

# Effect of Increasing Nutrient Availability on Lemna Gibba Populations

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## **Abstract**

Populations of the aquatic plant *Lemna gibba*, or duckweed, require sunlight and nutrients to synthesize biomass and reproduce. Nitrogen, potassium, and phosphorus provide nourishment to duckweed populations. These elements often play significant roles in research focused on determining *Lemna gibba* nutrient requirements. In this experiment, we explored varying nutrient availability and its effect on *Lemna gibba* populations. We aimed to determine if *Lemna gibba* populations living in aquatic environments exposed

to increased nutrient availability could be classified as density-independent. Our research shows strong evidence that when populations of Lemna gibba have access to larger supplies of nutrients, these populations can be classified as density-independent. Overall, our results indicate that the effect of varying nutrient availability has a significant effect on the rate at which a population of Lemna gibba grows.

## **Introduction**

Populations of the small flowering [1] aquatic plant Lemna gibba, or duckweed, synthesize biomass and grow when they absorb solar energy and nutrients respectively [2]. Lemna gibba increase their population sizes by asexual reproduction [3]. The Lemna gibba plant consists of leaves, called thalli, and roots [1]. A frond is the progeny of a Lemna gibba plant. Division of the parent Lemna gibba plants root occurs when the parent plant has grown enough thalli to give rise to the frond [1, 3]. Thus, the Lemna gibba plant reproduces vegetatively [3]. When populations of Lemna gibba have access to optimal levels of nutrients, rapid vegetative reproduction is possible

[2]. On the contrary, if a community of these plants is subject to nutrient imbalances or nutrient deficiencies, the rate of vegetative reproduction is stunted [2].

Nitrogen, potassium, and phosphorus are some of the elements that plants need to grow. Studies of *Lemna gibba* nutrient requirements often focus on the effect of varying nitrogen, potassium, and phosphorus on *Lemna gibba* populations [2]. For instance, Gale *et al.* chose to use the chemical bonds  $NH_4NO_3$ ,  $KNO_3$ , and  $KH_2PO_4$  as macronutrients in a study of *Lemna gibba* population growth and density [4]. Furthermore, it has been discovered that increasing the amount of phosphorus and holding nitrogen levels fixed can cause populations of *Lemna gibba* to achieve higher growth rates compared to populations living in environments where phosphorus becomes scarce [5].

If water temperature, light, and nutrient resources are held at constant abundances, *Lemna gibba* population growth is exponential [2]. However, the work of Demirezen *et al.* confirmed that there is an inverse relationship between increasing *Lemna gibba* population density and the growth rate of a *Lemna gibba* population [6]. We asked: does *Lemna gibba* pop-

ulation density have an influence on the population's growth rate in a relatively undisturbed environment that exhibits nutrient abundance? That is, we wished to show that such populations of Lemna gibba would reach a carrying capacity. Therefore our hypotheses were

$H_0$  : All Lemna gibba populations living in aquatic environments are *density – dependent*,

$H_A$  : All Lemna gibba populations subject to increased nutrient availability are the only *density – independent* populations,

and thus  $H_0$  would be the conclusion that intraspecific competition for nutrients in each Lemna gibba population was strong enough such that the inverse relationship between population density and the growth rate of each Lemna gibba population would be observable. That is, each Lemna gibba population studied would converge to a carrying capacity.

# Materials and Methods

## *Experimental Design*

Each experimental unit was a cup where we added soil as nutrients. The amount of nutrients added to each cup was a 1/4 teaspoon of soil. Treatment groups received twice the nutrient dosage. Before each initial thalli population,  $N_0$ , was placed into each unit, mock pond water consisting of  $1.3 \text{ mM NaC}$ ,  $0.1 \text{ mM KCl}$ ,  $0.8 \text{ mM CaCl}_2$ ,  $0.2 \text{ mM NaHCO}_3$ , was added to each cup. All volumes of mock pond water were measured at  $200 \text{ mL}$ . The initial population range was  $4 \leq N_0 \leq 6$ . Initial populations of thalli were then placed into the cups. The cups were then placed on a laboratory light tray where the tray floor and fluorescent light tubes were separated by a distance of  $30 \text{ cm}$ . The duration of the experiment was set to be twenty-three days.

### *Data Collection*

Each day, we observed and recorded the number of new thalli and removed senescent individuals. The discrete growth factor and per-capita growth rates were calculated for each population corresponding to that particular day [1]. These rates were calculated by

$$\lambda = N_t/N_{t-1}, \tag{1}$$

$$dN/Ndt = \log(\lambda) = r, \tag{2}$$

where (4) is the discrete growth factor and (5) is the intrinsic growth rate [1]. However, Friday and Saturday observations were not taken. Due to this fact, extrapolations for  $\lambda$ ,  $r$ , and  $N$  were made using

$$\lambda = N_3/N_0 \quad (3)$$

$$r = \log(N_3/N_0) \quad (4)$$

$$\int dN/N = r/4 \int dt \quad (5)$$

$$N_t = N_0 \exp(tr/2) \quad (6)$$

where  $N_0 \leq N \leq N_t$ ,  $0 \leq t \leq 2 r/4$  in (5) and  $t = 1, 2$  in (6).

In compliment,  $r/4$  was introduced in (5) to account for data extrapolation error.

### *Statistical Methods and Models*

In  $H_A$ , we assume that (2) is true for these Lemna gibba populations. Under  $H_0$  we think that

$$dN/Ndt = r - (r/K)N, \quad (7)$$

the logistic growth model is acceptable [1]. To build up evidence against  $H_0$ , we built a sequence of simple linear regression models where we

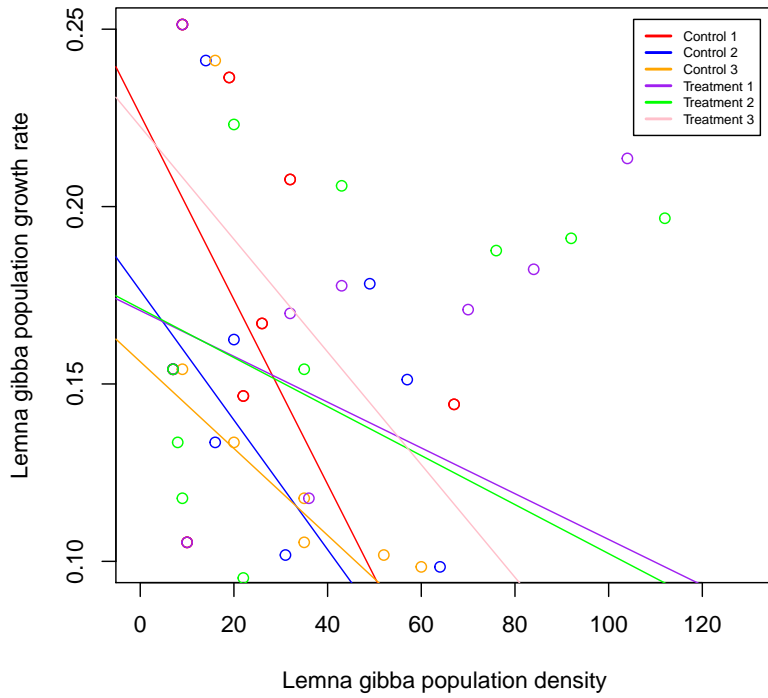
wished to predict  $r$  from  $N_t$  from each Lemna gibba population data set. This was appropriate since  $r$  and  $N$  are both quantitative variables [7]. Finally, we chose to do this because simple linear regression models have the ability to summarize strong, weak, or no relationship between these types of variables [7]. The general form of each model will be

$$E(r | N_t) = \hat{\beta}_0 + \hat{\beta}_1 N_t \quad (8)$$

where  $\hat{\beta}_0$  and  $\hat{\beta}_1$  are statistics estimated from the Lemna gibba data sets [8]. The significance of this is that if we compare (7) and (8), we see that for each population  $r \approx \hat{\beta}_0$ ,  $\frac{r}{K} \approx \hat{\beta}_1$ , and  $K \approx -\frac{\hat{\beta}_0}{\hat{\beta}_1}$  when  $r = 0$  and solving for  $N_t$ . Note that we bootstrapped each model so that  $r$  and  $K$  would be statistically significant and accurate estimates [8]. This was necessary due to the fact that models made errors that did not have constant variance, did not follow a  $N(0, \sigma_\epsilon)$  p.d.f, and our sample sizes were small [8].



# Results



**Figure 1: Effect of nutrients on Lemna gibba populations living in aquatic environments.**

The red, blue, and orange lines in (Fig. 1) are simple linear regression models of  $r$  vs.  $N_t$  for each population of Lemna gibba living in aquatic environments without increased nutrient availability. The purple, green, and

pink lines in (Fig. 1) are simple linear regression models of  $r$  vs.  $N_t$  for each Lemna gibba population living in aquatic environments with increased nutrient availability. (Fig. 1) shows that all Lemna gibba populations living in aquatic environments without increased nutrient availability ceased growth at low population densities. (Fig. 1) also shows that all Lemna gibba populations living in aquatic environments with increased nutrient availability were predicted to achieve a growth rate of zero at significantly higher population densities.

Cup	$\hat{\beta}_0$	$\hat{\beta}_0$ p-value	$\hat{\beta}_1$	$\hat{\beta}_1$ p-value	K
Control One	0.2258724	0.0000055	-0.0026040	0.0018470	87
Control Two	0.2135843	0.0000114	-0.0027094	0.0038400	79
Control Three	0.2788230	0.0014137	-0.0038335	0.0141681	73
Treatment One	0.17067874	0.0013214	-0.0006450	0.2183721	N/A
Treatment Two	0.1712556	0.0001470	-0.0006907	0.1939150	N/A
Treatment Three	0.1520366	0.0047008	-0.0012561	0.2293496	N/A

**Table 1: Estimated growth rates and carrying capacities for Lemna gibba populations living in aquatic environments.**

The red, blue, and orange rows in (Table 1) contain estimated growth rates and carrying capacities for each Lemna gibba population living in aquatic environments without increased nutrient availability. The purple, green, and pink rows in (Table 1) contain estimated growth rates and carrying capacities for each Lemna gibba population living in aquatic environments with increased nutrient availability. All growth rates were estimated by bootstrap simulations of each simple linear regression model. The slopes and intercepts,  $\hat{\beta}_1$  and  $\hat{\beta}_0$ , of each simulated model were concluded to be significantly different from zero for  $p$  values  $< 0.05$ . Carrying capacities that could be calculated were calculated by  $K = \frac{\hat{\beta}_0}{\hat{\beta}_1}$ .  $K = N/A$  means that the carrying capacity for the Lemna gibba population could not be determined.

*Effect of nutrient availability on Lemna gibba populations*

We modeled the effect of varying nutrient availability between the control and treatment groups of the Lemna gibba populations we studied. We found that the inverse relationship between population growth rate and population density was much stronger in all control groups of Lemna gibba compared to the treatment groups of Lemna gibba in this experiment. Further analysis confirmed that there was no inverse relationship between popu-

lation growth rate and population density in all treatment groups of Lemna gibba. We drew this conclusion due to the fact that it was not possible to compute a carrying capacity for these populations at the end of the experiment. This was due to the fact that each  $\beta_1 \approx \frac{r}{K}$  were not significantly different from zero for each simple linear regression model associated with the Lemna gibba treatment groups.

## Discussion

In this study, we found that there is no inverse relationship between the growth rate and population density of Lemna gibba populations living in aquatic environments with increased nutrient availability. On the contrary, we found that this inverse relationship is present when populations of Lemna gibba live in aquatic environments without nutrient increased nutrient availability. Therefore our results indicate that our alternative hypothesis is true. That is, Lemna gibba populations subject to increased nutrient availability were the only density-independent populations in this study. Our findings do not agree with the study done by Demirezen *et al.* However, Debusk and Ryther also conducted a study on Lemna gibba populations and the relationship

between population density and population growth rate [9]. They concluded that high population growth rates are achievable when populations of *Lemna gibba* exhibit low population densities [9]. This implies that our treatment group population densities,  $N_{23}$ , were low enough to offset the inverse relationship described by Demirezen *et al.* agreeing with the conclusion made by Debusk and Ryther.

Since we rejected our hypothesis,  $H_0$ , this may suggest that duration of the experiment was not long enough to observe carrying capacities for each population of *Lemna gibba* living in aquatic environments with increased nutrient availability. One way to estimate these carrying capacities would be to simulate  $r$  vs.  $N_t$  until each population density,  $N_t$ , is high enough to yield  $r = 0$ . Thus, these  $N_t$  would be the carrying capacities of these *Lemna gibba* populations. Otherwise, the duration of the experiment must simply be extended until carrying capacities would be observable. On other topic, we may not have observed carrying capacities of the *Lemna gibba* populations living in aquatic environments with increased nutrients in this study strictly due to chance alone. That is, our statistical analysis may have been unable to detect an inverse relationship between *Lemna gibba* population growth rates and population densities. This may have been due to poor model structure,

experimental design, or disregard of variables that play a significant role in the inverse relationship between *Lemna gibba* population growth rates and population density.

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