UTILIZATION OF COMMON DUCKWEED (*LEMNA MINOR* L.) AND GIANT DUCKWEED [*SPIRODELA POLYRHIZA* (L.) SCHLEID.] AS A BIOINDICATOR UNDER HYPEREUTROPHIC CONDITION

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ABSTRACT

This research aimed: 1) To evaluate the effect of a hypereutrophic environment on the growth of common duckweed (*LEMNA MINOR* L.) and giant duckweed [*SPIRODELA POLYRHIZA* (L.) Schleid.]. 2) To evaluate the use of common and giant duckweed for early detection of a hypereutrophic environment. In experiments within a controlled hypereutrophic environment, these free-floating aquatic plants had been cultured in modified 1/10 strength Hoagland's solution adjusting the level of total nitrogen (TN) to 2 and 5 mg/l and the total phosphorus (TP) to 0.9 mg/l. The experiments were conducted for 12 days under 12 hours/day of illumination (86μE/m^2/s).

The results showed that the salinity and pH of the solution were in the optimal range for plant growth throughout the research. Microalgae that appeared in the solution during the experiment had no effect on the growth of common and giant duckweeds. The total nitrogen and total phosphorus of the solution were between 2.26 to 5.68 mg/l and 0.02 to 0.95 mg/l, respectively. Fresh weight, dry weight, and number of fronds of both species increased during the study. However, the growth of common and giant duckweeds were decreased when the total nitrogen was elevated from 2 to 5 mg/l. Thus, both duckweeds may possibly be used as bioindicators for hypereutrophic conditions.

KEY WORDS: BIOINDICATOR / DUCKWEED / GROWTH / LIMIT FACTOR / HYPEREUTROPHICATION

79 pages

การใช้แหนเป็ดเล็ก (*LEMNA MINOR* L.) และแหนเป็ดใหญ่ [*SPIRODELA POLYRHIZA* (L.) Schleid.] เป็นตัวบ่งชี้ทางชีวภาพภายใต้สภาวะไฮเพอร์ยูโทรฟิเคชัน

(UTILIZATION OF COMMON DUCKWEED (*LEMNA MINOR* L.) AND GIANT DUCKWEED [*SPIRODELA POLYRHIZA* (L.) SCHLEID.] AS BIOINDICATOR UNDER HYPEREUTROPHIC CONDITION)

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วท.ม. (การวางแผนสิ่งแวดล้อมที่ยั่งยืน)

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บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อ 1) ประเมินผลกระทบต่อการเจริญของแหนเป็ดเล็ก (*LEMNA MINOR* L.) และแหนเป็ดใหญ่ [*SPIRODELA POLYRHIZA* (L.) Schleid.] ในสภาวะไฮเพอร์ยูโทรฟิเคชัน 2) ประเมินการใช้แหนเป็ดเล็กและแหนเป็ดใหญ่เพื่อตรวจสอบสภาวะไฮเพอร์ยูโทรฟิเคชันล่วงหน้า ในการทดลองภายใต้สภาวะไฮเพอร์ยูโทรฟิเคชันที่ควบคุม พืชลอยน้ำอิสระทั้ง 2 ชนิดนี้ถูกเพาะเลี้ยงในอาหารสูตรดัดแปลงของ Hoagland ความเข้มข้น 1/10 เท่า ซึ่งมีการปรับให้ระดับไนโตรเจนรวม (TN) มีค่าเท่ากับ 2 และ 5 มิลลิกรัม/ลิตร และฟอสฟอรัสรวม (TP) มีค่าเท่ากับ 0.9 มิลลิกรัม/ลิตร ทำการทดลองเป็นเวลา 12 วัน ภายใต้การให้แสง 12 ชั่วโมง/วัน (86 $\mu E/m^2/s$) ผลการทดลองพบว่า ความเค็มและความเป็นกรด-เบสของสารละลายมีค่าอยู่ในช่วงที่เหมาะสมต่อการเติบโตของพืชตลอดการวิจัย

สาหร่ายขนาดเล็กที่เกิดขึ้นในสารละลายระหว่างการทดลองไม่มีผลต่อการเติบโตของแหนเป็ดเล็กและแหนเป็ดใหญ่ สำหรับค่าไนโตรเจนรวมและฟอสฟอรัสรวมจะมีค่าอยู่ในช่วง 2.26-5.68 มิลลิกรัม/ลิตร และ 0.02-0.95 มิลลิกรัม/ลิตร โดยน้ำหนักสด น้ำหนักแห้งและจำนวนใบของพืชทั้งสองชนิดเพิ่มขึ้นในระหว่างการศึกษา อย่างไรก็ตาม การเติบโตของแหนเป็ดเล็กและแหนเป็ดใหญ่ลดลง เมื่อไนโตรเจนรวมเพิ่มขึ้นจาก 2 ไปเป็น 5 มิลลิกรัม/ลิตร ดังนั้นแหนเป็ดทั้ง 2 ชนิดนี้อาจใช้เป็นตัวบ่งชี้ทางชีวภาพสำหรับสภาวะไฮเพอร์ยูโทรฟิเคชันได้

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INTRODUCTION

1.1 Background and significance of problem

Eutrophication is currently one of the important environmental problems in the world. Understanding the mechanisms of water eutrophication will help us to prevent and remedy for water eutrophication. However, the decentralization of administration and environmental management to locality is complicated. It is necessary that local people should have appropriate knowledge and understanding in environment management of locality to support the consideration in strategic planning and measures in prevention and solving or assist in carefully and efficiently use the resource. Monitoring of eutrophication problem by local people may employ the simple thing in community.

In 1992, the Enhancement and Conservation of National Environmental Quality Act (Region of Environmental Office 7, 2543) determined that there must be a decentralization of administration and environmental management from central government to local regions through political machinery and planning process for the purpose that local people should involve in environmental protection and remediation of their locality. Generally, eutrophication could be indicated by the amount of phytoplankton or primary productivity in the form of chlorophyll a which is more than 100 mg/m^3 (Nadwell, 2002). Nevertheless, the cost and perceptive of chlorophyll a analysis in laboratory may not make local people clearly understand of eutrophication problem. Thus, to enhance local people involving in proper eutrophication problem solving, it is essential to find a suitable tool which provide the accurate information for local people to understand the rising of problematical process and to independently approach on problem management easier.

As above information, it is necessary to study and compare the indication of some hydrophytes in hypereutrophic condition by emphasize on understanding and indication of the level of hypereutrophication in order to make local people realizing on the important of water quality monitoring before their water resource getting to hypereutrophic condition. So, those aquatic plants should have many relevant properties such as easily to find in locality, low cost, rapid growth, easily to observe the growth and low sensitivity. From the mentioned characteristic, these appearances are conformed to the plants in family Lemnaceae: common duckweed (*Lemna minor* L.) and giant duckweed [*Spirodela polyrhiza* (L.) Schleid.]. These aquatic macrophytes have simple structures (Hillman, 1978; Kaojarern.1986), free floating and easily find in water resource in Thailand for all season. Thus, it is possible to use these free floating hydrophytes to study their growth response in hypereutrophic condittion and to evaluate the potential using as bio-indicator for hypereutrophic state which local people can undoubtedly employ to monitor the eutrophication problem in the future without laboratory analysis needed. By this way, it would be the suitable mean for local people ability to manage their community for sustainable environment. This approach is appropriate in cost and technology which local people could operate it independently and rapidly.

1.2 The objectives of this study were as follows

1.2.1. To evaluate the effect of hypereutrophic environment on growth of common and giant duckweeds.

1.2.2 To evaluate the use of common and giant duckweed for early detection on hypereutrophic environment.

1.3 The hypothesis of this study

Fresh weight, dry weight and number of fronds had the statistical significant difference on hypereutrophic condition.

1.4 Scope of this study was as follows

1.4.1 There studies were carried out at laboratory in the Faculty of Environment and Resource Studies, Mahidol University.

1.4.2 Solution used in this study was prepared from modified 1/10 strength Hoagland's solution (Hoagland et al., 1950)

1.4.3 Aquatic plants used in this study were collected from Krathumban, Samut Sakhon province.

1.4.4 Environmental factors in this study were light intensity, pH, salinity, temperature, volume of solution.

1.5 Conceptual framework

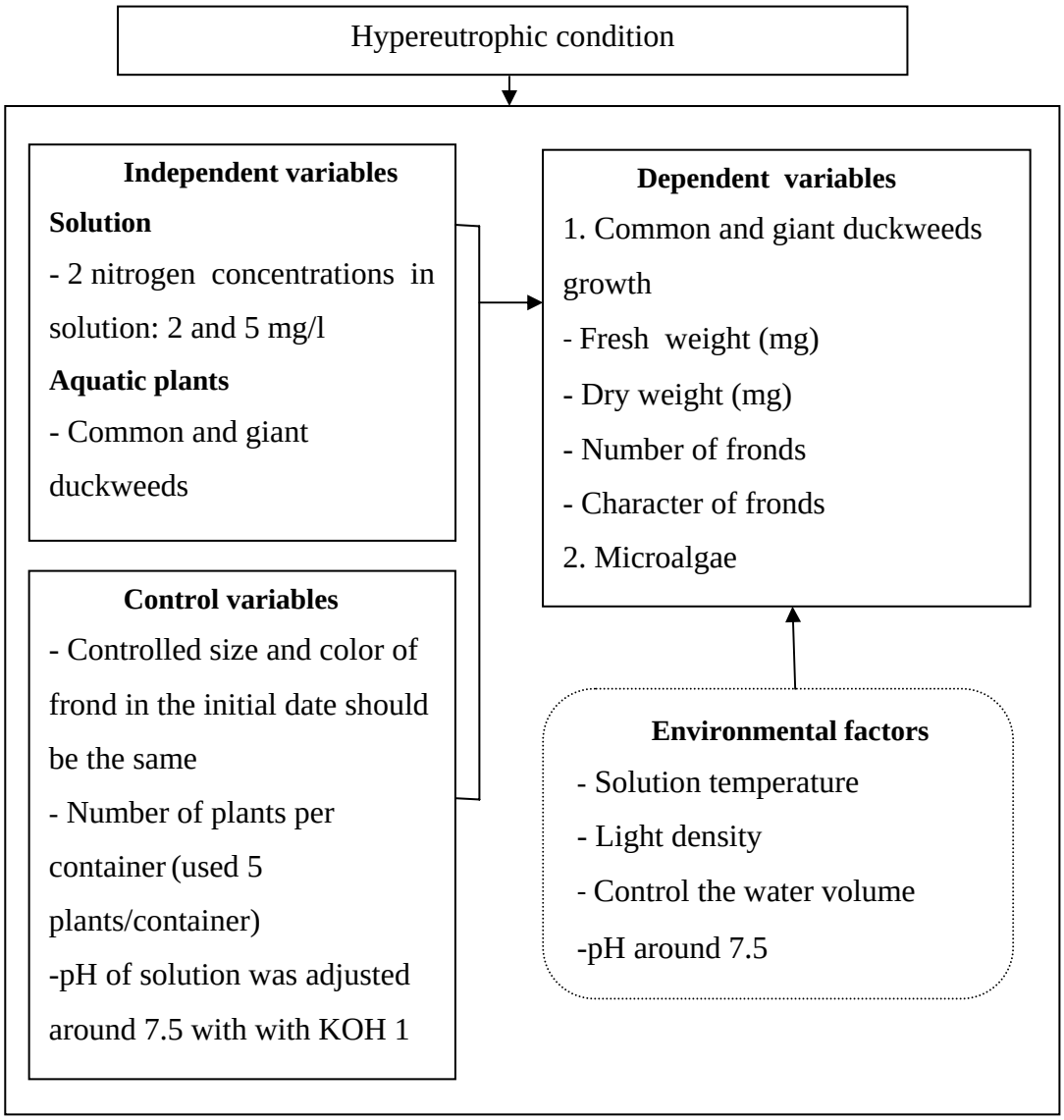

Figure 1-1 Conceptual framework

1.6 Studying variables and factors

1.6.1 Independent variables

1.6.1.1 Nitrogen concentration in the solution were 2 and 5 mg/l

1.6.1.2 Common and giant duckweeds

1.6.2 Dependent variables

1.6.2.1 Common and giant duckweeds growth such as fresh weight (mg), dry weight (mg), number of fronds, color and character of fronds, microalgae (chlorophyll a)

1.6.3 Controlled variables

1.6.3.1 Common and giant duckweeds at starting time

a) Controlled size and color of frond the initial date should be the same

b) Number of plant per a container (used 5 plants/container)

1.6.3.2 Phosphorus concentration 0.9 mg/l

1.6.3.3 Time measurement

1.6.4 Environmental factors

1.6.4.1 Establishment experiment at laboratory in Faculty of Environment and Resource Studies, Mahidol University.

1.6.4.2 Solution temperature

1.6.4.3 Light intensity

1.6.4.4 Water volume

1.6.4.5 Salinity

1.6.4.6 pH

1.7 Define of words used in this study

1.7.1. Hypereutrophic condition is the condition that very high nitrogen and phosphorus concentrations in water and the water body is overcrowded with algae. Hypereutorphic lakes are the most biologically productive lake, and support large amount of plants, fish and other animals, lethal effect will greatly arise to most living organisms in aquatic bodies and can be distinguished when nitrogen concentration is more than 1.2 mg/l and phosphorus is more than 0.1 mg/l.

1.7.2. Duckweed is aquatic plants which float or just beneath the surface of water. They are common and giant duckweeds**.**

1.7.3 Growth is the increase of duckweed under hypereutrophic condition.

CHAPTER II
LITERATURE REVIEW

2.1 Eutrophication or too much nutrient condition

Eutrophication is level of nitrogen, phosphorus and salinity that common duckweed *(Lemna minor)* and giant duckweed [*Spirodela polyrhiza* (L.) schleid.] can be used to indicate too much nutrient condition and consequently excessive growth of both duckweeds is exposed.

2.1.1 Eutrophication indicators

Eutrophication indicators which gathered by Gavin (1972) and Hopper (1969) are related to the research that implemented these indications in 3 aspects, such as nutrient indicators, biological indicators and productivity indicators. Eutrophication in the water bodies is considered as follows.

2.1.1.1 Nitrogen and phosphorus are basic nutrient of plants. Generally, human activities release nitrogen and phosphorus into the river. Nitrogen and phosphorus quantity and characteristic have changed all the time, thus the measurement must be in available form so that changing can be observed. Besides, other ions for example chloride, sulphate, sodium, potassium, calcium, etc. are also used as indicators since these ions can be received from waste water and agricultural activity and their form and quantity are stable.

2.1.1.2 Biological indicators depend on the occurrence and the lost of species which are not able to indicate the rate and level of eutrophy.

2.1.1.3 Productivity is the value that has enough sensitivity for indicators.

However, nutrients analysis in term of chemical aspect is also an important parameter and necessary for water quality assessment though it has some limitation.

2.1.2 Status of nutrient specification in ideal manner

A lake's trophic state is a measure of its "biological productivity", which, simply, is a measure of how many plants and animals are in a lake. Four trophic states are recognized by lake scientists (Table 2-1).

2.1.2.1 Oligotrophic lakes are very low in nutrients, so few algae grow and the water is very clear. Oligotrophic lakes are biologically less productive lakes (they have the lowest level of biological productivity), and support very few plants and fishes.

2.1.2.2 Mesotrophic lakes are moderately productive lake, with slightly green water.

2.1.2.3 Eutrophic lakes are productive lakes with murkier water, and/or lots of plants.

2.1.2.4 Hypereutrophic lakes are very high in nutrients and their water is very clouded with algae. Hypereutrophic lakes are the most biologically productive lakes, and support large amounts of plants, fishes and other animals.

Table 2-1 Modified trophic state classification by Total phosphorus and Total nitrogen source (Barnes et al., 2005; Canavon and Siver, 1995; CTDEP, 2002; Smith et al., 1999; Vollenweider, 1986)

Trophic classification	N (mg/l)	Total-P (mg/l)
1. Oligotrophic	>0.35	>0.01
2. Mesotrophic	0.35-0.65	0.01-0.03
3. Eutrophic	0.65-1.2	0.03-0.1
4. Hypereutrophic	>1.2	>0.1

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2.1.3 Nutrients

Nutrients are component that encourage and sustain the growth and development of aquatic plants. They are a major cause of eutrophication such as nitrogen in the form of nitrate and ammonium or phosphorus in the form of phosphate. Commonly, plants need nitrogen and phosphorus more than other nutrients such as sulphur, potassium, calcium, and magnesium. According to the requirement of these two elements over others, nitrogen or phosphorus can be used as a limiting nutrient which capable of controlling the eutrophication rate in the water (Randall, 1993; Thomann, 1998).

2.1.3.1 Nitrogen (Dennis et al., 1989)

The nitrogen cycle is more complicated than the phosphorus cycle. Nitrogen can exist in either oxidized forms, usually nitrate (NO_3-N) or nitrite (NO_2-N), or reduced forms, including ammonia (NH_3-N) and organic nitrogen. Atmospheric nitrogen (N_2) can also be used as a nutrient source by some species of algae, and decomposition processes can produce various other reduced forms of nitrogen. Of these various forms, NH_3, NO_3-N, NO_2-N, are readily available to aquatic primary producers for metabolic uptake. Ammonia-nitrogen (NH_3-N) is a metabolic by-product of the decomposition of organic material, such as proteins. In most healthy freshwater systems, ammonia is present in low concentrations, usually less than 1.0 mg/L; it is the preferred form of nitrogen for uptake by algae and plants. In highly eutrophic water bodies, particularly those that become devoid of oxygen (anoxia), much higher ammonia concentrations may be present. High ammonia concentrations can prove lethal to organisms if the pH of the lake or pond is greater than 8. Under these conditions, the un-ionized form of ammonia begins to replace ammonium ion (NH_4^+) as the predominant form of ammonia, and the un-ionized form is particularly toxic to fish.

Nitrate-nitrogen (NO_3-N) is readily utilized by algae and plants; however, it must be reduced before it can be metabolically used. Therefore, most algae and plants prefer ammonia to nitrate. The concentration of this nutrient, particularly when measured over a prolonged time scale, can shed a great deal of information on the productivity and trophic status of a lake. Although the amount of nitrate present at any given time is a function of the extent of metabolism in a water

body, the typical concentration for a relatively "healthy" lake is less than 0.05 mg/l. In eutrophic water bodies, the concentration of nitrate is usually low in the upper layers due to uptake and utilization by algae. In the deeper parts of the lake, the concentration of nitrate will be greater due to the decomposition of organic material.

Nitrite-nitrogen (NO_2-N) is typically present at very low concentrations (less than 0.005 mg/l). Seasonal changes in the concentration of nitrite generally follow a pattern similar to that of nitrate. High concentrations of nitrite may be indicative of inputs from septic systems or sewage treatment plants.

2.1.3.2 Phosphorus (Dennis et al., 1989)

Phosphorus is an essential element for both plant and animal life. It is importance stems from the fact that it is usually available in a form amenable to bio-uptake and at a concentration relatively much lower than that of other essential elements. As a result, it is usually limiting.

Some of the important external sources of phosphorus are fertilizers, septic leach ate, sewage effluent, detergents and soaps, particulate material transported by storm water, and even precipitation. Lake sediments, particularly those that are highly organic or mucky can serve as an internal source of phosphorus loading, especially if the overlying waters become devoid of oxygen. The decomposition of dead algal cells or aquatic weed tissue is another internal source of phosphorus.

Of the various forms of phosphorus, the two most commonly measured are total phosphorus (TP) and orthophosphate (PO_4). Total phosphorus represents the sum of all phosphorus forms, including dissolved and particulate organic phosphates from algae and other organisms, inorganic particulate phosphorus from soil particles and other solids, polyphosphates from detergents, and soluble (dissolved) orthophosphates. Soluble orthophosphate is the form most important to plant life as it is readily available for bio-uptake. The soluble orthophosphate concentration in unproductive lakes is usually less than 0.007 mg/l. Much higher concentrations are measured in eutrophic water bodies, particularly toward the bottom of the lake. Although only a small fraction of total phosphorus is available for use by primary producers, its measurement is of great importance. Numerous models exist which enable investigators to determine the trophic status (the degree of

eutrophication) based on the concentration of TP. In general, the following productivity TP concentration relationship can be utilized to categorize lakes.

2.1.4 pH of water

pH is derived from the word 'positive potential of the hydrogen ion'. The pH of water is generally water quality indicator. It is important because it is a major factor and has an impact on chemicals and bio-reaction products. pH of pure water is 7.0 while pH of natural water is 4-9, but mostly natural water is a bit alkali. (กรรณิการ์, 2544). Salts, acids and bases are normal components of natural water and will deviate the neutral pH form. Changes in the pH of the lake are affected by photosynthesis as well. This can occur because during photosynthesis plants fix carbon dioxide (CO_2). After CO_2 is fixed, the lake's buffering system is altered in accordance with the decreasing of H^+ concentration. This circumstance leads to the increasing of pH. Under condition of excessive productivity, pH of eutrophic water is around 7.31-8.23 (ธงชัย, 2543)

2.1.5 The consequence of eutrophication

2.1.5.1 Nutrient quantity which affects concentration of dissolved inorganic nitrogen and phosphorus. Generally, N/P ratio in dissolved form that appropriate to phytoplankton growth is 16:1, which is called red field ratio. Thus, variation of N/P ratio that different from red field ratio will be a limiting factor to primary productivity as the following explanation.

If the component of algae is $C_{106}H_{263}O_{110}N_{16}P$, it can be calculated that 1g nitrogen is able to produce algae 16 g while 1 g phosphorus can produce algae 113 g (Randall, 1992).

2.1.5.2 The direct and indirect change of water quality

a) Change of DO

b) Change of pH

c) Change of ammonia form: Free ammonia (NH_3-N) is toxic to fish while ionized-ammonia (NH_4^+-N) or ammonium ions is fixed on to soil particles which is an unavailable form to plants

d) Effect to the living organism in the water

The direct toxicity to fish from free ammonia depends on pH of the water. Ammonia ionizes below pH 7.4 to ammonium which is less toxic to fish. Above pH 8.0 most ammonia is ionized, and so becomes more toxic. It was found that at pH 7.5 and temperature 30°C, ionized-ammonia is 2.5% of all ammonia. When pH values raise to 9 and 10, this ionized-ammonia also increase to 45% and 89%, respectively.

For the indirect effect, dissolved oxygen in water has reduced and affect to the distribution of blue green algae. Therefore, food to chain destruction will take place and toxin from the dead algae will spread and harm other aquatic organisms as well as bird, terrestrial animals and human.

2.1.6 The problem of eutrophication in Thailand.

The system of waste water treatment emphasizes to eradicate only BOD (Biochemical Oxygen Demand) and can not dispose of nitrogen and phosphorus. In Thailand, phosphate has been used in 2 major activities, one is fertilizer and the second is industry (feed, detergent, instant food, beverage and seafood). Phosphorus in the water body can set off eutrophication problem, for example phosphorus in Chao Phraya river from Ayutthaya to the estuary is 0.02 mg/l (Table 2-2). With this concentration, the eutrophication crisis can undoubtedly appear in standstill water.

Table 2-2 Phosphorus values from different sources of water in Thailand (ธงชัย, 2543)

Sources of water	Phosphorus (mg/l)
Chao Phraya river (from Ayutthaya to the estuary)	0.02
Fresh water in communities (Kaen Nakhon Swamp and Thungsang Swamp in Khon Kaen; Lomtakong Dam in Nakhon Ratchasima)	0.1 -0.5
Channel ditch in Bangkok	5.9-36.2
Bangpakong river, Mae Klong river, Tha Jeen river	0.01-0.39
Songkhla lake	0.014-0.086
Laem Chabang deep sea port.	0.01-0.015
Small reservoirs in Khon Kaen and Maha Sarakham	1.63 in the dry season 0.27 in the rainy season

Early in 2000, the problem of fast growing phytoplankton in estuary of Choa Phraya river at Samut Prakan occurred covering more than 3 km along the border of estuary. Results of water analysis from Department of Fisheries were shown in Table 2-3. It was revealed that DO (dissolve oxygen) was higher beyond normal while carbon dioxide was down to zero around noon. Nevertheless, the DO was obviously low at night time. This data revealed that, under these conditions, phytoplankton were able to process very much photosynthesis (ธงชัย, 2543). These findings were related to the amount of chlorophyll a which was about 330-407 mg/m^3. Furthermore, the nitrogen and phosphorus concentration in this area were quite high, especially phosphorus which was around 0.56-0.7 mg/l.

Table 2-3 Analysis of water after the rapid increasing of algae at Samut Prakan, January, 4, 2000 (ธงชัย, 2543)

Time	pH	DO	CO_2	NO_2^-	NO_3^-	NH_3	Ortho PO_4	T- PO_4	Chlorophyll a
02.30	7.31	4.9	-	-	-	-	-	-	-
12.05	8.34	14.2	0	0.007	0.018	0.075	0.13	0.56	407
12.55	8.23	11.4	0	0.009	0.019	0.130	0.14	0.70	330

Note: All units are in mg/l, except pH and chlorophyll a (mg/m^3)

The problem of hyper-nutrient or eutrophication in water can threaten aquatic resource because nutrient will enhance phytoplankton growth very quickly. Afterward, this rapid rising has many effects to water resources, such as decreasing in clarity, increasing in the alteration in DO and pH. Besides, this inappropriate environmental condition can initiate phytoplankton death and precipitation to the bottom of water. Subsequently, the demand of oxygen will increase and lead to the anoxia. In low oxygen condition, hydrogen sulfide is produced, all benthic animals will die and most of the aquatic animal will decrease. Thus, in conclusion, eutrophication condition extremely has many effects to the ecosystem.

2.1.7 Administration of eutrophication

2.1.7.1 Seeking for the origin of the nutrient

2.1.7.2 Reducing nutrients from discharged sewage into surface water

2.1.7.3 Using legislative measure

2.1.7.4 Investigating water quality as it is an important issue and essential basic knowledge to manage eutrophication problem

2.1.7.5 Researching and studying of aquatic plant utilization to reduce the amount of aquatic plants

2.1.7.6 Educating and publicizing to the citizen

As above information, this research precisely conforms to the administration of eutrophication. The aquatic macrophytes in Lemnaceae family were used to develop a tool for indicating eutrophication which local people can apply for self-directed water quality investigation. This will be beneficial for water quality

monitoring and investigation, seeking for source of the nutrient discharge to manage quality of water efficiently.

2.2 The manipulation of eutrophication indicator (สำนักงานนโยบายและแผนทรัพยากรธรรมชาติและสิ่งแวดล้อม, 2549)

2.2.1 Conceptual framework in manipulation of eutrophication indicator: This will be an important tool to monitor and investigate water quality that risk for eutrophication.

2.2.2 Types of eutrophication indicator: There are 3 kinds of eutrophication indicator.

2.2.2.1 Indicating before eutrophication: This pressure can be positive or negative to the environment.

2.2.2.2 Indicating water quality at transition point that likely risk to eutrophication: This can be estimated from quantity, quality and distribution of eutrophication since most indicators are unable to specify the amount of eutrophication accurately. In addition, those indicators are really expensive, so that pressure indicator is preferably used to evaluate the level of eutrophication.

2.2.2.3. Indicating water quality after transition point that likely risk to the occurrence of eutrophication: This indicator purposely shows the impacts on environment or cause of eutrophication problem.

These 3 kinds of eutrophication indicator can explain the future direction of environmental situation, any supplementary or critical problem and any policy or procedure to solve those problems directly. Hence, the appropriate selection of indicator is absolutely important.

2.2.3 Development and selection of eutrophication indicator

There are 3 major steps to develop and select the eutrophication indicator.

2.2.3.1 Step number 1: The concept of eutrophication indicator must be done firstly. This is to indicate eutrophication for integrative environmental planning in the city and rural area as the following conceptual framework.

a) Conceptual framework

The major point has to be considered about the arrangement of environmental indicator is that conceptual framework of eutrophication indicator must distinctively be selected and developed. The first thing should be contemplated is what is the initial conceptual framework, what is the obligation of this indicator, what level or purpose to be measured, who will implement this indicator, what or whom that achieved indicator should be compared with.

The conceptual framework that used in this research is Pressure–State–Response Framework (PRS). PRS is developed by Organization for Economic Co-operation and Development (OECD) in order to using for analysis of national and international level. It is suitable for evaluation in regional and international level. The analysis does not want many details and there are 3 groups of indicatorsas follows.

- State variable is water quality that changes because of the increasing of people that accelerate the production of agriculture and industry which affect water quality in eutrophication aspect.

- Pressure variable is the technology using for accelerate the production to meet the need of consumer, such as using excess fertilizer to increase agricultural product that leading to eutrophication.

- Response variable is the source of eutrophication indicator for eutrophication awareness.

b) Thing that needed to indicate

Considering on establishing eutrophication indicator is aimed to support the decision on policy administration and specification, strategic planning and preventive measure, resolution of eutrophication as well as eutrophication awareness and assessment.

c) Indicator user

The objective of establishing eutrophication indicator is to assist person who involves in the action plan for community environment, such as provincial office, provincial environment office, city municipal, municipality,

municipal district, subdistrict administrative organization and local people on using eutrophication indicator in their community.

2.2.3.2 Step number 2: Criteria for eutrophication indicator selection.

Practically, it is difficult to specify standard indicator owing to the principle demand of indicator user. Different user may need dissimilar indicator. However, the way to obtain an effective usable indicator is the selection of some indicators and possibly in the minimal number. In general, there are extensively 6 criteria for indicator selection as follows.

a) Indicator must relate directly to eutrophication.

b) Indicator must relate to the indicator users. Different user may need dissimilar indicator. Thus, it is really important to consider on the target group for selecting the indicator. The local community may just need to know whether there is eutrophication which unlike the environmental protection agency that needs the precise value.

c) The selected indicator must be designed clearly and should consider directly to the group of users. The clarity of this point may depend on the difference of each group whereas the research needs the clearness of indicator for local people on the communication.

d) Indicator must be created from data that convenient for collection and not too expensive.

e) Indicator must be accuracy and reliable and always used the same procedure and scale. However, the indicator's manipulator should have indicator estimation that is mostly approximate to the ideal indicator.

f) Indicator must cover the dimension of the spatial and time because the impact on environment from economic activities less appear to the effect area.

2.2.3.3 Step number 3: Searching the data and making the database.

Indicator's manipulator must search and explore the sources of information and update the data processing in order to execute the raw data to usable

information and attempt to avail this data and information wisely. Nevertheless, effective indicator development needs high budget and speciality.

2.3 Lemnaceae

Duckweed is the word that used for plant in the group of aquatic vascular angiosperm. It is a small floating plant which generally grown on the swamp or pool. Typically, they are free floating and gathering as a sheet covering on the water surface (Hillman, 1978). Les et al. (2002) classified plants in Lemnaceae family into five genera which are *Lemna, Spirodela, Landoltia, Wolffia* and *Wolffiella* and contained more than 38 species.

2.3.1 Type and characteristic of Lemnaceae

2.3.1.1 Common Duckweed

Common name : Common duckweed

Genus : *Lemna*

Species : *Lemna minor* L.

Thallus resembles an egg and 2-4 mm of wideness. Lower epidermis of leave is not violet and each leave has only 1 root. The species of *Lemna* that generally found are *Lemna minor* (Figure 2-1) which can increase and cover water surface quickly. The length of frond is 2.0 mm while *Lemna gibba* has the length of frond 2.5 mm. Both types of *Lemna* have similar shape of frond which curve and flat but different in air chamber patent at the bottom side of frond. The number of air chamber at bottom side of *Lemna gibba* is 7-8 whereas *Lemna minor* has 13-15 air chamber (Pieterse, 1981). For *Lemna triculca*, it has the protruded connecting joint that looks like the bird's tail and arranges in T-shaped or cross on water surface. Each connected leave of this *Lemna* also has only 1 root (Prescott, 1980).

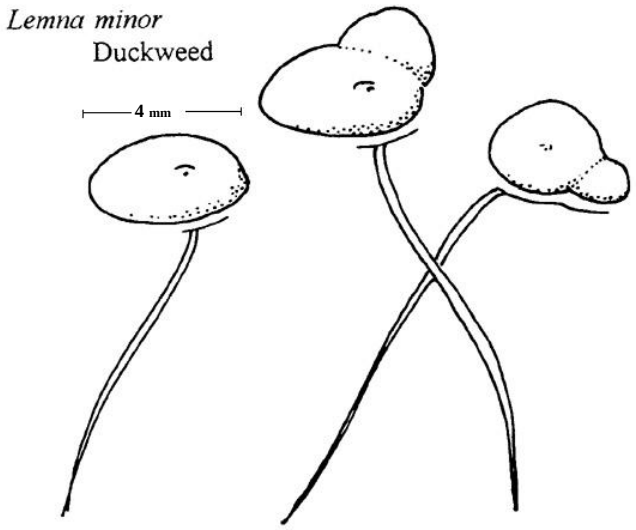

Figure 2-1 Picture of *Lemna minor* L. (Britton and Brown, 1913)

2.3.1.2 Giant duckweed (large duckweed)

Common name : Giant duckweed or Greater duckweed

Genus : *Spirodela*

Species: *Spirodela polyrhiza* (L) Schleiden

Thallus has 2 leaves or less. There is a connected joint in the center. The length of frond is 3.5 to 150 mm. Lower epidermis of leave is violet. *Spirodela polythiza* (Figure 2-2) is the dominant species of genus *Spirodela*. It has different shapes depending on the connection and has a lot of roots. It is the biggest duckweed in Lemnaceae and can develop and spread on water surface quickly (Hillman, 1978; Prescott, 1980; Rejmankova, 1990).

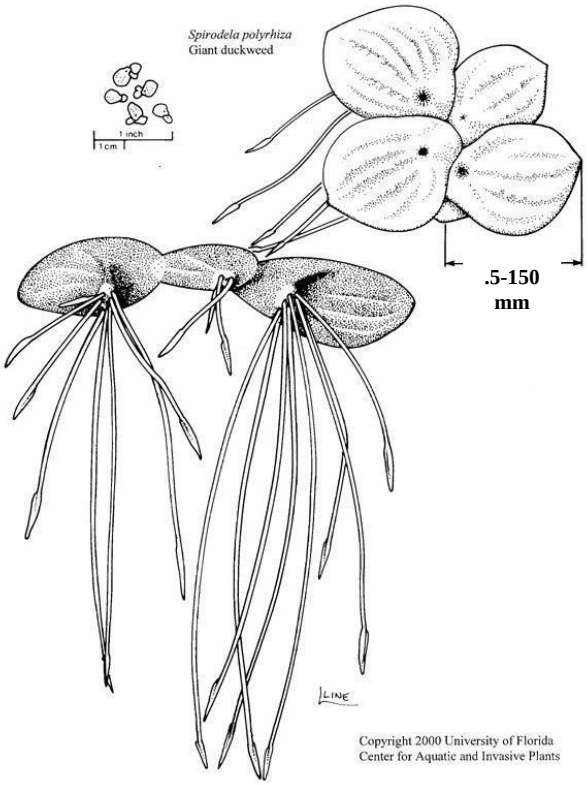

Figure 2-2 Picture of *Spirodela polyrhiza* (L) Schleiden (University of Florida, 2008)

2.3.1.3 Dotted duckweed

Common name : Dotted duckweed

Genus : *Landoltia*

Species : *Landoltia punctata*

Thallus is flat and oval shape, mostly asymmetry, dark green on upper epidermis of leave and reddish violet at the edge of leave. It has 1-5 roots and 3-5 mm length of frond. New frond develops from side chamber on both sides of leave. The flower is blooming from inside cuplike spathe. Dotted duckweed (Figure 2-3) typically has 2-6 fronds (Les, 2002).

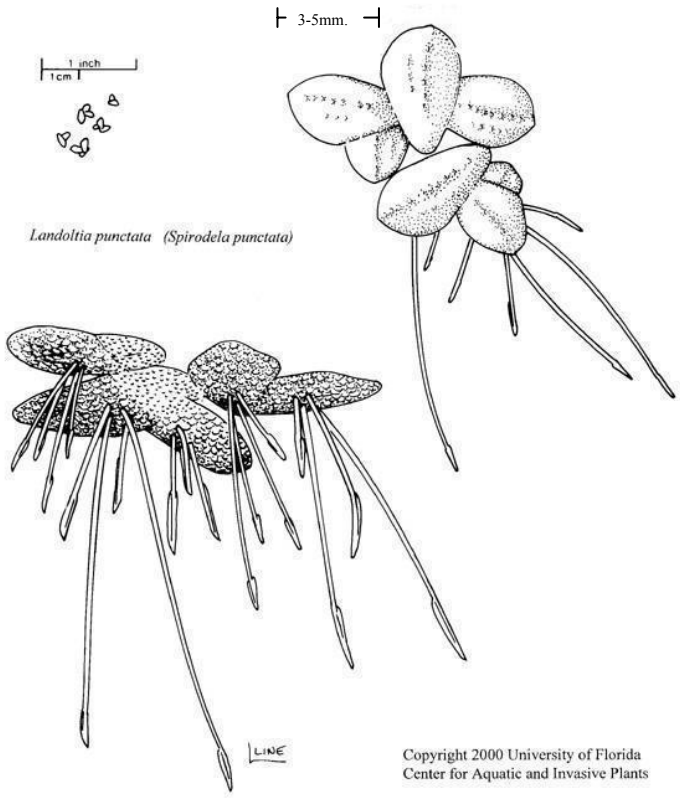

Figure 2-3 Picture of *Landoltia punctata* (University of Florida, 2008)

2.3.1.4 Water meal

Common name : Water meal

Genus : *Wolffia*

Species : *Wolffia columbiana*

Thallus is round shape, 2 mm width or less. It is the smallest plant in Lemnaceae and spreads as green particle on water surface. Flower is very small. It often mixes up with other plants in Lemnaceae. The species of *Wolffia* (Figure 2-4) that normally found are *Wolffia punctata* and *Wolffia arrhiza* Bhanthumnavin. *Wolffia punctata* has flat thallus and black dot on the frond (Rejmankova, 1990) while *Wolffia arrhiza* thallus is rootless. *Wolffia arrhiza* is the smallest flowering plant in the world. The size of this plant is as small as a pinpoint, 1

mm width and 1.5 mm length. It is a source of human food and called "water egg". (Bhanthumnavin, 1971; NAS, 1976; Hillman, 1978).

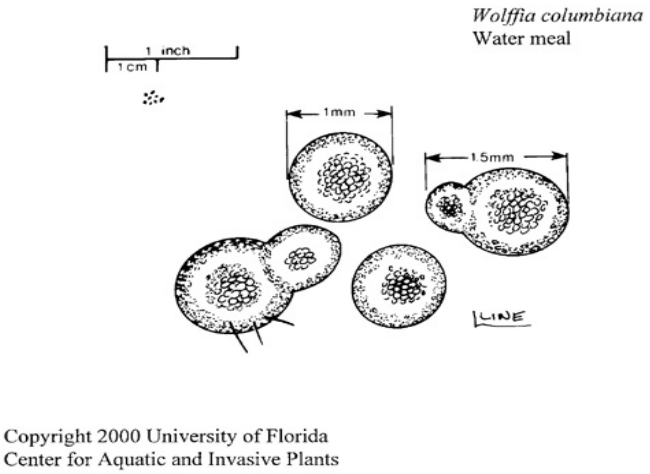

Figure 2-4 Picture of *Wolffia* (University of Florida, 2008)

2.3.1.5 Mud-midget

Common name : Mud-midget

Genus : *Wolffiella*

Species : *Wolffiella floridana*

Thallus is small, flat and star shape. It commonly floats and covers on water surface. It is 4-8 mm length. *Wolffiella floridana* (Figure 2-5) is the dominant species found in this genus. *Wolffiella floridana* has strap shaped and rootless (Prescott, 1980).

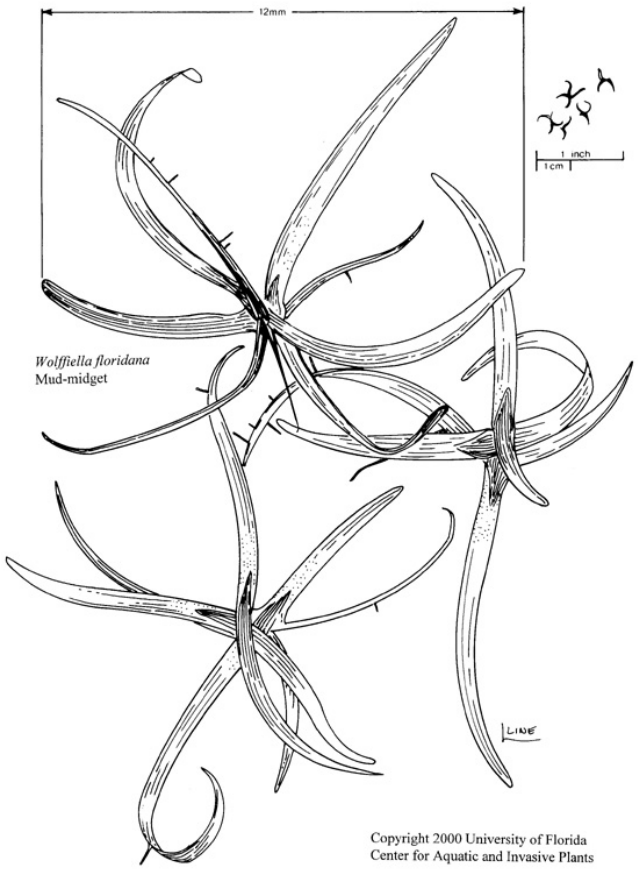

Figure 2-5 Picture of *Wolffiella floridana* (University of Florida, 2008)

There are 3 genera in Lemnaceae found in Thailand, such as *Lemna, Spirodera* and *Wolffia*. In this research, *Lemna* and *Spirodera* are chosen for the experiment because both duckweeds are easily found in natural water resource every season while *Wolffia* is simply found during the rainy season. The size of *Wolffia* is quite small and inappropriate to apply as eutrophication indicator for the visual observation.

2.3.2 Botanical characteristic

Lemnaceae is small floating monocotyledon (Hillman, 1978; Oron, 1986). It has simple structure. Leave is called "frond" which appear as one or more green thallus (Hillman, 1976). Frond may consist of 1 to 10 fronds (NAS, 1976) and each frond can divide into 10-20 fronds (Hillman, 1978; Zirschky, 1988).

Generally, duckweed is often found in the standstill water resource, such as swamp or isolated pond which has the leave and manure nearby. During rainfall, these compost and manure will flow into that water resource and accelerate duckweed

2.3.3 Factors that influence the growth

2.3.3.1 pH and salinity

Duckweed can live in pH level between 5 to 9 (Radić et al., 2010). Duckweed growth is also promoted up to salinity concentration of 1.3 ppt. (Omar and Balla, 2009)

2.3.3.2 Light and temperature

Duckweed can grow well in the light condition. For the experiment, a cool white fluorescent should be used with the light intensity not lower than 86-125 $\mu E/m^2/s$ (Mkandawire et al, 2005)**.**

Temperature that suitable for duckweed growth is between 20-30°C and inappropriate temperature for growth of duckweed is between 35-40°C. However, duckweed can live at cold and minimum temperatures at 7 °C (Zirschky, 1988 and Öbek and Hasar, 2002).

2.3.3.3 Toxin and mineral

PCB's and ethylene can be accumulated in and harmful to duckweed during growth. Nitrogen deficiency in wastewater or filamentous algae or fungus can reduce the growth of duckweed as well (Zirschky, 1988).

Duckweed starts growing slowly if there are other weeds, such as water hyacinth and hydrilla living in the same area. This occurs because of the competition for water surface zone (ดำรงชัย, 2542; กฤษดา, 2543). Bluckley (1993) reported that duckweed can grow well in the water that has high copper concentration. Besides, duckweed can grow faster than other plants under optimum conditions, for example, plenty of essential nutrients in the water, sun light and suitable temperature (Skillicom, 1993).

2.3.3.4 Wind and wave

Appropriate condition for the growth of duckweed is calm wave and standstill pond (Daubs, 1973).

2.3.4. Reproduction and distribution

Duckweed (NAS, 1976; Prescott, 1980) floats broadly on water surface in the tropical, subtropical and temperate areas. It can grow rapidly because of its short life cycle, well adaptation to the environment and less pest and disease. It also grows well in waste water and can be harvested easily (Cully, 1973; Oron, 1986; Stanley, 1976). Duckweed has both sexual and asexual reproduction but mostly asexually replicate by budding. New frond will produce from 2 pockets in each side of mother frond and still attach to the mother frond. This group of frond is called "colony". In life cycle, each mother frond can reproduce daughter frond 10-20 times or more in 1-2 months (Zirschky, 1988). The number of fronds production depends on many factors. For *Lemna* and *Spirodela*, frond will produce each time at only one side of frond.

The sexual reproduction of duckweed is by flower, fruit and seed production as in other flowering plant. Flower of duckweed is originated at the side of leaf and has white stalk that emerge from the water like fungus filament. At the terminal, there are 2 attached round knob which will be split into 4 parts for pollination. Pollen has light yellow-lime or white colors. Number of anther is 1 or 2. Carpel looks like vase and may not found in every flower. Male and female flower usually occur in the same plant which increase the opportunity for fertilization and seed production.

NAS (1976) reported that *Lemna minor*, *Spirodela polyrhiza*, and *Spirodela punctata* can increase twice in number within 3 days or less by double division of frond. When *Lemna minor* was cultured in the area of 6 square centimeter for 55 days, they grew and cover water surface in 3.2 rai (1/2 hectare). Moreover, Bhanthumnavin and McGarry (1971) found that *Wolffia arrhiza* can increase the number 4 times within 4 days or less in laboratory. Under optimum condition, this plant which had been harvested about 50 percent of water surface could spread and cover that area (biomass increased 2 times) within 3-4 days. This result was related to the work of Eberius (2001) who used 3 days to study initial met density that appropriate for duckweed growth rate. It was revealed that the density rely on richness or amount of organic matter in water resource (ดำรงชัย, 2542; กฤษดา, 2543).

2.3.5 Measurement of duckweed growth

2.3.5.1 Growth curve (Eberius, 2001)

Growth curve for plant or animal as well as duckweed can be divided into different phases (A-D phase) as shown in figure 2-6.

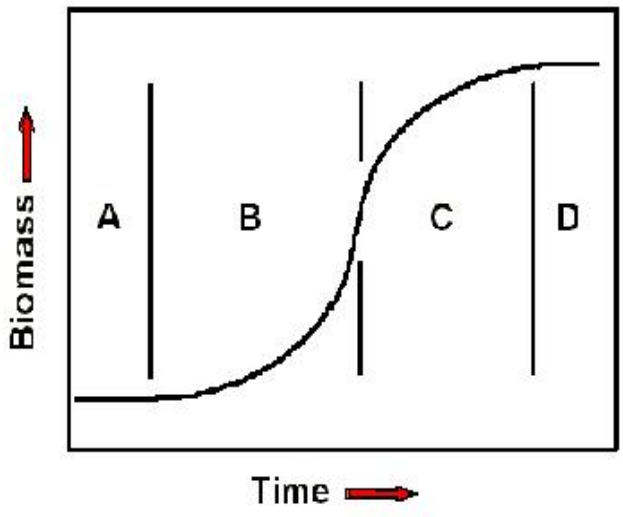

Figure 2-6 Graph shows duckweed growth (Eberius, 2001)

The duration and extent of each phase will depend on the organism and environmental conditions. For example, if plants at phase D are transferred to fresh medium state, the lag phase (A) will be longer than the case of fronds at Phase C. From fronds growth at Phase B, plants that transferred to fresh medium state will likely skip the lag phase. If the growth medium is more concentrated, plants at the phase B will increase at exponential growth for twice with a longer period and a greater biomass production.

If starting with a young duckweed frond, the growth will be very nearly exponential. However, exponential growth must continue to reproduce later, and it is stopped sometime.

2.3.5.2 Fronds count (Landolt, 1987)

The most common method of measuring growth of duckweed is to count of fronds. The standard procedure is to count every visible frond, even the

tip of new small fronds, even just beginning to emerge from the pocket of the mother frond (Figure 2-7).

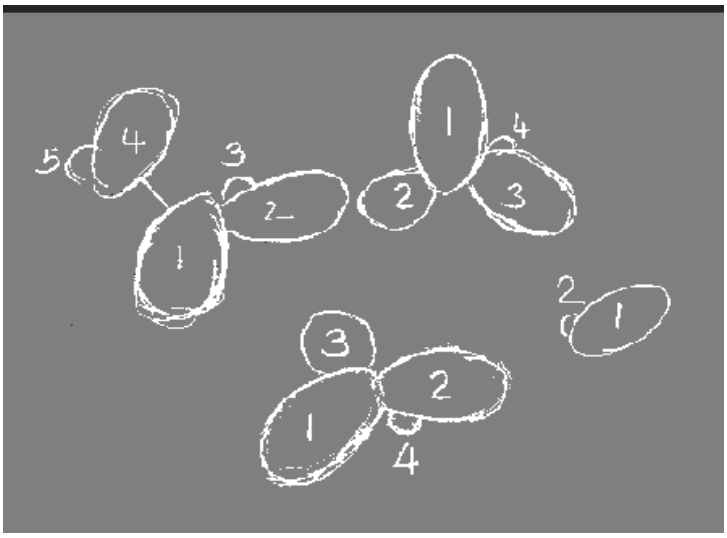

Figure 2-7 Drawing shows an example with several fronds in different orientations and stages of growth, and with two plants connected by a stipule (Landolt, 1987)

A magnifying glass or a stereomicroscope (10x is good) is necessary instrument for fronds counting. It is easy to miss fronds or count them twice. Placing a square-ruled sheet of paper can help to reduce counting errors. It is also possible to automate frond counting using video image analysis.

2.3.5.3 Fresh and dry weighting (Landolt, 1987)

Weighting is an obvious measurement of plant growth. Fresh or wet weight is measured after blotting the plants with a soft towel to remove the free moisture, then they are weighed immediately. Since duckweed lacks a cuticle on lower surface, it will be dry in a short time. Weight water of duckweeds is between as 86% and 97%, so small variations in drying will have a major impact on results, however, fresh weights are a useful means of estimating the biomass.

Dry weight is a more acceptable growth measurement, especially the end point of experiment. Dry weight of duckweed is just 3% to 14% of fresh weight, so (for laboratory-scale experiments) an accurate milligram is accuracy and necessary unit for experiment.

Dry weight is affected by plants contaminating, placing them on a pre-weighed and plants number assessment. Then plants will be dried in a hot oven at the temperature about 105 ºC for 12 hours (Wood, 1975).

2.3.6 Using duckweed as ecology, biology and physiology monitor.

There are 3 reasons to use duckweed as ecology, biology and physiology monitor as follows.

2.3.6.1 Growth rate of duckweed was generally around 0.1-0.5 g/g/day. *Lemna gibba* growth rate was 0.13-0.23 g/g/day while *Lemna minor* could grow from 6.4 square centimeter to almost 5,000 squares meter in 55 days (Nwo, 1992). The reason why duckweed has high growth rate was it use small energy from photosynthesis to form and maintain the structure.

2.3.6.2 New frond was not attached to form colony or complex structure. It connected to mother frond for 2-3 generations and then separate from the original frond (Hillman, 1978; Kaojarern, 1986).

2.3.6.3 Duckweed has no part of woody tissue and most cells were like young and old leave (Kaojarern, 1986).

Various kinds of duckweed could absorb the nutrients from the waste water. Nutrients were absorbed by roots and the lower part of frond. Since duckweed had high growth rate, it could absorb a lot of substances, for example, *Lemna minor* and *Lemna triculca* absorbed boron more than other aquatic weeds 10 times. Clarket cited by Kaojarern (1986) reported that duckweed had bioremediation activity to eliminate toxin from waste water. Duckweed was high tolerance and harvested easier than water hyacinth. Therefore, duckweed was suitable for waste water treatment more than water hyacinth and the genus that had been used for waste water treatment were *Lemna, Spirodela and Wolffia* (Zirschky, 1988) and it can also be applied for ecological indicator and others.

2.3.7 Chemistry element and nutrition of duckweed

Duckweed had high moisture which around 90-95% water (Oron, 1986; Haustein, 1990). After it was dried and analysis of chemistry element, the chemical elements were different depending on species and water resource (Rusoff, 1980). Culley and Epps (1973) notified that duckweed in natural fresh water contained protein 14-24.4 percent of dry weight and fiber 9.5-16.6 percent of dry weight.

Haustein et al. (1990) reported that duckweed had high protein as same as soybean residue (40 percents of dry weight). From those data, it can summarize that nitrogen in the water changed to protein nitrogen**,** each kind of duckweed had different level of protein which depends on species, harvesting time, temperature, environment and nutrient concentration (Cully, 1973; Chang, 1997; Hillman, 1978).

2.3.8 The advantage of duckweed

2.3.8.1 Using duckweed for waste water treatment

Properties of aquatic plant that appropriate for waste water treatment are easy harvest, less water, high protein, low fiber and lignin, well mineral absorption, short life cycle and harvesting period, safe for human and pets, easy processing and less pest. These mentioned properties are exactly specified to the plant in Lemnaceae.

Recently, scientist tried to implement duckweed to eliminate heavy metal in the water. Chattopadhydy and Konar (1990); Dirilgen and Inel (1994); Srivastar et al. (1994); Gard and Chandra (1994) reported that duckweed can be used to detect heavy metal level in the water. Sinha et al. (1994) stated that *Spirodela polythiza* could absorb and accumulate iron and manganese effectively whereas Srivastar et al. (1994) revealed that *Spirodela* could absorb chromium or nickel 10-53% from this mixture. Chattopadhydy and Konar (1990) discovered that *Spirodela polythiza* increased dissolution of oxygen (DO) and available phosphorus as well as decreased the base-solution, heavy metal and carbon dioxide.

2.3.8.2 Using duckweed as animal feed (Hillman, 1978; Haustein, 1990)

a) Using duckweed as fish food
b) Using duckweed as poultry feed
c) Using duckweed as pig food
d) Using duckweed as ruminant feed

2.3.9 Nutrient solution (ยงยุทธ, 2546)

Nutrient solution has an essential element for growth and reproduction of plant. It helps the plant grow normally. If there are any mineral deficiency, plant is

completely unable to develop. Nutrient requirement of plant is specific and other elements can not replace.

Considering on the advantage of elements, plant scientist and ecologist attempt to link the biochemical or physiological property of element and the complex dynamic of ecosystem as well as variation in environment. Then, the concept of related element administration should be developed which can be classified into 5 groups (Grass, 1988).

Group 1: Element that essential for plant physiological process has an important role of plant growth, for example, nitrogen and phosphorus.

Group 2: Element that essential for plant growth and likely to obtain from soil in a sufficient rate for survival, such as potassium, calcium, magnesium, iron, sulphur, manganese, copper, zinc, boron, molybdenum, sodium and chlorine.

Group 3: Element that is non-essential for plant physiological process but help the plant to survive in ecosystem, such as silicon (selenium).

Group 4: Element that is non-essential for plant survival but help the plant to transfer some nutrients in food web, such as cobalt and selenium.

Group 5: Element that is poisonous for the plant if it is excess and become a trouble in ecosystem and agriculture sector, for example, aluminum, iron, manganese, lead, zinc, chromium, magnesium, sodium, chlorine and sulphur.

Scientist can prove that development of plant biochemistry is an important part to verify the function in methabolism of that element as shown in table 2-4.

Table 2-4 Essential elements of plant

Element	Plant	Author (s)	B.E.
N, P	every kind of plant	Ville	2396-2430
Ca	every kind of plant	Salm-Horstmer	2399
K	every kind of plant	Biner and Lucanus	2408
S	every kind of plant	Biner and Lucanus	2409
Mg	every kind of plant	De Saussure von Raaumer Wilstaetter	2347 2426 2449
Fe	every kind of plant	Sacchs	2403
Mn	every kind of plant	McHargue	2465
B	every kind of plant	Sommer and Lipman	2469
Zn	every kind of plant	Sommer and Lipman	2469
Cu	every kind of plant	Sommer, Lipman and McKinney	2474
Mo	every kind of plant	Amon and Stout	2482
Cl	multi-cellular plant	Broyer et alii	2482
Co	blue-green algae or cyanobacteria, *Rhizobium*	Holm-Hansen et alii	2503
V	Green algae	Ahmed and Evan Reisoneuer	2503 2503
Na	blue-green algae or cyanobacteria, *Atriplex spp*	Allen and Amon Brown and Wood	2479 2500
Ni	multi-cellular plant	Brown et alii	2530

From the information of Jeffrey (1988) and Pomerran (1965), Hoagland's solution was chosen to use the experiment because it had more advantage than Delmer's solution and Knop's solution since both solutions were lack of some essential elements for plant, such as molybdenum, copper, zinc, boron and manganese which necessary for all kind of plant.

2.3.10 Related Literature works

2.3.10.1 Organism and hypereutrophication

Under the hypereutrophic environment, a number of organisms such as sessile animals, heterotrophic nanoflagellates and ciliates and cyanobacteria had been examined and be regarded as biological indicator species of hypereutrophic condition as the following detail.

Kajiwara et al. (1997) quantitatively examined the sessile animals and monitoring of water quality in Dokal Bay, northern Kyushu, Japan, from 1991 to 1992 for four time to evaluate the water condition in the bay, Kitakyushu heavy and chemical industry area. In this study, seventy four species of sessile animals ware collected, which include *Mytilus galloprovincialis, Limnoperna forlunei, Crassosirea gigas, Mytilopsis sallei, Balanus amphitrite, Balanus trigonus, Balanus eburneus, Ciona inteslinalis and Styela plicata*. These nine representative species in the bay showed characteristic distribution and seasonal occurrence patterns. Eutrophic level of water in Dokai Bay was classified according to the occurrence of the sessile animals in the bay. Water condition of the inner most and central parts of the bay were classified as hypereutrophic and eutrophic levels. The results also found that six dominant species, including a mussel *M. galloprovincialis*, were useful as biological indicator organisms of hypereutrophic level of coastal water.

Nakano et al. (1998) followed seasonal changes in abundance of bacteria, heterotrophic nanoflagellates (HNF), ciliates and crustaceans, and consumption of bacteria by the protozoans, to investigate trophic interactions among these organisms in a hypereutrophic pond from March to October 1997. Densities of HNF and ciliates were high and attained a maximum of 1.4 X 10^5 and 3500 cells/ml, respectively. However, the high densities decreased as chlorophyll concentration increased. Since the predominant phytoplankton species was *Mycrocystis aerugjnosa* (Cyanophyceae), toxin produced by the alga possibly affected growth of protozoans. Not only HNF but also ciliates were important consumers of bacteria, and consumption of bacteria by ciliates varied at the same level as that of HNF from August to October. Bacterial turnover rate (%/d) due to consumption by the protozoa ranged between 5.6 and 112 (mean 25), and there were significant relationships

between densities of bacteria and specific ingestion rates (bacteria protozoan cell^{-1} h^{-1}) of the protozoans. These results suggested that the food linkage between bacteria and the protozoans was substantial in the pond.

Chomérat et al. (2007) carried out this study in order to understand the seasonal variations in the phytoplankton composition and biomass, and to analyse the influence of environmental parameters such as salinity, nutrients and climate on the seasonal succession of species. The phytoplankton was permanently dominated by cyanoprokaryotes, probably because of high availability of nutrients, low light penetration in the water column and frequent turbulent mixing induced by wind. The two most abundant species *Planktothrix agardhii* (in winter–spring) and *Pseudanabaena limnetica* (in summer) had low light requirements and were well adapted to a high mixing frequency. The ecological success of Oscillatoriales observed in the Bolmon lagoon was a perfect example of a shift to the "turbid stable state" as proposed for freshwater shallow lakes only. This work demonstrated that hypereutrophic Mediterranean lagoons could function very similarly to shallow lakes at higher latitudes; but the warmer climate and higher irradiances were probably responsible for differences in the seasonal pattern of species dominance.

2.3.10.2 Duckweed as phytoremediator

There are many reports showed that duckweed had the ability in phytoremediation which useful for reduction of the environmental problem and would be relevant for environmental indication as the follows.

Amporn (2538) studied the poisonous of cadmium and lead on greater duckweed (*Spirodela polyrhiza* L.) and common duckweed (*Lemna perpusillia* Torr.) to find the impacts of cadmium and lead on weight and chlorophyll-a of duckweed. *Lemna* perpusillia Torr. was cultured in 0.3, 0.6, 0.9, 1.2 and 1.5 mg/l cadmium solution while *Spirodela* polyrhiza L. was cultured in 2, 8, 14, 20 and 26 mg/l cadmium solution. Moreover, both species were also cultured in 12, 20, 28, 36 and 44 mg/l lead solution. The main solution used in the experiment was 1:10 Hoagland solution and the results had been examined at 24, 48, 72 and 96 hours, respectively. It was found that the weight and chlorophyll quantity of duckweed reduced when cadmium and lead concentration and time course increased. *Lemna perpusillia* Torr. was sensitive for cadmium less than 0.3 mg/l while *Spirodela*

polyrhiza L. was sensitive for cadmium less than2 mg/l. Both species were sensitive for lead equally.

Saranya (2539) investigated the effects of poison cadmium on duckweed (*Spirodela polyrhiza* L.) and water lettuce (*Pistia stratiotes* Linn.) in separated and coexisted conditions. The conditions of experience were 1:10 Hoagland solution, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 mg/l cadmium concentration, 24, 48, 72 and 96 hours for experimental determination. The result illustrated that both separating and coexisting of duckweed and water lettuce had the same outcome. The amount of chlorophyll, weight and survival rate of each species obviously increased.

Waranusantigul P. (2001) examined the elimination of nickel and lead by greater duckweed [*Spirodela polyrhiza* (L.) Schleid]. The result revealed that removal percentage of nickel and lead was highest in first 2 days of experiment at 58-80% and 68.25-91%, respectively. When duration of experiment and nickel and lead concentrations increase, removal percentage of nickel and lead by greater duckweed significantly decreased. Elimination percentage of lead was higher than that of nickel.

2.3.10.3 Duckweed and eutrophication

For the plant species, duckweed which admiringly used in many environmental aquatic studies due to the small size, rapid growth, extensive distribution, short life duration and steadiness to environmental changes were expectantly treated in the research for early detection on eutrophic status as well.

Fre´de´ric et al. (2006) examined the effect of mat density on duckweed (*Lemna minor*) growth under controlled eutrophic conditions: 12.5 hour a day light exposure and 342 mol/m^2/s^1 light intensity at 20 $^{\circ}$C. The plant growth was carried out in Hoagland medium for 7 days without harvesting. The results revealed a maximal biomass growth rate of 88 g-dry/m^2 (1470 g-wet/m^2) at an optimal initial mat density of 45 g-dry/m^2 (750 g-wet/m^2), with removal rates for nitrogen (N) and phosphorus (P) of 483mg-N/m^2/d and 128mg-P/m^2/d, respectively. A mathematical model that took into account the mat density was developed in order to simulate the growth of *Lemna minor* under controlled eutrophication. Based on experiments carried out, the model exhibited a reliability of 89%. The model remained to be validated at the full-scale level.

Lasfar et al. (2007) studied the change of duckweed mat density impacts by temperature, photoperiod and concentration of phosphorus and nitrogen that affects on the growth of duckweed under eutrophication condition. The result demonstrated that duckweed intrinsic growth rate is mainly affected by temperature and photoperiod. However, for the nutrient (P–N) concentrations usually found in wastewater, the intrinsic growth rate is not affected. The results obtained in this research had permitted to determine some mathematical relations which correlate the intrinsic growth rate of duckweed *Lemna minor* with the variations of the parameters; temperature, photoperiod and P–N concentrations. These relations were then incorporated into a global model to predict the growth of duckweed. The model was calibrated using data from laboratory experiments carried out during the present study, and validated using experimental data from the literature. In both cases (calibration and validation) the results yielded a deviation less than 0:03/d within a confidence interval of 95%.

Lasfar et al. (2007) also indicated the effects of the parameters on duckweed growth which were:

The maximum intrinsic growth rate was observed at an optimal temperature and photoperiod of 26 ºC and 12–13 hours, respectively.

The intrinsic growth rate was strongly inhibited at temperatures below 8 ºC or above 35 ºC.

The photoperiod was inversely proportional to the number of attached leaflets and directly proportional to size of the leaflets.

The extent of the separation and the development of the leaflets were affected by the light–dark ratio.

The intrinsic growth rate was inhibited for photoperiods less than 7 hours and greater than 16 hours.

CHAPTER III
METERDOLOGY AND METHODS

Common duckweed (*Lemna minor* L.) and giant duckweed [*Spirodela polyrhiza* (L.) Schleid.] using in this research were collected from Krathumbaen, Samut Sakhon province and conducted under hypereutrophic condition as the following steps.

3.1 Determining and classification of variables

Variables in this study were classified into 3 categories.

3.1.1 Independent variables

3.1.1.1 Nitrogen concentration in the solution were 2 and 5 mg/l

3.1.1.2 Common and giant duckweeds

3.1.2 Dependent variables

3.1.2.1 Common duckweed and giant duckweed growth

Growth of common and giant duckweeds was measured from the changes of the following indices.

a) Fresh weight (mg)

b) Dry weight (mg)

c) Number of fronds

d) Character of fronds

3.1.2.2 Microalgae

In this experiment, microalgae were measured to see the effect of these organisms on common and giant duckweeds growth. The number of microalgae was detected by the amount of chlorophyll a.

3.1.3 Controlled variables

3.1.3.1 Color and size of common duckweed and giant duckweed on the initial date should be the same.

3.1.3.2 Number of plant per a container (used 5 plants/container)

3.1.2.3 Phosphorus concentration (0.9 mg/l)

3.1.2.4 Time to investigated the experimental condition

a) Light intensity, total nitrogen (TN) and total phosphorus (TP) were detected on the initial and last days

b) Solution and room temperatures, number of frond and character of frond were assessed every day

c) Chlorophyll a, pH, salinity, fresh weight and dry weight were determined on day 0, 4, 8 and 12 of experiment

3.1.2.5 Modified 1/10 strength Hoagland's solution

3.2 Environmental parameters

All of the experiments were carried out at laboratory in Faculty of Environment and Resource Studies, Mahidol University.

3.2.1 Solution temperature

The temperature which appropriate for growth of duckweed was between 20-30 ºC (Zirschky and Reed, 1988; Öbek and Hasar, 2002). Thus, it was necessary to verify the solution temperature throughout the experiment.

3.2.2 Room temperature

In addition to the solution temperature, room temperature was required to evaluate during the experiment as well.

3.2.3 Chlorophyll a

Chlorophyll a content of the solution was the representative of microalgae number. It was spectrophotometrically measured (Thermo Spectronic, GENESYS 10 UV spectrophotometer) according to Strickland and Parson (1972).

3.2.4 Light intensity and duration

The optimal light intensity range for duckweed growth was around 85-125 μE/m^2/s (Mkandawire et al., 2005), so that the experimental units were kept under 12 hours of illumination with cool white fluorescent lamps at 86 μE/m^2/s and 12 hour of darkness.

3.2.5 pH

The pH of solution was adjusted to around 7.5 with KOH 1 N and HCl 1 N and investigated by pH meter (Mettler Toledo, SevenGo™ SG2-FK).

3.2.6 Salinity

Salinity of the solution was determined using conductivity meter (Mettler Toledo, SevenGo™ SG3-FK2).

3.2.7 Water volume

To make up for evaporation losses, distillated water was added to adjust the nutrient solution level daily to 2,000 ml if necessary.

Salinity and pH of the solution were determined using conductivity meter (Mettler Toledo, SevenGo™ SG3-FK2) and pH meter (Mettler Toledo, SevenGo™ SG2-FK), respectively.

3.3 Experimental procedures

3.3.1 Container

In this study, cylindrical glass container was used as a sub-unit of experimental set. The size of container was shown in Figure 3-1. Black paper was also used to cover a container around the outside of solution level to reduce the disturbance of microalgae.

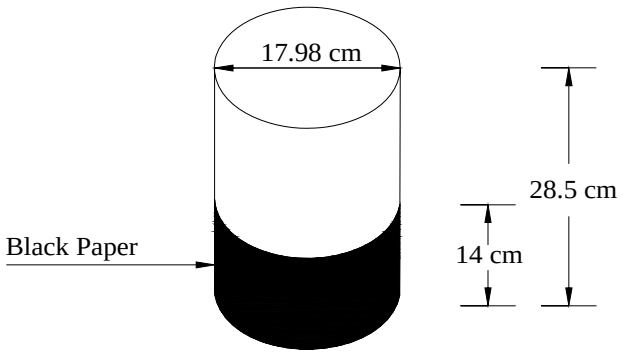

Figure 3-1 Container used in the experiment.

3.3.2 Experimental units

Under control condition in the laboratory, all experimental units were placed as illustrated in Figure 3-2 and 3-3.

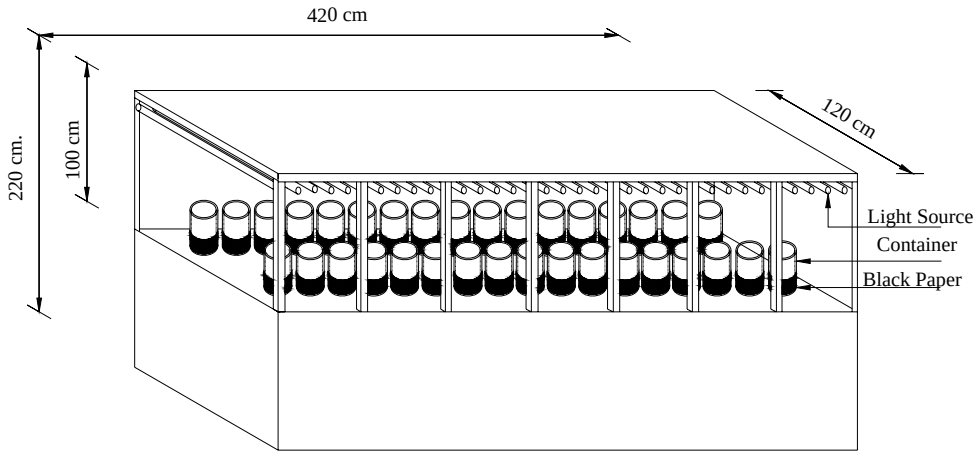

Figure 3-2 Setting of experiment units.

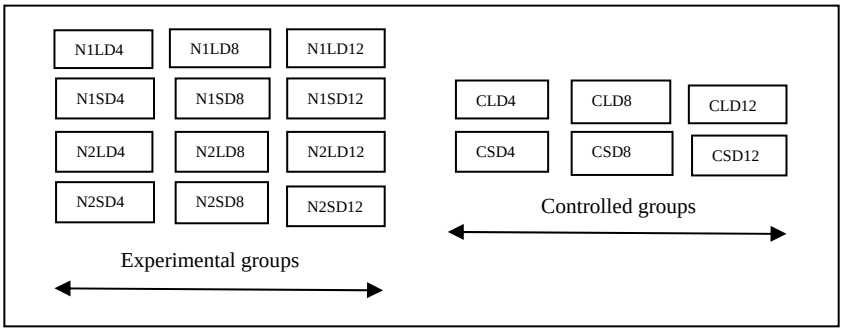

Figure 3-3 Experimental groups and control groups in experiment. Each unit had 3 replications. N1 is nitrogen 2 mg/l, N2 is nitrogen 5 mg/l, C is Control set, L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively.

3.3.3 Nutrient solution

Nutrient solution using in control groups was modified 1/10 strength Hoagland's solution (Hoagland et al., 1950). For experimental groups, the total nitrogen (TN) and total phosphorus (TP) in the solution were adjusted to 2 and 5 mg/l for TN and 0.9 mg/l for TP.

3.3.4 Experimental plants

a) Common duckweed and giant duckweed from natural water in Krathumbaen, Samut Sakhon province were collected.

b) Plants were separately placed on an aluminum sieve (20.2 cm diameter, 0.1 cm pore size) and washed with clean running tap water for 15 minutes and repeatedly (3 times) washed in distilled water to remove unwanted matter.

c) Each plant (1 frond/plant) was moved into a cylindrical glass container (28.5 cm height X 17.98 cm diameter) containing 1,000 ml of modified 1/10 strength Hoagland’s solution.

d) Plants were kept under 12 hours of illumination with cool white fluorescent lamps at 86 µE/m2/s light intensity (detected by Lux meter, Lutron, Lx-101) and 12 hours of darkness for 7 days.

e) Record the character of plants such as color of frond, size of frond every day for 7 days.

f) When the plants had been familiar with new environment and increased in number rapidly, one gram of each species was transferred into an empty fish tank (26.5 cm width X 50 cm length X 32 cm height) filling with 4,000 ml of modified 1/10 strength Hoagland's solution under the same light source and intensity for 2 weeks.

g) After obtaining enough amount of the experimental plants, each species were rinsed with clean running tap water (15 minutes) on an aluminum sieve (20.2 cm diameter, 0.1 cm pore size) and soaked in distilled water 3 times to remove undesired microalgae.

h) Subsequently, five plants (1 frond/ plant) of common and giant duckweeds were cultivated independently in a cylindrical glass container (28.5 cm height X 17.98 cm diameter) loading with 2,000 ml of modified 1/10 strength Hoagland's solution (pH 7.5) and illuminated with cool white fluorescent lamp (86 µE/m2/s) 12 hours/day for 12 days as a control group.

i) For an experimental group, the same feature and number of plants had been done as mentioned above, except the total nitrogen (TN) and total phosphorus (TP) in the solution were adjusted to 2 and 5 mg/l for TN and 0.9 mg/l for TP using spectrophotometric procedures (Jasco, V530 UV/VIS spectrophotometer) as stated by Singh et al.(2003) and Zhou and Struve (2004), respectively.

3.4 Growth measurement

3.4.1 Fresh weight

The fresh weight of plants was obtained on day 0, 4, 8 and 12 by placing duckweeds on absorbed paper for 5 minutes to absorb the water and weighed them on the scales (Landolt and Kandeler, 1987).

3.4.2 Dry weight

After gaining the fresh weight, plants were moved into hot air oven at 105ºC for 24 hours. Then, they were transferred into desiccators and left inside for 2 hours. Later, they were weighed on the scales (Landolt and Kandeler, 1987).

3.4.3 Fronds

Fronds were counted and observed (size of frond 0.1 mm.) the character on day 0, 4, 8 and 12 of experiment (Landolt and Kandeler, 1987).

All parameters measuring in this research are shown in the table 3-1.

3.5 Statistical analysis

3.5.1 Descriptive statistics were mean and standard deviation used for data description of water quality analysis from experiment such as solution temperature, amount of chlorophyll a, pH, total nitrogen, total phosphorus and also for fresh weight, dry weight and number of fronds.

3.5.2 Statistical analysis was performed by factorial method to study the utilization of common and giant duckweeds as bioindicator under hypereutorphic condition. Each experiment had been done with 3 replications of 2 different nitrogen concentrations. Means were analyzed using SPSS for Windows version 17. Three-way ANOVA was first carried out at the significance level of $P<0.05$ and then Least Significant Difference (LSD) procedure for comparison of means were made at $P<0.05$

Table 3-1 Parameters and methods of analysis

Parameters	Methods	Measurement date
1. Solution		
• Temperature	- Method of Zirschky and Reed (1988); Öbek and Hasar (2002)	-Every day
• pH	- Method of Mohammad et al. (1997)	-0, 4, 8 and 12 day(s)
• Salinity	- Method of Strickland and Parson. (1972)	-0, 4, 8 and 12 day(s)
• Chlorophyll a	- Method of Strickland and Parson. (1972)	-0, 4, 8 and 12 day(s)
• TP	- Method of Zhou and Struve (2004)	-0 and 12 day(s)
• Nitrate	- Method of Singh et al. (2003)	-0 and 12 day(s)
2. Plants		
• Biomass	Landolt (1987)	-0, 4, 8 and 12 day(s)
• Fronds	Landolt (1987)	-0, 4, 8 and 12 day(s)
3 Light intensity	- Method of Mkandawire et al. (2005)	-0 and 12 day(s)

CHAPTER IV
RESULTS AND DISCUSSIONS

After common duckweed (*Lemna minor* L.) and giant duckweed (*Spirodela polyrhiza* (L.) Schleiden had been grown under controlled hypereutrophication, the present results could be divided into 2 parts as follows.

4.1 Experimental conditions

The experimental conditions which may affect the common and giant duckweeds grown under controlled hypereutrophic state were solution temperature, pH, salinity, microalgae, total nitrogen (TN) and total phosphorus (TP).

4.1.1 Solution temperature

All this study, all solution temperatures were measured at 9.30-10.00 am. and 2.30-3.00 pm to check the different of temperature during the day time. It was found that solution temperature in the afternoon was higher than one in the morning (Table 4-1).

Table 4-1 Statistical value of solution temperature during the experiment

Descriptive statistics	Solution temperature (^{o}C)	
	9.30-10.00am	2.30-3.00pm
Mean±S.D.	24.85±0.85	26.85±1.10
Minimum	23.5	25.1
Maximum	26.4	28.5

4.1.2 Microalgae

The number of microalgae was detected by using 3.2.3. The result showed that chlorophyll a content in the solution was less than 0.08 mg/m^3 (Table 4-2).

Table 4-2 Statistical value of Chlorophyll a content in the solution during the experiment

Descriptive statistics	Chlorophyll a (Mean±S.D. in mg/m^3)		
	Control	Set1	Set2
Mean±S.D.	0.0171±0.0182	0.0275±0.0266	0.0141±0.0147
Minimum	0	0	0
Maximum	0.02	0.08	0.04

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively.

4.1.3 pH

In this study, pH value of the solution was determined and found that it was in between 6.18 and 7.63 (Table 4-3).

Table 4-3 Statistical value of pH of the solution during the experiment

Descriptive statistics	pH (Mean ±S.D.)		
	Control	Set1	Set2
Mean±S.D.	7.02±0.3	6.93±0.55	7.07±0.41
Minimum	6.58	6.18	6.45
Maximum	7.46	7.59	7.63

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively.

4.1.4 Salinity

The salinity of the solution found was in between 0.10 and 0.14 ppt. (Table 4-4)

Table 4-4 Statistical value of salinity of the solution during the experiment

Descriptive statistics	Salinity (Mean±S.D. in ppt.)		
	Control	Set1	Set2
Mean±S.D.	0.13±0.004	0.135±0.005	0.12±0.014
Minimum	0.13	0.13	0.1
Maximum	0.14	0.14	0.14

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively

4.1.5 Total nitrogen (TN)

Total nitrogen of the solution was determined on initial and last day of experiment. The result revealed that total nitrogen notably rose in all treatments (Table 4-5).

Table 4-5 Statistical value of total nitrogen exposed to different solution on initial and last day of experiment.

Experimental Unit	Total nitrogen (Mean ± S.D. in mg/l)		
	Control	Set1	Set2
L0	3.28±0.08	2.26±0	5.08±0
L12	3.39±0.08	4.05±0.35	5.47±0.56
S0	3.28±0.08	2.26±0	5.08±0
S12	4.42±0.51	4.30±0.56	5.68±0.22

Note : L is common duckweed, S is giant duckweed, the number 0 and 12 represent day 0 and 12, respectively.

4.1.6 Total phosphorus (TP)

Though total phosphorus of the solution was also examined on initial and last day of experiment as will as total nitrogen, the result was absolutely different. The amount of total phosphorus obviously reduced in all treatments (Table 4-6).

Table 4-6 Statistical value of Total phosphorus exposed to different solution on initial and last day of experiment.

Experimental Unit	Total phosphorus (Mean ± S.D. in mg/l)		
	Control	Set1	Set2
L0	0.93±0	0.95±0	0.95±0
L12	0.3±0.002	0.22±0.01	0.73±0.03
S0	0.93±0	0.95±0	0.95±0
S12	0.05±0.04	0.08±0.01	0.02±0.01

Note : L is common duckweed, S is giant duckweed, the number 0 and 12 represent day 0 and 12, respectively.

4.2 Fresh weight, dry weight and number of fronds

4.2.1 Fresh weight

Fresh weight of common and giant duckweeds increased throughout the experiment (Table 4-7 and Figure 4-1). Nevertheless, giant duckweed had higher fresh weight than common duckweed.

Table 4-7 Statistical value of fresh weight exposed to different solution during the experiment.

Experimental Unit	Fresh weight (Mean±SD in mg)		
	Control	Set1	Set2
L0	2.3±0	2±0	2.3±0
L4	4.6±2.26	2.6±0.7	5.96±1.36
L8	11.25±2.19	4.23±2.83	11.23±0.99
L12	51.45±7.85	242.1±30.59	86.55±9.45
S0	11.3±0	8.75±0.06	11.3±0
S4	23.9±5.80	44.07±18.06	33.93±1.29
S8	68.6±8.06	88.1±33.78	90±6
S12	177.5±33.52	369.73±59.74	277.05±5.15

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively.

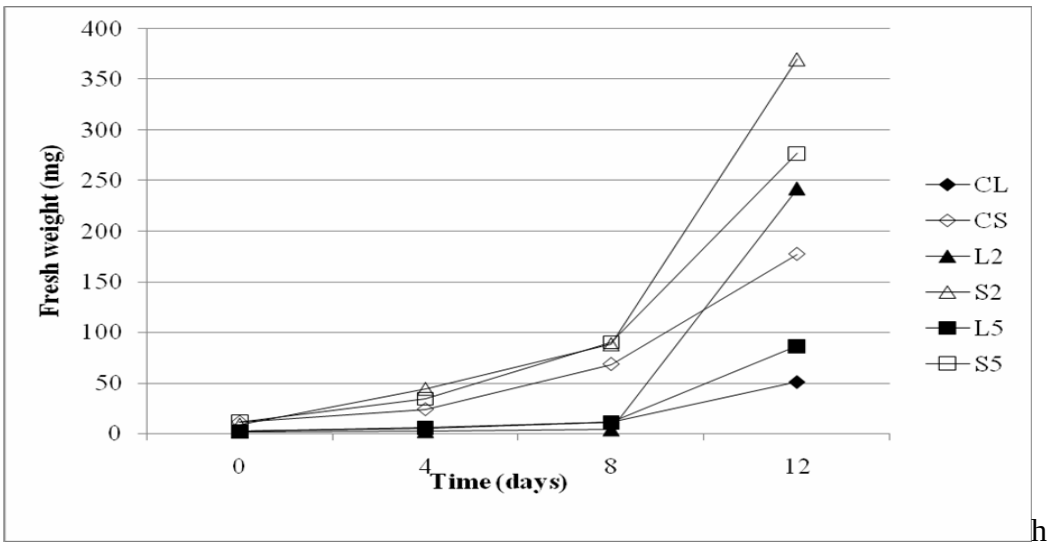

Figure 4-1 Fresh weight of duckweeds during the experiment (C is control set, L is common duckweed, S is giant duckweed, the number 2 and 5 represent total nitrogen at 2 and 5 mg/l, respectively).

For statistical analysis, it was found that means of fresh weight under different nitrogen concentrations, plants and time (days) had certain significant differences at $P<0.05$ (Table 4-8).

Table 4-8 Statistical test by Three-way ANOVA for the difference of means of fresh weight under different nitrogen concentrations, plants and time (days).

Sources	Type III sum of squares	df	Mean sqaure	F	Sig
Nitrogen concentration	32415.119	2	16225.559	6.983	0.002
Plant	76030.501	1	76030.501	32.722	< 0.001
Day	436492.085	3	145497.362	62.619	< 0.001

Subsequently, means of each set were compared using Least Significant Difference (LSD) procedure at $P<0.05$ as shown in Table 4-9 to Table 4-11.

Table 4-9 Comparison of means between fresh weight of different nitrogen concentrations by LSD procedure (Set 1 and 2 represent total nitrogen at 2 and 5 mg/l, respectively).

Set(l)	Set(J)	Mean difference (l-J)	Sig[a]
Control set	Set 1	-51.692*	<0.001
	Set 2	-20.929*	0.137
Set 1	Set 2	30.763*	0.031

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 4-10 Comparison of means between fresh weight of different plant species by LSD procedure.

Plant(l)	Plant(J)	Mean difference (l-J)	Sig[a]
Common duckweed	Giant duckweed	-8.375*	0.000

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 4-11 Comparison of means between fresh weight of different time (days) by LSD procedure (the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively).

Day(l)	Day(J)	Mean difference (l-J)	Sig[a]
0	4	-2.831	0.293
	8	-7.581*	0.006
	12	-29.786*	< 0.001
4	8	-4.750	0.080
	12	-26.956*	< 0.001
8	12	-22.206*	< 0.001

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

The result from comparison of means revealed that fresh weight between different nitrogen concentrations gave statistical difference at $P<0.05$, except control set and 5 mg/l total nitrogen (Table 4-9). Fresh weight between common and giant duckweed were also statistically significant difference at $P<0.05$ (Table 4-10). In case

of time (days), fresh weight among those groups had found significant difference at $P<0.05$, excluding day 0 and day 4 as well as day 4 and day 8 (Table 4-11).

4.2.2 Dry weight

Dry weight of common and giant duckweeds gave the same tendency as fresh weight (Table 4-12 and Figure 4-2). However, both fresh weight and dry weight decreased when total nitrogen of the solution increased.

Table 4-12 Statistical value of dry weight exposed to different solution during the experiment.

Experimental Unit	Dry weight (Mean±SD in mg)		
	Control	Set1	Set2
L0	0.3±0	0.1±0	0.3±0
L4	0.45±0.21	0.3±0.1	7.67±1.53
L8	1.85.±0.07	0.67±0.5	9.5±1.55
L12	8.35±1.49	40.23±9.46	10.87±0.66
S0	1.8±0	1.9±0	1.8±0
S4	3.7±0.85	5.97±1.23	5.2±0.4
S8	11.6±1.56	13.67±5.51	14.5±1.10
S12	28.4±6.22	53.1±9.48	44.07±5.10

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively.

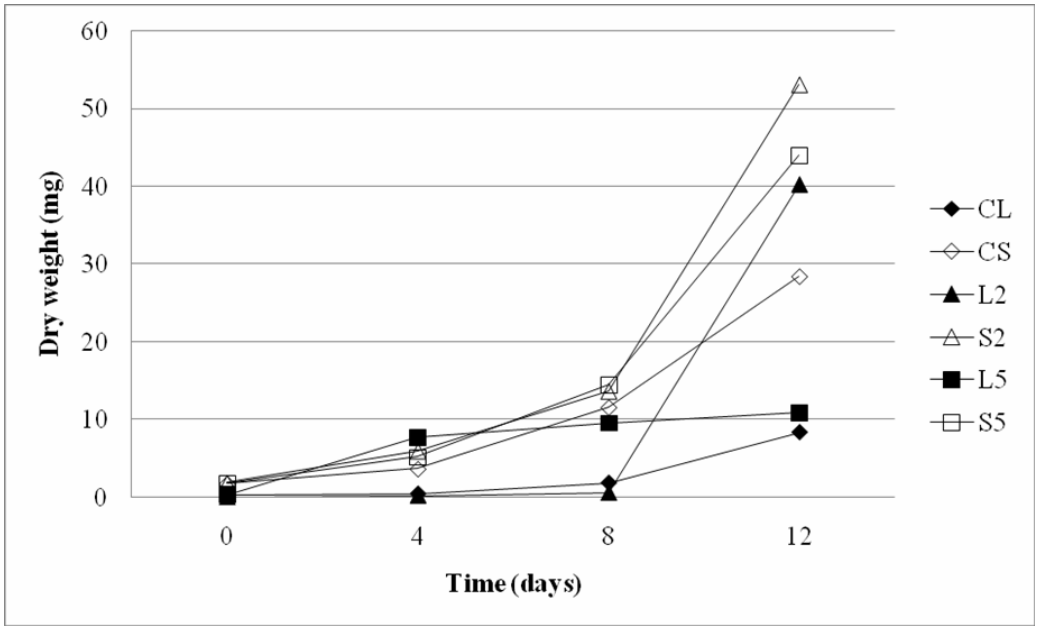

Figure 4-2 Dry weight of common and giant duckweeds exposed to different solution during the experiment (C is control set, L is common duckweed, S is giant duckweed, the number 2 and 5 represent total nitrogen 2 and 5 mg/l, respectively).

Having statistical analysis, the result illustrated that means of dry weight under different nitrogen concentrations, plants and time (days) gave undeniable significant differences at $P<0.05$ (Table 4-13).

Table 4-13 Statistical test by Three-way ANOVA for the difference of means of dry weight under different nitrogen concentrations, plants and time (days).

Source	Type III sum of squares	df	Mean sqaure	F	Sig
Nitrogen concentration	680.320	2	340.160	5.305	0.007
Plant	1373.317	1	1373.317	21.417	< 0.001
Day	9877.232	3	3292.411	51.344	< 0.001

Consequently, means of each set were compared using Least Significant Difference (LSD) procedure at $P<0.05$ as revealed in Table 4-14 to Table 4-16.

Table 4-14 Comparison of means between dry weight of different nitrogen concentrations by LSD procedure (Set 1 and 2 represent total nitrogen 2 and 5 mg/l, respectively).

Set(l)	Set(J)	Mean difference (l-J)	Sig[a]
Control set	Set 1	-7.448*	0.002
	Set 2	-4.681*	0.047
Set 1	Set 2	2.767	0.236

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 4-15 Comparison of means between dry weight of different plant species by LSD procedure.

Plant(l)	Plant(J)	Mean difference (l-J)	Sig[a]
Common duckweed	Giant duckweed	-8.375*	< 0.001

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 4-16 Comparison of means between dry weight of different experimental days by LSD procedure (the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively).

Day(l)	Day(J)	Mean difference (l-J)	Sig[a]
0	4	-2.831	0.293
	8	-7.581*	<0.001
	12	-29.786*	< 0.001
4	8	-4.750	0.080
	12	-26.956*	< 0.001
8	12	-22.206*	< 0.001

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Having comparison of means, the result showed that dry weight between different nitrogen concentrations gave significantly statistical difference at $P<0.05$, except total nitrogen at 2 and 5 mg/l (Table 4-14). Dry weight between common and giant duckweed were statistically significant difference at $P<0.05$ too (Table 4-15).

For time (days), dry weight among those groups had also shown statistical difference at $P<0.05$, excluding day 0 and day 4 as well as day 4 and day 8 (Table 4-16).

4.2.3 Number of fronds

Number of fronds of common and giant duckweeds obviously elevated throughout the experiment (Table 4-17 and Figure 4-3). Nonetheless, common duckweed had noticeably higher number of fronds than giant duckweed.

Table 4-17 Statistical value of Number of fronds exposed to different solution during the experiment.

Experimental Unit	Number of fronds (Mean±SD)		
	Control	Set1	Set2
L0	5±0	5±0	5±0
L4	14.5±0.71	27.67±6.66	14±1
L8	46.±1.41	101.33±8.33	43.33±1.53
L12	194.5±9.19	289.67±160.32	254.67±67.90
S0	5±0	5±0	5±0
S4	16.5±0.71	23±6.66	17±1
S8	47.5±3.54	56.67±14.19	66.7±5.69
S12	133.5±20.51	194.67±23.46	164±50.67

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively.

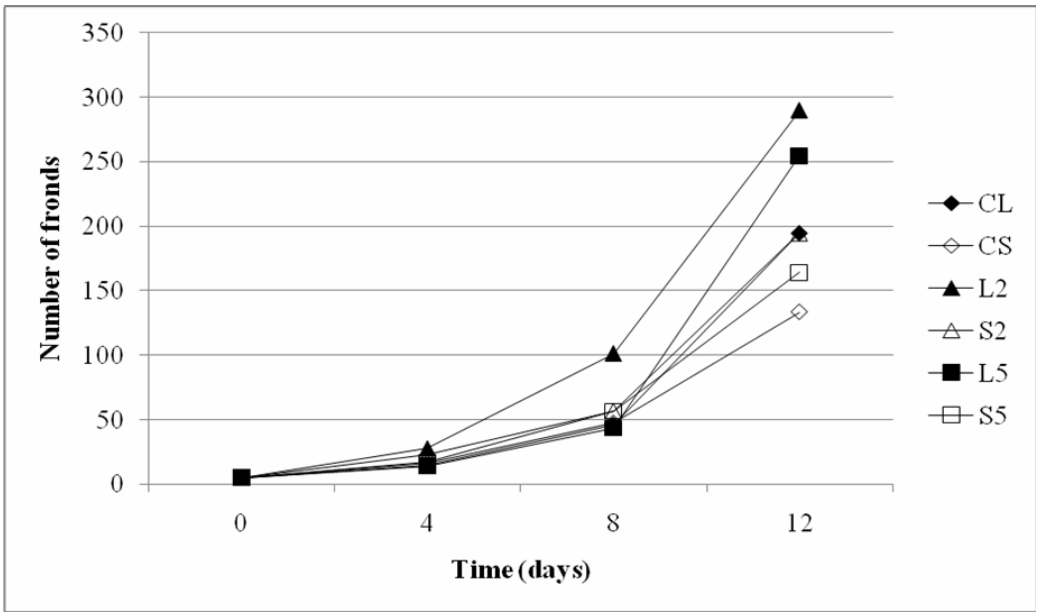

Figure 4-3 Number of fronds of common and giant duckweeds exposed to different solution during the experiment (C is control set, L is common duckweed, S is giant duckweed, the number 2 and 5 represent total nitrogen 2 and 5 mg/l, respectively).

For statistical analysis, it was noticed that means of number of fronds under different nitrogen concentrations, plants and time (days) had certified significant differences at $P<0.05$ (Table 4-18).

Table 4-18 Statistical test by Three-way ANOVA for the difference of means of number of fronds under different nitrogen concentrations, plants and experimental days.

Source	Type III sum of squares	df	Mean sqaure	F	Sig
Nitrogen concentration	10975.443	2	5487.772	3.400	0.039
Plant	9531.202	1	9531.202	5.905	0.018
Day	454217.890	3	151405.963	93.801	< 0.001

Afterward, means of each set were compared using Least Significant Difference (LSD) procedure at $P<0.05$ as demonstrated in Table 4-19 to Table 4-21.

Table 4-19 Comparison of means between number of fronds of different nitrogen concentrations by LSD procedure (Set 1 and 2 represent total nitrogen 2 and 5 mg/l, respectively).

Set(l)	Set(J)	Mean difference (l-J)	Sig[a]
Control set	Set 1	-30.058*	0.012
	Set 2	-12.142	0.299
Set 1	Set 2	17.917	0.127

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 4-20 Comparison of means between number of fronds of different plant species by LSD procedure.

Plant(l)	Plant(J)	Mean difference (l-J)	Sig[a]
Common duckweed	Giant duckweed	23.011*	0.018

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 4-21 Comparison of means between number of fronds of different experimental days by LSD procedure (the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively).

Day(l)	Day(J)	Mean difference (l-J)	Sig[a]
0	4	-13.783	0.307
	8	-53.583*	<0.001
	12	-200.167*	< 0.001
4	8	-39.800*	0.004
	12	-186.383*	< 0.001
8	12	-146.583*	< 0.001

Note : Based on estimated marginal means: * = The mean difference is significant at the 0.05 level, a = Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Following comparison of means, it was determined that number of fronds among different nitrogen concentrations had statistical difference only between control set and 2 mg/l total nitrogen at $P<0.05$ (Table 4-19). Number of fronds between common and giant duckweed had also found significant difference at $P<0.05$ (Table 4-20). On comparison of experimental days, number of fronds among those groups were significantly statistical difference at $P<0.05$, excluding day 0 and day 4 (Table 4-21).

4.3 Discussions

4.3.1 Solution temperature

Having solution temperature had been investigated at 9.30-10.00am and 2.30-3.00pm during experiment, it was found that temperature was between 23.5 $^{\circ}$C and 28.5 $^{\circ}$C (Table 4-1). This temperature range had no effect to the growth of

common duckweed and giant duckweed as Zirschky and Reed (1988) and Öbek and Hasar (2002) discovered that the temperature which appropriate for the growth of duckweed was between 20 and 30 ºC. However, Ansari and Khan (2009) came across that the most suitable range of temperature for remediation of eutrophic water using giant duckweed was between 25 to 30 ºC. When harvested regularly duckweed plants may be of use in counteracting eutrophication in affected water bodies. In this research, the low temperature, less than 25 ºC, was obtained because the experiment had been done during winter. Nevertheless, fresh weight and dry weight of both duckweeds increased throughout the experiment (Figure 4-4 and 4-5) indicating that this cool temperature had no effect to plant growth.

4.3.2 Chlorophyll a

Chlorophyll a was suitable as one of the biological indicators to show the trend of eutrophication of lake (Khan and Ansari, 2005). Generally, chlorophyll a content of eutrophic and hypereutrophic states of lake are more than 9 and 25 mg/m^3, respectively (Smith et al., 1999). In this experiment, the amount of chlorophyll a represented the number of microalgae. The result in Table 4-2 showed that chlorophyll a increased during the research period. The highest amount of chlorophyll a was 0.08 mg/m^3. This quantity of chlorophyll a during experiment of the solution was quite low. This result illustrated that there were an insignificant number of microalgae which should not disturb the growth of common and giant duckweed in the experiment.

4.3.3 pH

The pH value from all treatments was between 6.18 and 7.63 (Table 4-3, Figure 4-1). Mostly, they were not significantly difference among groups. Radic´ et al. (2010) stated that duckweed grew rapidly between pH 5 and 9. Moreover, Öbek and Hasar (2002) supported that duckweed was among the most vigorously growing plants on earth. Typical pH range for duckweed growth was 4.5-7.5 and growth was completely inhibited only at pH values higher than 10. Thus, the pH range between 6.18 and 7.63 in this research should promote the growth of both organisms.

4.3.4 Salinity

All salinity was also shown in between 0.10-0.14 ppt (Table 4-4, Figure 4-2) though out the experiment . At this level, it should be advantage to common and giant duckweeds as supported by Omar and Balla (2009) who revealed that duckweed

growth was promoted up to salinity concentration of 1.3 ppt. Due to result of salinity was very low in all treatments during the experiment, suggested that low salinity had no effect to the growth of both duckweeds. Moreover, desalination by duckweed could occur up to 25% of the initial total salinity independent on salinity content (Omar and Balla, 2009).

4.3.5 Total Nitrogen (TN)

Total nitrogen of the solution on initial and last day of experiment was a bit different. The result revealed that total nitrogen notably rose in all treatments (Table 4-5). This may be occurred follow as ;

4.3.5.1 Because of the decomposition of microalgae and duckweed which died during the experiment in the container.

4.3.5.2 Another reason was the total nitrogen values in this research might increase from nitrate synthesis Van der Steen et al. (1998) found that total nitrogen in NO_3 form in duckweed pond system effluent increased from not detectable to 2 mg/l whereas total nitrogen in NH_4 form reduced from 40 mg/l to 24 mg/l. Thus, it might be possible that total nitrogen in NO_3 form in this experiment could elevate in all treatments.

4.3.6 Total Phosphorus (TP)

Total phosphorus of the solution on initial and last day of experiment was absolutely different from total nitrogen. It was observed that the amount of total phosphorus obviously reduced in all treatments (Table 4-6). It was known very well that duckweed had capability in phosphate removal from secondary effluents. Öbek and Hasar (2002) found that the initial phosphate concentration decreased from 15 mg/l to 0.5 mg/l at the end of an 8 days period whereas van der Steen et al. (1998) revealed that total phosphorus in PO_4 form in duckweed pond system effluent decreased from 17 mg/l to 10 mg/l. Therefore, total phosphorus in this experiment had no effect to the growth of common and giant duckweeds.

4.3.7 Fresh weight and dry weight

Fresh weight and dry weight of common and giant duckweeds had the same tendency responding to hypereutrophic environment as presented in Table 4-7

and 4-8 and Figure 4-3 and 4-4. Increasing in total nitrogen content from 2 mg/l to 5 mg/l, both fresh weight and dry weight decreased. These data illustrated that there should be some effect of the high level of hypereutrophic state (5 mg/l TN) to common and giant duckweeds growth than the lower one (2 mg/l TN). As a result, it is possible to use the growth of common and giant duckweeds for early detection on hypereutrophic status. Nevertheless, fresh weight and dry weight of common duckweed were higher than giant duckweed in all experimental days. This probably occurred because giant duckweed typically had the bigger size than common duckweed.

4.3.8 Number of fronds

Frond number of both free-floating common and giant duckweeds (Table 4-9 and Figure 4-5) was related to fresh and dry weights (Figure 4-4 and 4-5). It obviously elevated throughout the experiment. However, common duckweed showed noticeably higher number of frond than giant duckweed. This possibly happened because common duckweed normally was the smaller size than giant duckweed. Öbek and Hasar (2002) found that duckweed reached a doubling of frond number in 4 days under laboratory conditions (24 °C, 12 hours dark and light photo period). In this experiment, number of frond of common and giant duckweeds was higher than double in 4 days. This may occurred because hypereutrophic condition was more nitrogen and phosphorus than normal one.

CHAPTER V
CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on this experiment, common and giant duckweeds were classified as tolerant aquatic macrophytes according to their response to an eutrophication pressure (Penning et al., 2008). As a consequence, both plants could survive well under controlled hypereutrophic environment in this research. Nonetheless, the growth pattern of both floating duckweeds reduced under the rising of total nitrogen or more hypereutrophic state suggesting that common and giant duckweeds could be one of the bioindicator species for hypereutrophic condition. It is also interesting to have a further study and to compare these findings with the natural hypereutrophic environment.

5.2 Recommendation

5.2.1 To study and develop the appropriate method for dry weight investigation of common and giant duckweed, such as proper length of time and temperature in obtaining the correct dry weight.

5.2.2 Frequently record the fresh weight, dry weight and number of fronds to find the most suitable indication point that envisage the change of growth and be implemented for the future use.

5.2.3 To study the growth of these aquatic plants using nitrogen at the concentration 1.2-2 mg/l which is the starting point of hypereutrophic circumstance to see the possibility on early hypereutrophic indication.

5.2.4 From the experimental result, nitrogen concentration increased from the beginning. Thus, nitrogen fixation of plant should intentionally be examined.

5.2.5 In this experiment, the depth of solution was fixed in one level. Therefore, various levels of solution should be manipulated to observe the appropriate level of solution for plant growth (static condition).

5.2.6 Since there were only 2 experimental species in this study, it would be more useful if a number of plant species was investigated to find the best of bioindicator.

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APPENDICES

APPENDIX A

Prepared Nutrient solution

Prepared nutrient solution concentration (modify recipe for full strength Hoagland's solution) (Hoagland et.al., 1950)

Chemical	Formular	g/l	Concentration	Amount (ml) in nutrient Solution 1 L
Macronutrient:				
1.Potassium dihydrogen phosphate	KH_2PO_4	136.09	1M	1
2. Potassium nitrate	KNO_3	101.10	1M	5
3. Calcium nitrate	$Ca(NO_3)_2$	164.10	1M	5
4. Magnesium sulfate	$MgSO_4$	120.39	1M	2
Micronutrient*:				
1. Boric acid	H_3BO_3	2.86	0.5mg,B/ml	1
2. Manganese chloride tetrahydrate	$MnCl_2.4H_2O$	1.81	0.5mg,Mn/ml	
3. Zinc sulfate heptahydrate	$ZnSO_4.7\ H_2O$	0.22	0.05mg,Zn/ml	
4. Copper sulfate pentahydrate	$CuSO_4.5H_2O$	0.08	0.02mg,Cu/ml	
5. Molybdic acid monohydrate	$H_2MoO_4.H_2O$	0.02	0.01mg,Mo/ml	
Fe-EDTA** (Fe 5 mg/ml)				1

Note : *Chemicals mixing (1-5) dissolved in the liquor 1,000 ml vacuum micronutrient approx 1 ml dilute the liquor 1,000 ml

** Prepared by the Dissolved disodium ethylenediaminetetraacetate ($Na_2C_{10}H_{14}O_8N_2.H_2O$) number 2 g in liquor 50 ml from the heated solution Ferric Chloride ($FeCL_3.6H_2O$) number 3.5 g shake to dissolve the salt all together

APPENDIX B

Water quality

Table B-1 Mean and Standard Deviation of solution and room temperature during the experiment

Day	Solution temperature during 9.30-10.00 a.m. (°C)	Room temperature during 9.30-10.00 p.m. (°C)	Solution temperature during 14.30-15.00 a.m. (°C)	Room temperature during 14.30-15.00 p.m. (°C)
1	25	30	27.3	30.2
2	25	28.8	28.5	30.5
3	24.8	29.1	28.5	31
4	25	29	26.8	30
5	23.8	27.8	25.1	27
6	24.1	28	25.1	27
7	24.1	27.5	26.2	30
8	25.3	28	26.8	31
9	23.5	28.5	27.3	32
10	25.7	27.5	26.1	31.1
11	25.5	28	27	31.5
12	26.4	29.5	27.5	30.5
Mean	24.85	28.48	26.85	30.33
Minimum	23.5	27.5	25.1	7
Maximum	26.4	30	28.5	32
S.D.	0.85	0.81	1.10	1.29

Table B-2 Mean and Standard Deviation of pH value of the solution during the experiment

Unit	pH (Mean ±S.D.)		
	Control	Set1	Set2
L0	7.46±0	7.51±0	7.46±0
L4	6.96±	6.18±0.71	6.81±0.02
L8	6.88±0.04	6.6±0.21	6.82±0.08
L12	6.82±0.2	7.59±1.26	6.45±0.07
S0	7.46±0	7.51±0	7.46±0
S4	6.97±0.02	6.66±0.16	6.93±0.04
S8	7.04±0.37	6.41±0.27	7.63±0.22
S12	6.58±0.02	6.98±0.46	7.02±0.35

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively.

Table B-3 Mean and Standard Deviation of salinity of the solution during the experiment.

Experimental Unit	Salinity (Mean±S.D. in ppt.)		
	Control	Set1	Set2
L0	0.13±0	0.13±0	0.12±0
L4	0.13±0	0.14±0	0.12±0
L8	0.13±0	0.13±0.01	0.14±0.01
L12	0.13±0.01	0.14±0	0.1±0
S0	0.13±0	0.13±0	0.12±0
S4	0.13±0	0.14±0	0.12±0
S8	0.13±0	0.13±0.01	0.13±0
S12	0.14±0.01	0.14±0.01	0.1±0

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and12 represent day 0, 4, 8 and 12, respectively

Table B-4 Mean and Standard Deviation of chlorophyll a content of the solution during the experiment.

Experimental Unit	Chlorophyll a (Mean±S.D. in mg/m^3)		
	Control	Set1	Set2
L0	0±0	0±0	0±0
L4	0.01±0.002	0.02±0.004	0.003±0.001
L8	0.02±0.004	0.04±0.01	0.01±0.01
L12	0.02±0.01	0.08±0.04	0.04±0.004
S0	0±0	0±0	0±0
S4	0.029±0	0.01±0.003	0.01±0.001
S8	0.004±0.02	0.03±0.003	0.02±0.001
S12	0.054±0.03	0.04±0.02	0.03±0.01

Note : L is common duckweed, S is giant duckweed, the number 0, 4, 8 and 12 represent day 0, 4, 8 and 12, respectively.

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