE. Results suggest that N availability plays a critical role in expediting the onset of offshoot production in *P. crassipes*. Optimal N levels (around 20 mg L^{-1}) appeared to promote the earliest offshoot development. Beyond this optimal concentration, increasing N rates resulted in diminishing offshoot emergence. In contrast, limited N availability delayed growth, prolonging the time required for offshoot production.

Plants that received P exhibited earlier offshoot production compared to those without P application, though the difference in timing was less pronounced (Table 3). Plants with 1 mg P L^{-1} produced offshoots at 7 DAE, while the other required approximately 9 d. This implies that even low P levels can accelerate offshoot production. Phosphorus is essential for key biological processes such as DNA and RNA synthesis, as well as cell membrane formation (Khan et al. 2023). Adequate P availability thus supports faster growth and reproductive development in *P. crassipes*, leading to earlier offshoot emergence.

Flowering was only observed in plants that received 20 mg N L^{-1}, which was identified as the optimal N rate for *P. crassipes* (Gaikwad and Gavande 2017). The application of this optimal N rate likely promoted rapid vegetative development, accelerating the transition to the reproductive phase and triggering earlier flowering (Kiba et al. 2011).

The lack of flowering in plants without optimal N may have been aggravated by elevated temperatures during the experimental period. Daily temperatures ranged from 25 to 35 °C, exceeding the reported optimum growth temperature of *P. crassipes*, which was between 25 to 30 °C (Kriticos and Brunel 2016). Heat stress at these higher temperatures likely interfered with the plants' metabolic and physiological processes, which could have hindered the flowering response. Heat stress can disrupt key functions in plants, such as hormonal balance and nutrient uptake, thereby affecting processes like reproductive development and flower formation (Taiz et al. 2015).

Number and biomass of *Pontederia crassipes* offshoots

Different rates of N and P had no effect on the number of offshoots produced. Moreover, different rates of P also had no effect on weight per offshoot of *P. crassipes* (Table 4). Offshoots without N had the least weight. This result was expected, as insufficient N availability can impede leaf and

Table 3 Days to offshoot production of *Pontederia crassipes* in response to different rates of nitrogen and phosphorus

Nutrient	Rate	Offshoot production*
	mg L^{-1}	DAE
Nitrogen	0	25 a
	10	12 b
	20	7 c
	30	12 b
	40	12 b
	50	12 b
Phosphorus	0	14 a
	1	7 b
	3	9 b
	5	9 b
	7	14 b
	9	9 b

n=12; DAE - days after emergence. Means within each column for the same nutrient, followed by the same letter are not significantly different based on Fisher's Protected LSD ($\alpha \geq 0.05$).

Table 4 Offshoot biomass of *Pontederia crassipes* in response to different rates of nitrogen in plastic container conditions

Nutrient	Rate	Weight per offshoot*
	mg L^{-1}	g
Nitrogen	0	3.13 b
	10	14.33 a
	20	6.22 ab
	30	11.56 a
	40	9.30 ab
	50	5.83 ab

*Means within a column with the same letter are not significantly different based on Fisher's Protected LSD ($\alpha \geq 0.05$).

stem production in *P. crassipes*. Consequently, overall growth and development are restricted, which may result in fewer and lighter offshoots. In addition, the absence of N can hinder the growth and development of axillary buds responsible for offshoot production (Kiba et al. 2011), leading to a reduced number of offshoots. On the other hand, offshoots with N, regardless of rate, were generally heavier than those without N. Sufficient N enables increased allocation of assimilates towards offshoot production and development, resulting in increased biomass (Irving and Mori 2021).

Overall, the growth of *P. crassipes* was strongly influenced by the availability of N and P, with a preferential uptake of P over N. Optimal N and P rates are crucial for promoting balanced growth, offshoot production, and flowering, whereas excessive nutrient levels can hinder these processes. Understanding these optimal nutrient thresholds will be essential for controlling *P. crassipes* populations. By managing N and P availability, it may be possible to limit the plant's invasiveness, preventing it from reaching densities that could disrupt native biodiversity, impair water quality, and harm ecosystem functions.

Cconclusions and Recommendations

Based on the results, *Pontederia crassipes* can effectively remove nutrients from water, absorbing 57% to 87% of applied N and 96% to 99% of P regardless of application rate. Optimal growth was achieved at 25 to 30 mg N L^{-1}, with diminishing returns and potential toxicity at higher levels. Leaf production peaked at 30 mg N L^{-1}, indicating that adequate N enhances leaf expansion, but excess amounts can disrupt nutrient balance. Both N and P accelerated offshoot production with the earliest offshoot emergence and flowering observed at 20 mg N L^{-1}, supporting reproductive development.

This study illustrates how *P. crassipes* efficiently utilizes N and P, contributing to its abundance. The findings provide evidence that in areas with high N and P concentrations, P. *crassipes* will thrive and proliferate. Given this scenario, the development of strategies to manage overabundance of *P. crassipes* is crucial. Further research should focus on long-term studies to assess how varying nutrient concentrations influence the plant's growth, which would enhance understanding of its population dynamics and potential for invasive spread. Additionally, investigating the mechanisms behind nutrient uptake and assimilation could reveal the adaptive strategies of *P. crassipes*, offering insights into more effective management approaches.

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