The effect of Nitrogen on the growth rate and protein content of Lemna Minor

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This work is **not** a replacement for the Bagrut test.

Subject: The effect of nitrogen (N) on the growth rate and protein content of *Lemna minor*.

Research goal: Checking the feasibility of Lemna minor's protein content elevation using Nitrogen addition.

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Abstract

Duckweed is a flowering aquatic plant, also described as water lentils or water lenses; The common duckweed's scientific name is *Lemna minor*. Duckweed floats on or just beneath the surface of freshwater ponds, forming a mat-looking surface. Duckweed is a promising candidate to be used as a protein source in livestock feed because of its potential for high protein content; ability to grow quickly without using any fertile soil; and accessibility, for example duckweed can be grown in any freshwater body and can be grown in wastewater. Deforestation is a major issue in South America due to the cultivation of the current livestock crop feed, and finding sustainable ways to grow crop feed to combat the deforestation is necessary for the future of the planet. Duckweed, a crop that has a potentially high protein content, is grown in the water and takes up less space than soybean, for example. Duckweed can also be grown in wastewater, even benefitting from the nutrients of the decaying organisms in the water. Although duckweed offers promising applications, right now its cultivation techniques are yet to be optimized and in many instances contain less protein in the plant than soybean, which is a major crop used for livestock feed since it offers high concentrations of protein and its growing practices are well known. In this experiment, I grew duckweed in solutions with varying amounts of Nitrogen Phosphorus Potassium fertilizer (NPK) in hopes to maximize protein levels in the duckweed. Addition of NPK fertilizer at high amounts (6-16 mL/L) and growth period of one week was shown to increase protein levels up to 32% in dry weight of duckweed. This offers applications in agriculture for livestock feed because of high protein levels. In this experiment, studying protein in duckweed opens opportunities to further develop an effective livestock crop feed and ultimately reduce pollutants, land use and help mitigate the effects of deforestation.

1. Introduction and Literature review

Background:

Among the smallest of all flowering plants, common duckweed is a free-floating aquatic perennial that forms a floating mat on the water surface of many fresh bodies of water (including sewage). Common duckweed can be used to remove excess nutrients or toxic metals from bodies of water due to its ability to bioaccumulate or absorb toxic chemicals. *Lemna minor* is a fast-growing and robust plant whose easy harvesting process increases agricultural efficiency when used as feed for livestock. Currently, *Lemna minor* does not have better nutritional value than the feed being used currently. However, although current feed being used, namely soy, has dry weight protein content of up to 50-60% , it requires a lot of arable land in order to grow it. As a result, forests are being cut down in order to make way for crop feed. The main focus of this research paper is to assess the possibility of reaching a higher protein content in *Lemna minor*, in order to substitute current feed and prevent deforestation.

Literature Review:

Utilities:

Lemna minor has become prominent in agricultural practices and water treatment processes because it can absorb minerals in heavily polluted water such as that from sewage treatment facilities. The growing awareness of water pollution and its threat to the ecology of a region and agriculture has also focused attention on potential biological mechanisms for water treatment. In addition, one important characteristic of *Lemna minor* is its potentially high concentration of nutrients like: phosphorous, nitrogen, and potassium. These nutrients are critical for feeding livestock, particularly pig livestock. In general, water and land scarcity is becoming a primary limitation to population growth and is also preventing agricultural growth to feed the ever increasing population of the world. This is where duckweed comes in; duckweed has certain properties allowing for water cleansing and a substitute for feed crop grown on big quantities of land. (Leng 1999).

Growth Conditions:

Water Temperature:

Duckweeds grow at water temperatures ranging from 6 to 33° C. Growth rate increases with water temperature, but there is an upper limit of water temperature around 30° C when growth slows and ceases at even higher temperatures (Leng 1999). Since duckweed is sensitive to temperature fluctuations, there is a need for daily check ups, either mixing in cooler water to balance the heat, or simply stir the water to create circulation.

<u>pH in water:</u>

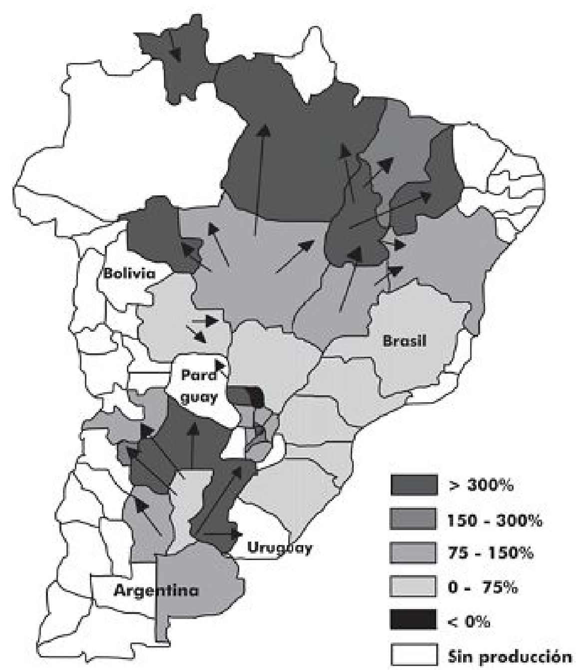
Duckweed survives at pHs between 5 and 9 but grows best over the range of 6.5-7.5. In this pH range, ammonia is present largely as the ammonium ion (NH4+) which is the most readily absorbed nitrogen form (Leng 1999).

<u>Mineral concentrations:</u>

When grown in water filled with other decaying plants that release beneficial materials, such as ammonium (NH_4^+), phosphorous (P), potassium (K) and sodium (Na), the growth rate of *Lemna minor* increases. Growth rate increases because of well balanced mineral levels that encourage photosynthesis, and ammonium provides nitrogen which is essential for all plant growth. (Leng 1999).

<u>Lemna minor as a Solution to Land Shortage:</u>

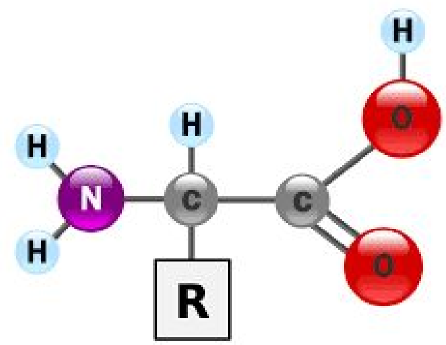
Figure 1 The expansion of the soy growth area in South America from 1995 to 2003. The arrows show the expected growth direction.



The value of duckweed as a feed resource for domestic animals increases with increasing crude protein content, which is the amount of protein in food after observing the nitrogen levels (Tremblay 2020). Currently, soybean is mainly used to feed livestock because of its high protein content, but soybean fields take up around 84.5 to 86.5 million acres of land (Hubbs et al. 2019) that used to be forests. South America is especially suffering from this problem as seen in Figure 1; since 2000, the soy cultivation growth area has grown by more than 10 percent a year (Coalition 2008). The total area dedicated to feed crop production amounts to 33 percent of total arable land (land that can be used to plough and to grow crops) (Steinfeld et al. 2006). Expansion of livestock production is a key factor in deforestation, especially in Latin America where the greatest amount of deforestation is occurring – 70 percent of previous forested land in the Amazon is occupied by pastures, and feed crops cover a large part of the remainder (Steinfeld et al 2006). In addition, soybean expansion and shipment have big impacts on the environment such as biodiversity erosion, pollution, and greenhouse gas emissions. The way that biodiversity is affected by soybean cultivation is because of deforestation and use of animal habitats for soy fields. Rainforests and savannas all over the words are home to millions of animals that now have to find a new area to settle in; some animals don't survive this change in habitat and therefore leads to erosion of biodiversity. This means that if protein content in *Lemna minor* can be raised, soy can be replaced as a main feed source, saving species, forests, reducing pollutants, and minimizing land use.

Nitrogen's effect on Duckweed:

Nitrogen (N) will be tested to elevate crude protein content in Duckweed because of its presence in the amino acids (see amino acid structure image right), the basic units which make a protein. Therefore, nitrogen seemed like the most optimal choice as an independent variable and in many other studies, nitrogen seemed to be the most effective variable to manipulate in the duckweed habitat in order to produce more protein.



2.Research Approach and Thesis

Research Approach:

It was observed in studies at the University of New England, Armidale, Australia, that the crude protein content of duckweed growing on diluted effluent from housed pigs increased with increased levels of nitrogen (N) from about 15% crude protein with trace levels of nitrogen (1-4 mg of nitrogen per liter (N/L)) to 37% between 10-15 mg of N/L. Above 60 mg of N/L a toxic effect was noticed perhaps due to high levels of free ammonia in the water (Leng 1999). Taking this information into account, if we manage to prove that *Lemna minor* can be mass produced with a very high crude protein content, it could replace soy and other crop feeds reducing land use in the future.

Research Question:

In which ways do nitrogen additions in duckweed cultivations affect the growth rate and protein content of duckweed.

Hypothesis:

A limited but increased addition of nitrogen (10-20 mg N/L) will increase the protein content in dry weight of duckweed.

3. Methods

Exp. 1.) The effect of nitrogen concentration on growth rate and protein content of *Lemna minor*.

- *Lemna minor* was cultured in 18, 4 liter plastic containers. With a solution made up of 3 liters of water, 3 ml of SHEFER NPK fertilizer (7% Nitrogen, 3% Phosphorus, and 7% Potassium) and varying amounts of $NaNO_3$ solution (corresponding to 0, 5, 10, 20, 40, 60 mgN/L).
- The experiment consisted of 6 treatments in 3 replicates (total 18 containers)
- Photos were taken every few days in order to track estimated growth. The containers were watered every few days to compensate for water loss due to evaporation.
- At the end of the experiment (1.5 weeks), the *Lemna* was filtered out of the containers and dried in the oven at 60 degrees celsius for two days, biomass was measured, and protein contents were measured by Ambar Laboratories.

Independent variables: Nitrogen content (mgN/L)

Dependent variables: Dry weight Crude protein content was measured in AMBAR laboratories, dry weight biomass was measured using a scale after drying

Environmental variables: temperature, pH and salinity was monitored throughout the experiments. Although these factors will not be controlled, they are expected to be similar in all treatments.

Replicates: each treatment will consist of 3 replicates. Each replicate will consist of hundreds of tiny plants (~10 grams).

Exp. 2.) The effect of NPK fertilizer on growth rate and protein content of Duckweed.

After reviewing the results of experiment 1, it was decided that NPK would be used for the rest of the experiments because the optimal NPK level had not been checked beforehand, which could influence the results of the $NaNO_3$ and make it inaccurate.

- Duckweed was cultured in 4 liter plastic containers with medium containing 3 liters of water and varying amounts of NPK fertilizer. The experiment consisted of 6 treatment groups corresponding to 0, 0.5, 1, 2, 4, 6 mL NPK/L) in 3 replicates for a total of 18 containers
- Photos were taken every few days in order to track estimated growth. The containers were watered every few days.
- After one week, the *Lemna* was filtered out of the containers and dried in the oven at 60 degrees celsius, biomass was measured, and protein contents were measured by Ambar Laboratories.

Independent variables: Nitrogen Phosphorus Potassium (NPK) fertilizer

Dependent variables: Crude protein content (dry weight) was measured in AMBAR laboratories, dry weight biomass was measured using a scale after drying

Environmental variables: temperature, pH and salinity was monitored throughout the experiments. Although these factors were not controlled, they are expected to be similar in all treatments.

Replicates: each treatment consisted of 3 replicates. Each replicate consisted of hundreds of tiny plants (10~ grams).

2.1

Due to the lack of watering and maintenance of *Lemna*, this treatment was not taken into account in the results. The results of the remaining treatments are still valid and shown.

2.2

Another treatment was done with proper *Lemna* maintenance in order to collect accurate results.

Exp. 3) The effect of growth period and NPK fertilizer on growth rate and protein content of Duckweed <u>over a longer period of time</u>.

After reviewing the results of Experiment 2, it was decided that the next experiment would be conducted with the same growing conditions but final measurements would be taken after a longer period of time. This was done to study if longer periods of fertilizer exposure would increase duckweed growth.

- The experiment consisted of 6 treatments in 3 replicates (total 18 containers)
- Duckweed was cultured in 18, 4 liter plastic containers. With medium containing 3 liters of water and varying amounts of NPK fertilizer (corresponding to 0, 0.5, 1, 2, 4, 6 mL/L).
- Photos were taken every few days in order to track estimated growth. The containers were watered every few days.
- At the end of the experiment (two weeks), the *Lemna* was filtered out of the containers and dried in the oven at 60 degrees celsius, biomass was measured, and protein contents were measured by Ambar Laboratories.

In experiment 3, variables and conditions are the same as experiment 2, except for the growing period which was two weeks instead of one.

Exp. 4) The effect of NPK fertilizer <u>at higher concentrations</u> on growth rate and protein content of Duckweed.

After reviewing the results of Experiment 3, it was found that there was continuous growth of duckweed at those specific concentrations. It was then decided that the same experiment would be conducted again but with higher concentrations of NPK fertilizer to test the upper limit of fertilizer content.

- The experiment consisted of 6 treatments in 3 replicates (total 18 containers)
- Duckweed was cultured in 18, 4 liter plastic containers. With medium containing 3 liters of water and varying amounts of NPK fertilizer (corresponding to 0, 2, 4, 6, 10, 16 mL/L).
- Photos were taken every few days in order to track estimated growth. The containers were watered every few days.
- At the end of the experiment (one week), the *Lemna* was filtered out of the containers and dried in the oven at 60 degrees celsius, biomass was measured, and protein contents were measured by Ambar Laboratories.

In experiment 4, the variables and conditions are the same as in experiment 3, except that the independent variable (NPK) concentrations are higher.

4. Results

Experiment 1

The effect of nitrogen content on growth rate and protein content of *Lemna minor*.

The Effect of $NaNO_3$ on Growth:

It was found that when *Lemna minor* was grown in treatments with around 40 mg of $NaNO_3$ addition the *Lemna* grew the fastest. Results of duckweed dry weight from experiment 1, presented in Figure 2, showed that as the amount of $NaNO_3$ increased, the average amount of dry weight (g) increases until 40 mg of $NaNO_3$. There is a logarithmic trendline and the R^2 = 0.906. The control group (0 mg) does not follow the trendline. And as the averages with the higher $NaNO_3$ additions increase, the standard deviation decreases (figure 2).

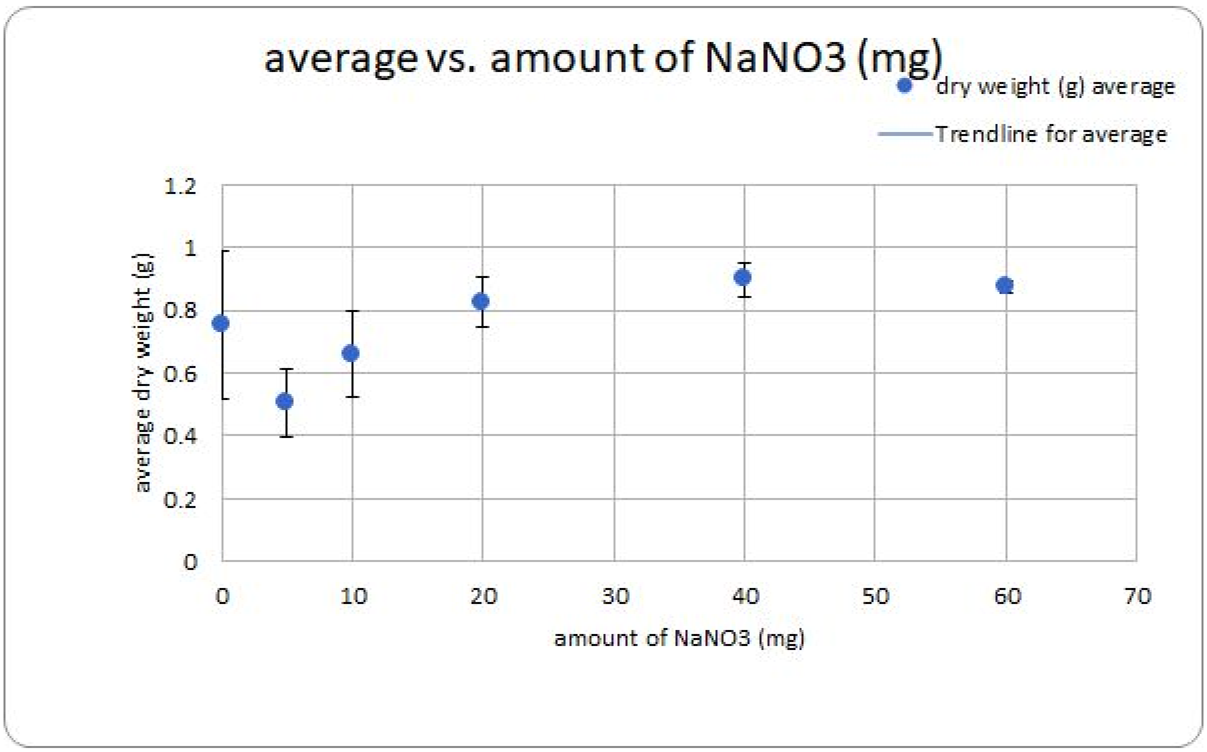


Figure 2: *The effect of $NaNO_3$ addition on the final dry weight of duckweed cultures. Each data point represents the average of three replicates. Error bars represent the standard deviation.*

The results of experiment 1 show that small amounts of $NaNO_3$ decrease the growth rate, but with an addition of more than 20 mg of $NaNO_3$ the growth rate increases higher than the control group until hitting the optimal addition of 40 mg of $NaNO_3$.

The Effect of $NaNO_3$ on Protein Content:

It was found that when *Lemna minor* was grown in treatments with more concentrated amounts of $NaNO_3$, the protein content decreased. Results of duckweed dry weight from experiment 1, presented in Figure 3, showed that as the amount of $NaNO_3$ increased, the average crude protein content (%) slowly decreased. There is a polynomial trendline and the $R^2 = 0.682$.

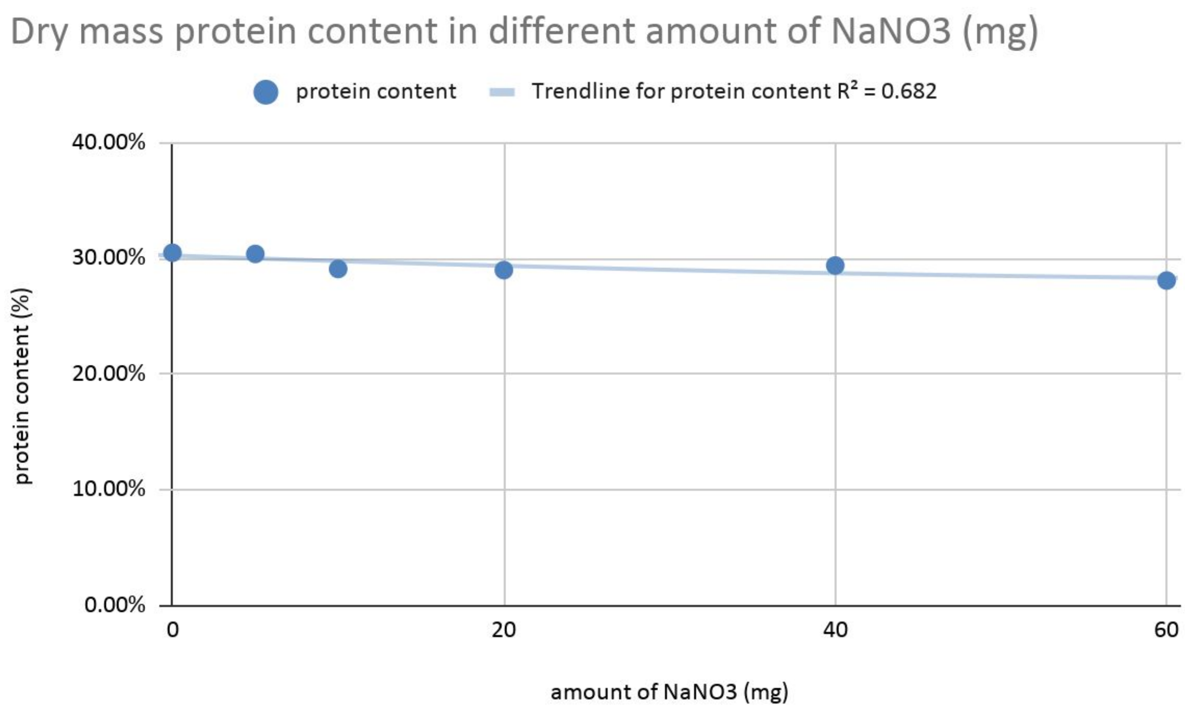


Figure 3: *The effect of $NaNO_3$ addition on the final crude protein content of duckweed cultures. Each data point represents the protein content (%) of three replicates combined.*

The results of experiment 1 show that there is a slow decline in protein content with additions of higher concentrations of $NaNO_3$ unlike the growth rate of the duckweed. The growth rate increased a little, just as the protein content decreased.

Experiment 2.1 and 2.2

The effect of NPK fertilizer on growth rate and protein content of Duckweed.

The Effect of NPK on Growth Rate:

Results of duckweed dry weight from experiment 2.2 yielded that between the amounts of 0 and 1 ml of NPK, the average dry weight decreases and when more than 1 ml of NPK is added the more the NPK increases the more the dry weight of the duckweed increases. In experiment 2.1, between the amounts of 0 and 2 ml of NPK, the average dry weight decreases, and when more than 2 ml of NPK is added the more the NPK increases, dry weight of the duckweed increases. There is a polynomial trendline and the R^2 = 0.8885. In the inaccurate experiment (2.1), the replicates with 1 ml/L do no follow the trendline.

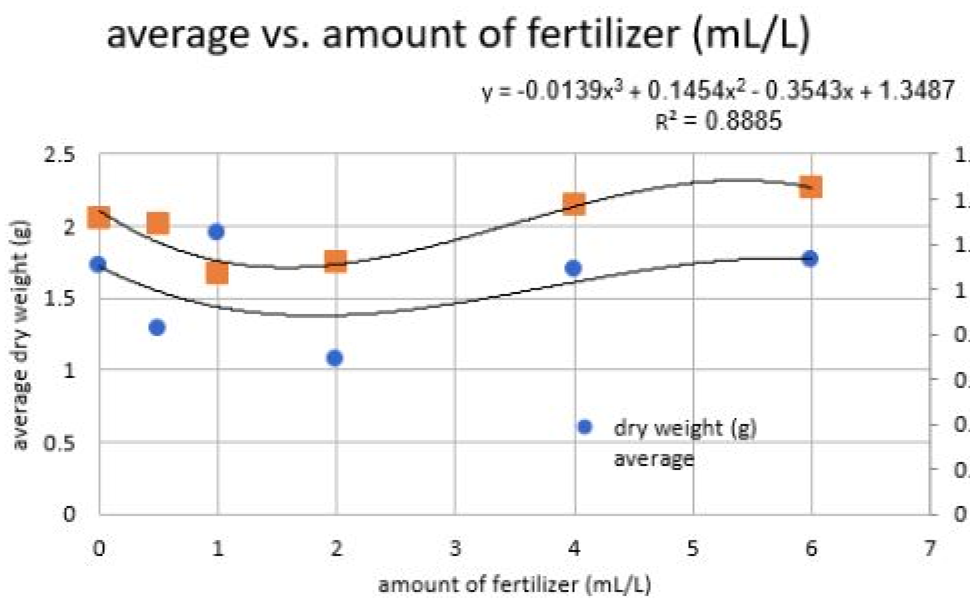


Figure 4: *The effect of NPK fertilizer addition on the final dry weight of duckweed cultures in experiment 2.1 and 2.2. Each data point represents the average of three replicates. Blue data points represent the averages of the inaccurate experiment (2.1) and the orange data points represent the averages of the revised, accurate experiment (2.2).*

The results of experiment 2.2 show that when the additions of NPK fertilizer are in small amounts, the duckweed does not grow as fast as when there is no NPK added or when there is a lot of NPK added.

The Effect of NPK on Protein Content in Experiment 2.2:

The samples from experiment 2.1 were not sent to AMBAR for protein measurement because of the inaccuracies. From experiment 2.2, it was found that when *Lemna minor* was put in a solution with higher concentrations of NPK the crude protein content increased. Results of duckweed dry weight from experiment 2.2, presented in Figure 5, showed that as the amount of NPK increased, the average crude protein content (%) increased apparently getting close to saturation at the highest NPK level (6ml/l). There is a logarithmic trendline and the $R^2 = 0.996$.

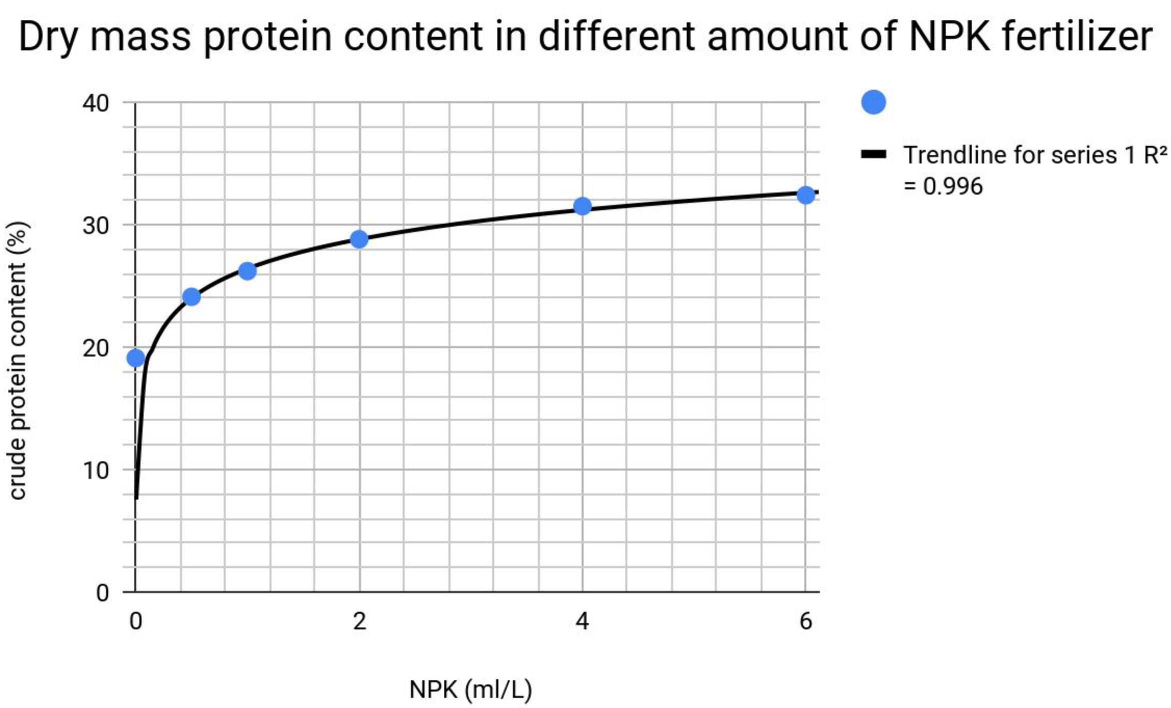


Figure 5: *The effect of NPK addition on the final crude protein content in duckweed cultures. Each data point represents the average protein content (%) of three replicates.*

The results from experiment 2.2 show that there is a constant increase in crude protein content levels in duckweed with more NPK fertilizer. The data shows no upper limit of NPK in this experiment in terms of maximum protein content in duckweed. The experiment shows that when there is a high concentration of NPK, both the growth rate and the protein content increases.

Experiment 3

The effect of growth period and NPK fertilizer on growth rate and protein content of Duckweed over a longer period of time.

<u>The Effect of NPK on Growth Rate:</u>

It was found that as the NPK concentrations increased, the growth rate of the duckweed decreased. Results of duckweed dry weight from experiment 3, presented in Figure 6, showed that as the amount of NPK increased, the average amount of dry weight (g) decreased. There is a polynomial trendline and the $R^2 = 0.41$. The control group (0 mg) and the fourth group (2 mL/L) do not follow the trendline.

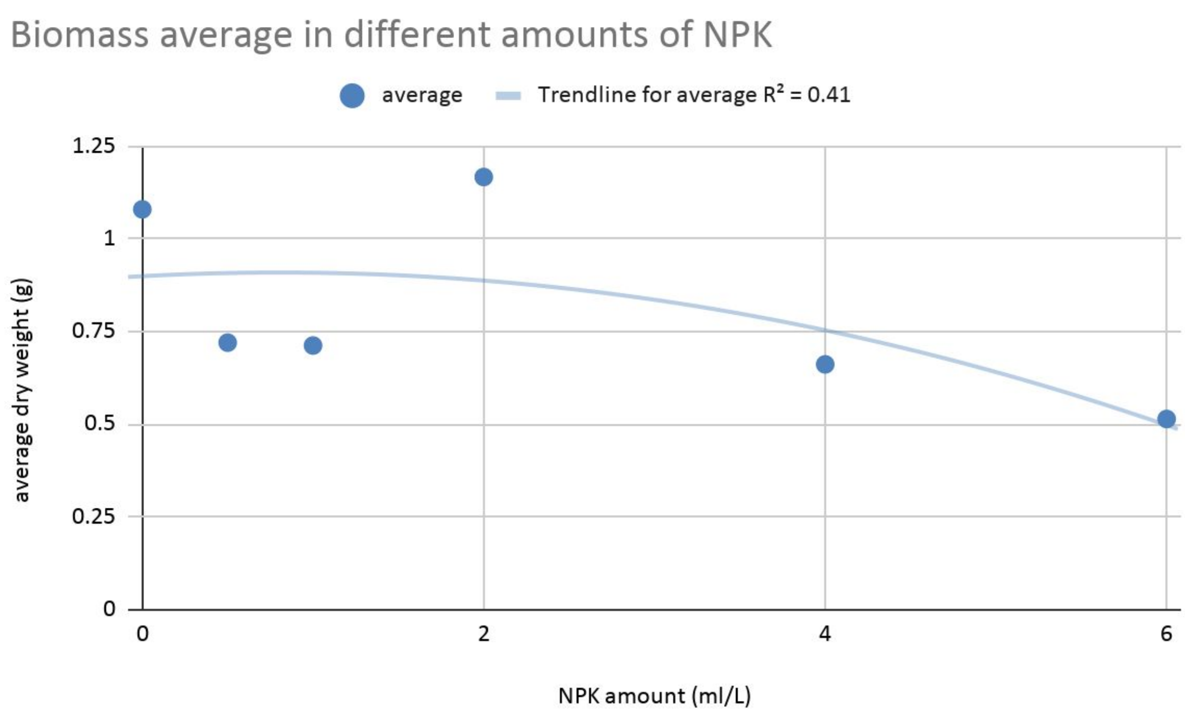


***Figure 6:** The effect of NPK addition on the growth rate of duckweed cultivations. The data points represent the average growth rates of three replicates.*

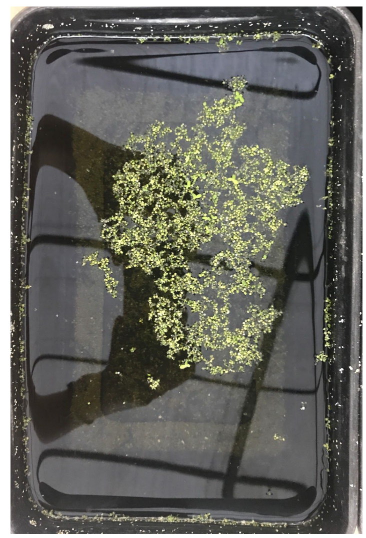
The duckweed that was used for this experiment was unhealthy and most of the duckweed in the cultivations were already dead, thus the experiment might not be fully reliable.

The Effect of Longer Growth Period on Growth Rate:

On the first day of experiment 3 a large percentage of the duckweed used for the experiment were already dead (appearance is white) (Picture 1). After one week, there is less duckweed on the surface of the water, but there is also more green, healthy duckweed in relation with dead, white duckweed (picture 2). On the last day of the experiment, there is a significantly smaller amount of duckweed in the container, but there is only green, healthy duckweed and no dead duckweed on the surface of the water (picture 3).

Picture 1: *Replicate 1 of the control group (0mL/L) on day one of the experiment.*

Picture 2: *Replicate 1 of the control group (0mL/L) on day seven of the experiment.*



Picture 3: *Replicate 1 of the control group (0mL/L) on day 15 of the experiment.*

Effect of NPK on Protein Content:

It was found that as NPK concentrations increased, crude protein content percentages also increased slowly. Results of duckweed dry weight from experiment 3, presented in Figure 7, showed that as the amount of NPK increased, the average crude protein content (%) slowly increased. There is a polynomial trendline and the R2 = 0.994.

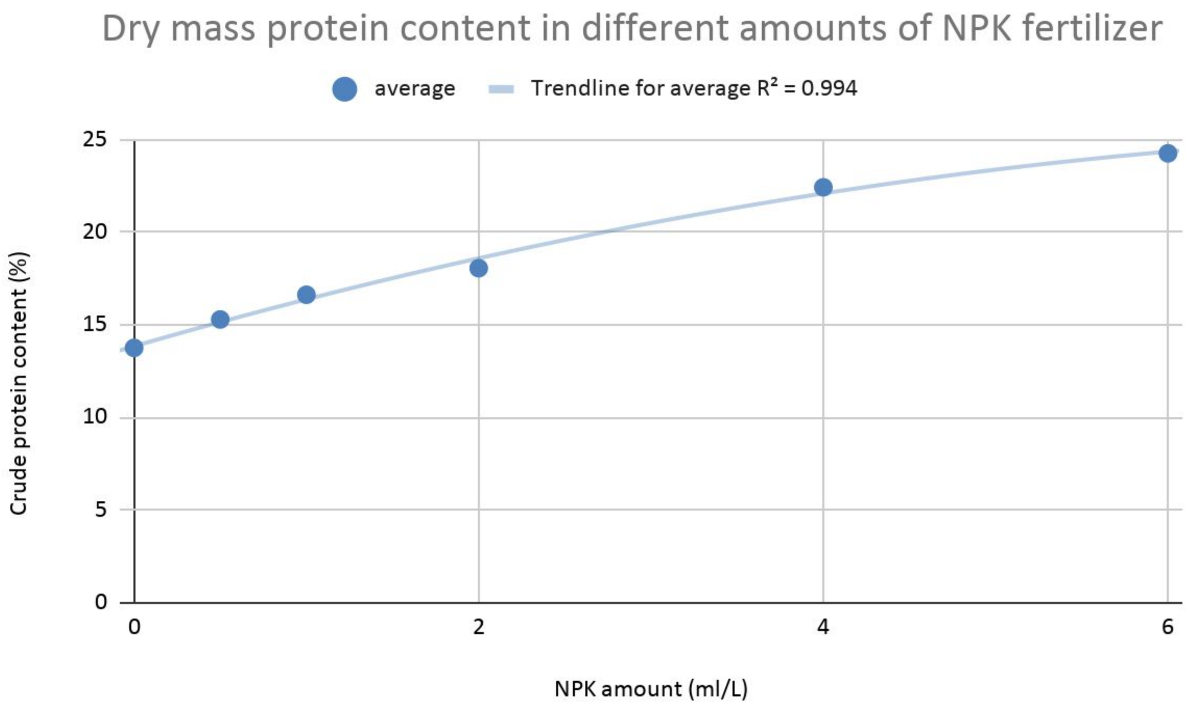


Figure 7: *The effect of NPK addition on the final crude protein content in duckweed cultivations. The data points represent the average protein content (%) of three replicates.*

The results from experiment 3 show that there is a constant increase of protein content in the duckweed cultivations. But the protein content in the control group was lower than in the other experiments, and therefore the highest average was lower than in experiment 2.2 (the effect of NPK on protein content) by 8 percent possibly because the duckweed was unhealthy,.

Experiment 4

The effect of NPK fertilizer at higher concentrations on growth rate and protein content of Duckweed.

<u>The Effect of Higher NPK Concentrations on Growth Rate:</u>

It was found that when NPK was added at higher concentrations, the growth rate increased until the upper limit of 2 ml/L of NPK. Results of duckweed dry weight from experiment 4, presented in Figure 8, showed that as the amount of NPK increased, the average amount of dry weight (g) increased until the upper level of 2 mL/L of NPK and then the average dry weight decreased gradually. There is a polynomial trendline and the $R^2 = 0.967$. The control group (0 mg) and the second group (2 mL/L) do not follow the trendline.

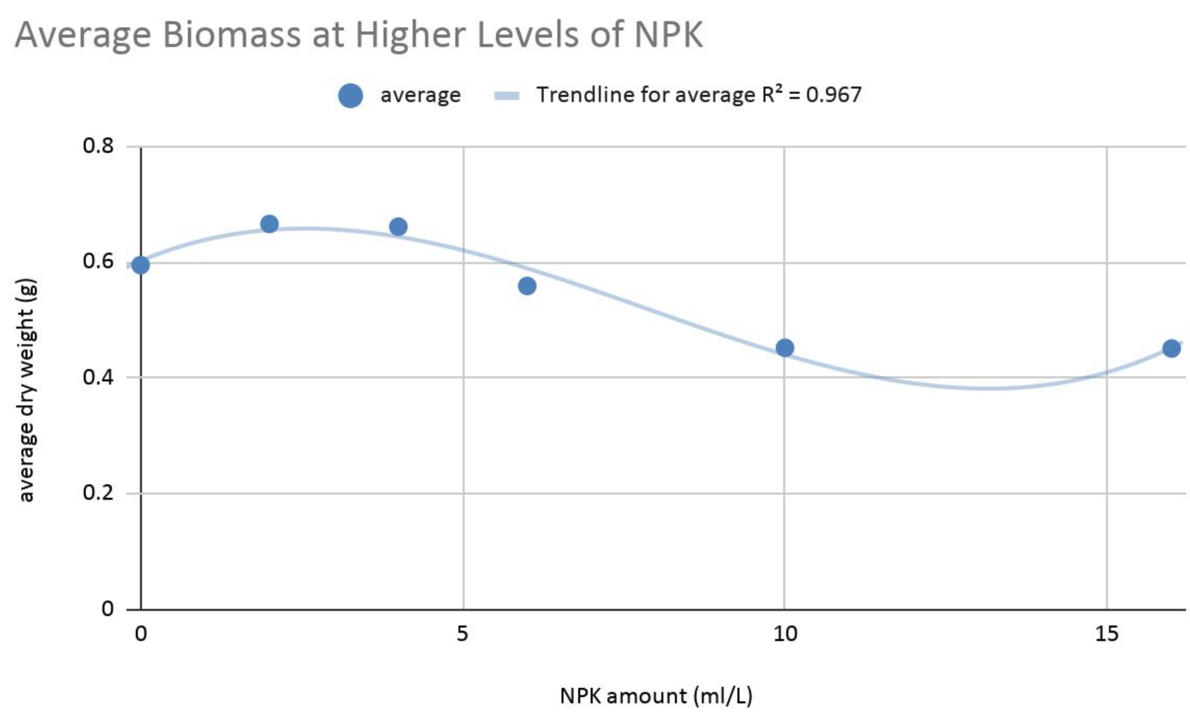


***Figure 8:** The effect of higher NPK addition on growth rate of duckweed cultivations. The data points represent the average dry weight of three replicates.*

The results of experiment 4 show that when there are extremely large concentrations of NPK fertilizer in the duckweed cultivations, the growth rate of the duckweed is slower, yet the protein content is higher.

The Effect of Higher NPK Concentrations on Protein Content:

It was found that as NPK concentrations increased, the final crude protein content in the duckweed also increased. Results of duckweed dry weight from experiment 4, presented in Figure 9, showed that as the amount of NPK increased, the average crude protein content (%) increased. There is a polynomial trendline and the R2 = 0.987.

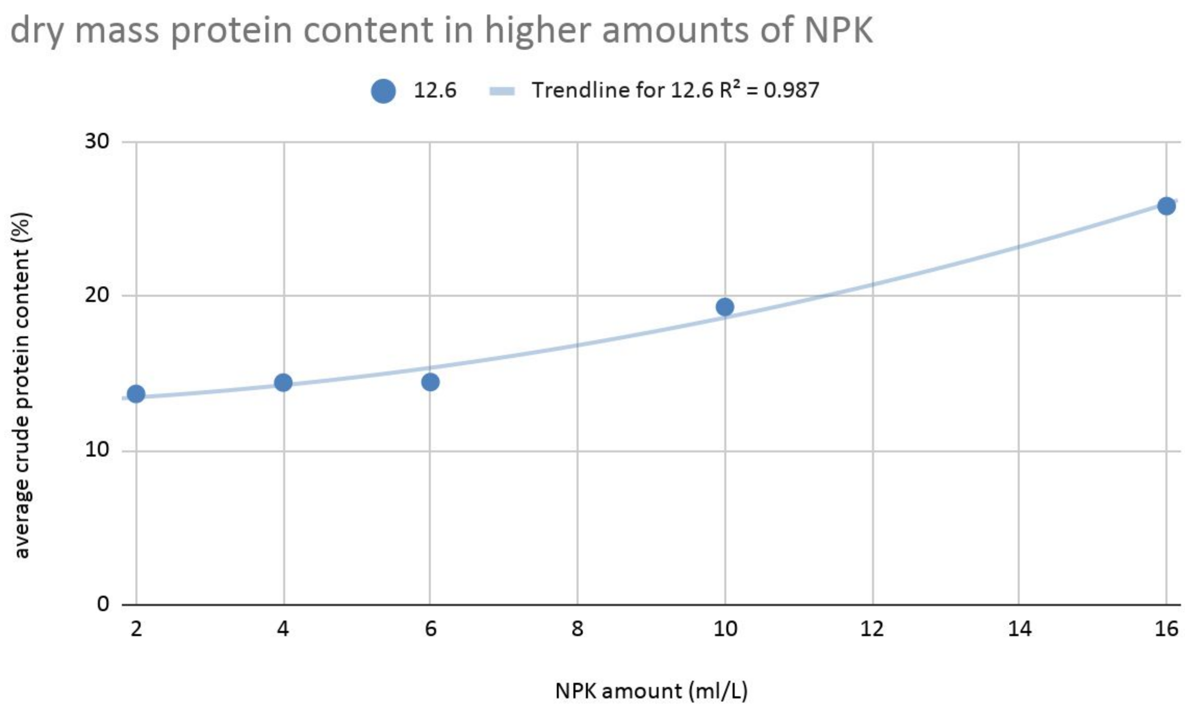


Figure 9: *The effect of NPK addition on the final crude protein content in duckweed cultivations. Each data point represents the average protein content (%) of three replicates.*

The results of experiment 4 showed that although there is a constant increase of the protein content in the duckweed cultivations, the average protein content wasn't higher than the average protein content from experiment 2.2 (the effect of NPK on protein content).

5. Discussion

This study was conducted in order to find if duckweed can be grown with higher protein levels on a large scale. Growing duckweed with a higher protein content on a large scale can be a substitute for soy and other feed crops taking up immense quantities of land on the planet. It can reduce the land use on Earth and also stop the erosion of biodiversity all around the globe. It can also allow for faster feed crop production as well as easy access to the plant, leading to a smaller carbon footprint created by crop transportation.

It was hypothesised that adding inorganic Nitrogen as well as other nutrients to *Lemna* growth medium will support enhanced protein production due to the high nutrient requirements needed to build protein molecules. The research was done in order to find out what is the optimal concentration of nitrogen and other elements in order to see what concentration can produce the highest protein content of the dry weight of the plant.

The Effect of $NaNO_3$ on Growth Rate and Protein Content:

The control group, with only 3 ml of NPK, had the highest protein content in the NaNO3 addition experiment (30.5%) which is really close to the highest percentage reached in all of the experiments (33.8%) in 6 ml/L of NPK and no $NaNO_3$. The difference in growth rate between the control group and the optimal concentration (~0.14 g) was insignificant , yet it was the fastest growth rate of all of the experiments. This finding suggests that $NaNO_3$ might be better for growth rate enhancement than only NPK additions.

There is a possibility that the use of $NaNO_3$ was improperly calculated due to the fact that it was unknown if NPK was used at a good concentration and if the fertilizer could affect the duckweed more than the $NaNO_3$. It is also possible that both the NPK fertilizer and the $NaNO_3$ is just too much nitrogen for the duckweed, and that is why the protein content gradually decreased with the addition of more $NaNO_3$.
These results contradict the hypothesis made in the beginning of the study, which proposed that as more nitrogen was added, the higher the protein content would be. Pure concentrations of sodium nitrate ($NaNO_3$) must not be the form of nitrogen that duckweed responds well to. It is also possible that protein synthesis mechanism in Lemna requires additional elements, and therefore addition of solely Nitrogen is not enough to allow higher protein production; this is supported by the results of experiments 2-3 where addition of a full nutrients suite (NPK+) did result in higher protein content.

The Effect of NPK Fertilizer on Growth Rate and Protein Content:

When NPK fertilizer is added to duckweed cultivations, there is no point in the results where the protein content decreases as levels of NPK concentration rise. This means that further testing must be conducted to evaluate the maximum protein level. So far the highest average protein content of three replicates reached in this study is 32.4% dry weight crude protein content. Several other studies around the world have shown that the crude protein content could even reach up to 40% protein content in dry weight (Leng et al 1995). In experiment 2, the percentage of protein jumped 13.3% when adding 6 mL/L of NPK fertilizer, but when adding 16 mL/L of NPK in the fourth experiment, the percentage only jumped 12.16% compared to the control group. The results from the experiments are unexpected because in both experiments, the protein content kept rising without an upper limit. Except, experiment 4 had higher concentrations of NPK but a lower protein content. It may be possible that the duckweeds used were in different conditions and therefore reacted differently to the different amounts of NPK. For example, the duckweed used in experiment 2 had a start off protein content of 19.1% and the duckweed used in experiment 4 had a start of protein content of 13.6%. This could mean that the duckweed in experiment 4 was different from the duckweed in experiment 2, explaining the lower protein content rise and level.

Environmental conditions could not be kept uniform for all of the experiments (that were conducted in different seasons in an out-of-the-lab setting) making the comparison across experiments problematic. However, within specific experiments all treatments were experiencing similar environmental conditions and therefore can be more reliably compared.

NPK fertilizer addition affected *Lemna* growth rates in an unpredicted manner characterized by a negative effect for low NPK additions (upto 2 ml/l) and positive effect for higher NPK concentrations (experiment 2, Fig. 4). Nevertheless, when an extended NPK range was tested, a different trend was revealed with an optimal NPK concentration of 2-4ml/l followed by a negative effect for higher NPK additions (Fig. 8). This trend coincides with the expected beneficial usage of NPK as nutrients when nutrients are scarce, and the toxic effect that some elements in the fertilizer may have when found in high concentrations. However, this trend was not consistent across all relevant experiments and should be tested again with repeated experiments (ideally conducted in controlled environmental conditions).

In normal levels of NPK fertilizer, the growth rate of the duckweed decreases in the solutions with small concentrations of NPK, but rise in higher concentrations. Further experiments would be necessary to assess why this is the case.

Overall, NPK manipulation conducted in this project successfully elevated Duckweed protein content from medium to the higher range known for Duckweed's protein content (Appenroth et al. 2017).

Following the findings presented here I recommend cultivating duckweed in a solution with multiple elements (not just Nitrogen) such as the Nitrogen Phosphorus Potassium fertilizer I used in most of the study. I also recommend growing Duckweed in high concentrations of NPK (6-16 mL/L). Also, the optimal growth period for Duckweed is one week, and no longer than two weeks.

Implementing these recommendations in full-scale commercial farms may improve Lemna production and it's potential to be used as an alternative protein source for both livestock and humans.

6. Conclusion

In this study, I tested whether the protein content in Duckweed could be increased using NPK fertilizer so that Duckweed could be applied to agriculture. The study was done over eleven months, and four experiments were conducted in this period of time. When 6 mL/L of NPK was added to Duckweed cultivations, the protein content reached 32% dry weight crude protein content. The current protein content of duckweed is 20-35% (Appenroth et al. 2017), but the potential crude protein content in duckweed is 35-43% (Leng et al 1995).

The results of the study show that as more NPK fertilizer is added over a short period of time, the higher the crude protein content is in Duckweed. These results are important because with further experimenting and research, Duckweed could possibly be used as a feed crop for livestock. However, this can only be done after immersive testing of the Duckweed in order to find the optimal growing conditions for the highest protein content and growth rate. My study merely pointed to future, more advanced studies in a guided direction. In future experiments, I recommend growing duckweed in high levels of NPK fertilizer over one week in hotter temperatures (around 20 degrees Celsius), as this when the duckweed flourished in experiment two. Also, I recommend when doing more than one experiment, with the goal of comparing results, to do both experiments at the same time so that the cultivations are grown in the same environmental variables.

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