EFFECTS OF MIX CULTURE AND MONOCULTURE OF THREE FRESHWATER FISHES SPECIES ON GREEN WATER PRODUCTION

CHUNG YUH FEI

DISSERTATION SUBMITTED AS PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR BACHELOR OF SCIENCE (HONOURS)

AQUACULTURE PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

April 2007

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: Effects of Mix Culture and Monoculture of Three freshwater fishes species on Green Water production.

Ijazah: Bachelor of Science ~~(Honours)~~ (Honours)

SESI PENGAJIAN: 2007.

Saya CHUNG YUH FEI.

(HURUF BESAR)

mengaku membenarkan tesis (LPS/Sarjana/Doktor Falsafah)* ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
[/]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[ ]	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 51, Lrg 1A King fisher · phase 2.

Nama Penyelia

Tarikh: 16/04.

Tarikh:

CATATAN: * Potong yang tidak berkenaan.

** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I declare that this dissertation is the result of my own independent work, except where otherwise stated.

April 2007

CHUNG YUH FEI
HS2004-3116

AUTHENTICATION

AUTHENTICATION BY MEMBERS OF DISSERTATION COMMITTEE

Signature

1. SUPERVISOR

(Dr. Sujjat Al Azad)

2. CO-SUPERVISOR

(Dr. Sitti Raehanah Binti Muhamad Shaleh)

3. EXAMINER 1

(Mr. Muhammad Ali Syed Hussien)

3. EXAMINER 2

(Mr. Kennedy Aaron Aguol)

4. DEAN

(SUPT/KS Assoc. Prof. Dr. Shariff A. K. Omang)

ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge Prof Saleem Mustafa, the Director of Borneo Marine Research Institute, UMS for his encouragement and support in this completion of this dissertation. I would like to extend my deepest gratitude to my supervisor, Dr. Sujjat al-Azad for his relentless effort and motivation which propelled me to complete my dissertation. I would like to thank Dr. Sitti Raehanah Muhamad Shaleh for her suggestion and motivation which guide me to complete my dissertation. I would like to thank Prof. Dr. Shigeharu Senoo, Mr. Kennedy Aaron Aguol and Mr. Muhammad Ali Syed Hussein for all his help, comments and suggestion on the completion of this dissertation. I would also like to thank Mr. Norazmi Osman and all staffs of UMS Hatchery for their support to provide me the facilities to complete the experiments that conducted in Hatchery.

I would like to thank Ms. Mok Wan Jye who had help and supported me. I am also greatly in debt for Mr. Ho Teck Yung, Ms Liew Sit Fun, Mr. Tan Chit Dah, Mr. Andrew Hee Chun Foon and Mr. Stanley Wong Vun Yee who helped and supported me in this dissertation. Last but not least I would like to thank for my course mates helped me and guide me to complete this dissertation.

ABSTRACT

This study was conducted to produce green water using different culture method with different fish species. This study compared the green water production that been produced by using either freshwater fish mix culture and mono culture. The targeted freshwater fish species are Red tilapia, African catfish and Common carp with starting green water concentration 0.05 million cells per milliliter. Mix culture contented red tilapia, African catfish and common carp as a unit. Red tilapia, African catfish and common carp also cultured separately as mono culture. The results show that the mix culture (2.20 million cells per mL) had the highest green water production, in mean values. However, red tilapia culture (1.76 million cells per mL) was the highest green water production among the mono culture method. Meanwhile, the lowest means value of phytoplankton were from the tank of African Catfish. This study suggested that mix culture with low feeding ratio was the best quantity producer in green water. Meanwhile, tilapia culture can be as alternative culture method to produce green water as in mono culture method.

ABSTRAK

Tesis ini telah dikendalikan untuk menghasilkan air hijau dengan menggunakan kaedah kultur berbeza dengan spesies ikan yang berbeza. Kajian ini membandingkan kaedah kulturan sama ada kulturan bersama atau mono kultur dari segi penghasilan kuantit air hijau yang berkualiti baik dan paling banyak. Ikan air tawar yang dipilih sebagai bahan kajian adalah ikan tilapia merah, ikan keli dan ikan karp biasa dengan permulaan kepekatan air hijau sebanyak 0.05 juta sel per mL. Kaedah kulturan bersama mengandungi ikan tilapia merah, ikan keli dan ikan karp biasa. Pada masa yang sama, ikan-ikan tersebut juga dikultur secara berbeza sebagai mono kultur. Keputusan menunjukan bahawa kaedah kulturan bersama (2.20 juta sel per mL) menghasilkan kuantiti air hijau yang paling banyak secara keseluruhan. Manakala, kaedah mono kultur ikan tilapia merah (1.76 juta sel per mL) menghasilkan kuantiti air hijau yang paling banyak dibandingkan dengan mono kultur ikan karp biasa (1.40 juta sel per mL) dan ikan keli (0.91 juta sel per mL). Kajian ini mencadangkan bahawa pengkulturan merupakan kaedah yang paling baik kerana dapat menghasilkan air hijau yang banyak dan baik. Manakala, kaedah mono kultur tilapia merah merupakan kaedah yang paling baik untuk menghasilkan air hijau yang banyak dan baik, jika dibandingkan dengan kaedah mono kultur spesis ikan air tawar yang lain.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF ABBREVIATIONS

km	Kilometer
km^2	Kilometer per square
cm	Centimeter
μm	micrometer
g	gram
°C	degree centigrade
%	percentage
mL	milliliter
L	liter
UMS	Universiti Malaysia Sabah
h	hour
mg/L	milligram per liter
DO	dissolved oxygen

Chapter 1

Introduction

1.1 Aquaculture and Malaysia

Aquaculture is the cultivation of the natural produce of water such as aquatic organism and others. This cultivation is a combination of science, art and business. Malaysia has advantages in doing aquaculture. Basically the advantages can be grouped into 2 groups, geographical advantages and social advantages. Malaysia has 23 million populations. This country has total area 329,758km^2 and 4,400km of shorelines. Malaysia yearly temperature ranging is around 30°C and total annual rainfall averages is around 200 cm.

Aquaculture has an important role as a source of food and protein (Fisheries Department, 2003). The total production of aquaculture in year 2002 is 191,843 tones output, this amount increase in the following year, 2003 of 196,874 tones or 2.63% over 2002. Freshwater aquaculture production in year 2003 is 26.0% of the total production or

51,189 tones. The three major species in freshwater aquaculture are Red Tilapia, Catfish and River Catfish, (Gulf States Marine Fisheries Commission, 2004).

1.2 Green water

Green water containing green algae, phytoplankton, which is a result of algal bloom. Experiments have shown that neither marine copepod nor freshwater cladoceran absolutely needs algae as food, (Sanders *et al.* 1996), suggesting that algae possess desirable qualities as zooplankton food. These marine or freshwater zooplanktons are important as first feed for larvae. So, the essential nutrient content in the zooplanktons have to be considered, because the nutrient will be absorbed by the larvae, shrimp, prawn or bivalve larvae.

Most aquaculturists prefer to produce green algae such as *Chlorella* sp. and *Nannochlopsis* sp. Pure culture of these species are very costly and needs extra care to prevent contamination from zooplanktons such as rotifer and ciliates. Besides, the pure culture needs extra space, because more surface area, means can receive more extra sunlight for green water activity. Also transparent tanks are more suitable for the culture as light can pass through the media.

1.3 Problems in culturing green water

Green water is produced either by using photobioreactor system or outdoor culture system. The outdoor culture systems are open air and easily to be contaminated. Photobioreactor system is more commonly used to define a closed system. This system is controlled parameter (carbon dioxide, water and light). Closed system can produce the green water that low percentages of contaminations, but this system are very costly and using an extra space and equipments. Therefore, most of the aquaculturists choose to use the outdoor culture system as green water supplement. Outdoor open culture system is considered as inexpensive green water culture system (Zmora and Ricmond, 2004) and produces a low cell density batch culture, few million cells per milliliter. However, outdoor system is easily contaminated by zooplankton such as rotifer and ciliates, that always been complained by aquaculturists. So, it requires good management practice to produce green water with out contamination by zooplankton. This study will conducted for produce green water in outdoor system.

1.4 Objectives

The main objective of this experiment was to produce green water effectively. Others objective are:

1. to determine how to produce higher green water cells/mL with different culture method.
2. to observe the best condition for green water growth.

Chapter 2

Literature Review

Freshwater green water usually refers to phytoplankton, free-floating and suspended plant and green micro algae from *Chlorella* of trebouxiophyceae class and chlorophyta of chlorophyceae class (Tomaselli, 2004). They present differently in shapes and forms, such as colonies, unicells (usually big sizes), filaments, free-swimming unicellular species, and others.

2.1 Green water in aquaculture

Many aquatic animals cannot synthesize certain long-chain fatty acids in high quantities high enough for growth and survive. Thus, most of the animals are depends upon algal food to supply the needs. Nontoxic freshwater and marine represent as primary food source for at least some stage in the life of most cultivated aquatic animals. Sanders. *et al*, (1996), suggested that algae possess desirable qualities as zooplankton food.

2.1.1 Green water growth and development

Growth and reproduction are important in each living organism, to ensure that their species are remains after their death. Growth defined as increase in size or value, the numbers of the phytoplankton. Yet, size affects more than just phytoplankton growth rates. Small cells can respond to a pulse of nutrients with a rapid burst of growth. Large cells, however, can take up and stored nutrients such as phosphate than can smalls cells (Sommer, 1985).

The growth and development of green water (phytoplankton) are relay on the energy that remains in the phytoplankton. This energy was produce through photosynthesis activities. When the energy is no longer cannot afford the phytoplankton cells maintenance or recovery, the phytoplankton growth would start declined and died as a result. But, some species will remain in dormant stage until favorable condition and thus, the phytoplankton will growth and reproduce again for new generation (Tomaselli, 2004).

2.2 Photosynthesis

Photosynthesis generally, is the synthesis of glucose from sunlight, carbon dioxides, and water. The byproduct of photosynthesis is oxygen that it is arguably the most important biochemical pathway known; nearly all life depends on it. Photosynthesis occurs in two stages. In the first phase light-dependent reactions or photosynthetic reactions (also called the Light reactions) capture the energy of light and use it to make high-energy molecules. During the second phase, the light-independent reactions (also called the Calvin-Benson cycle, and formerly known as the Dark Reactions) use the high-energy molecules to capture carbon dioxides (CO_2) and make the precursors of glucose (McConnaughey, 1994).

2.3 Green water pH and nutrients requirement

Green water photosynthesis influenced by certain factors, chemically or physically. Chemically such as pH, ammonium, nitrite, nitrate, phosphorus and iron while physically are dissolved oxygen, phytoplankton sizes, surface area and water quality. pH may influenced both photosynthesis and respiration, below equation is the equilibrium of carbonic acid system:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ + ATP \leftrightarrow H^+ + CO_3^-$$

From the equation above, carbon dioxides will removed due to photosynthesis and thus causing a shift to the left. Meanwhile, respiration which will release carbon dioxides and causing the equilibrium shift to the right. pH may increase in the morning due to photosynthesis activities (Dawes, 1981), when in the phase light-dependent reactions. pH decline during the night time or phase light-independent reactions, because of the respiration activities at night which releases CO_2 (Becker, 2004).

Phosphorus and nitrogen playing important role as nutrient requirement for photosynthesis plant growth (Dawes, 1981), even both elements appear in low concentration. Combined nitrogen is required by algae to manufacture amino acids, chlorophyll, and other nitrogen-containing organic compounds. Inorganic sources of combined nitrogen include ammonium and nitrate, but most of the algae usually are prefer on ammonium because can be used in a more direct as ion than nitrate in the biosynthesis of amino acid (Figure 2.1).

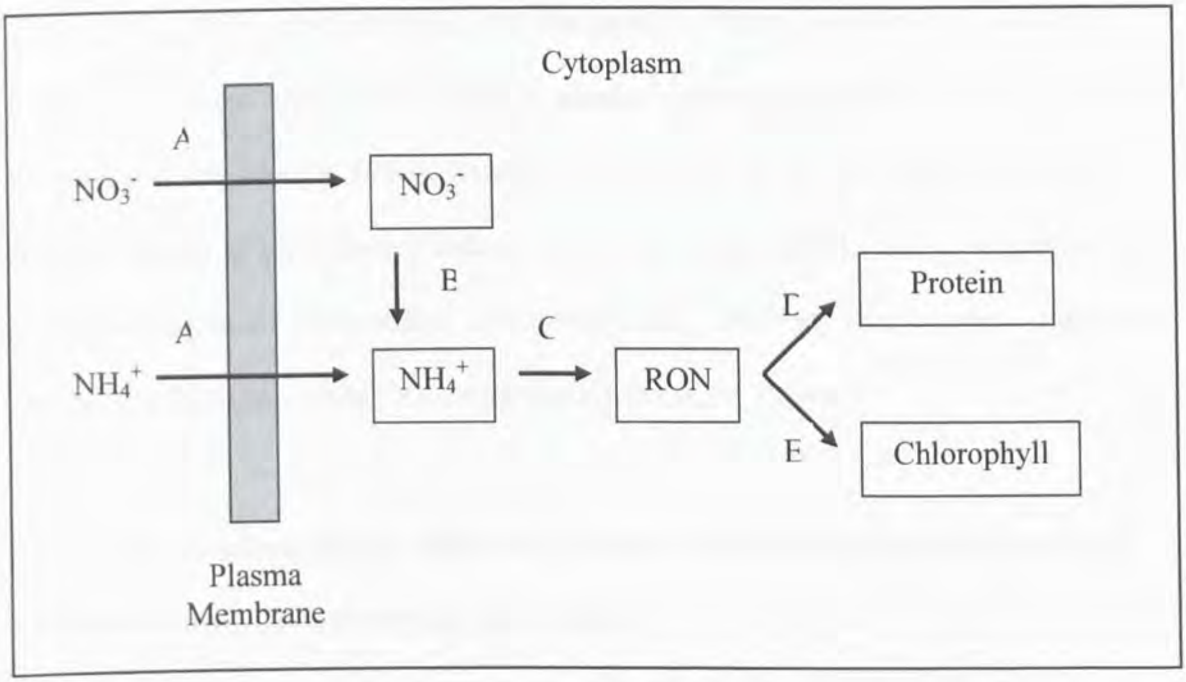

Figure 2.1: Comparison of algae cell utilization of ammonium and nitrate intake

As been shown at figure above, nitrogen is used to produce protein and chlorophyll, which chlorophyll is used for as photosynthesis agent. Algae uptake ammonium and nitrate by using membrane transport system (A). Ammonium can be directly converted to become reduced organic nitrogen (RON). In the other hand, nitrate utilization requires an additional enzymatic step (B) before changed to RON by enzyme system. This step needs and dependent on iron availability and molybdenum as cofactors. After utilized become ammonium, then it will utilized and resulting as protein and chlorophyll (C, D and E) (Grobbelaar, 2004).

Phosphorus is for growth and others cellular process, example deoxyribonucleic acid (DNA), biosynthesis of nucleic acids, energy as adenosine triphosphate (ATP), phospholipids, etc. The preferred form in which it is supplied to algae is as orthophosphate (PO_4^{2-}) and its uptake is energy dependent. Although algal biomass contains less 1% phosphorus (P), but it is one of the most important growth limiting factors in algae biotechnology. Due to P easily bound with other ions (e.g. iron) resulting in its precipitation and consequently rendering this essential nutrient unavailable for algae uptake, it is important (Grobbelaar, 2004).

2.4 Tilapia (*Oreochromis niloticus*), African Catfish (*Clarias gariepinus*) and Common Carp (*Cyprinus carpio*) mix culture

Catfish ponds are ideals ecosystems for polyculture because catfish is a manufactured and omnivores feed. This natural productivity is largely unused by catfish and could support substantial production of omnivore or herbivore fish or crustaceans. Mix culture between tilapia (plankton-feeder fish) can improve catfish pond water quality by altering plankton community dynamics (Boyd and Tucker, 1998). Tilapia in catfish pond is been used avoid environment-derived off-flavors and to control aquatic weeds, such as phytoplankton (Boyd and Tucker, 1998).

Both carp and tilapia were amongst the earliest freshwater fish to be cultivated in the history of aquaculture. Carp also have been mix with other warm water species fish in an attempt to achieve even higher yields in the studies that had been conducted before (Michaels, 1988). Those warm-water fish that been chosen for these studies mainly catfish and tilapia. In the same time, others warm-water species fish are also being considered and the study still in the progress. Nevertheless, tilapia shown the preferred sign to being more suitable cultured for polyculture or mix culture with carp (Dadzie, 1982). Common carp is a bottom feeder meanwhile tilapia is an omnivore feeder (mainly feed on plankton). Until today, there is no research on the combination on this three species of fish, mix culture of this three species together. Because most of the combination culture that been studies is either tilapia with common carp or tilapia with catfish.

2.5 Fish feces as fertilizer

Fish feces or waste react as fertilizer, as horse and chicken waste in agriculture, in aquaculture field because of the waste product is ammonia. This ammonia occurs either in ionized (NH_4^+) or un-ionized (NH_3) form, which are depending on the pH value of the water (Parker, 2002). Fish waste, which is ammonia resources, is vital as the importance of ammonium that been discussed before.

Chapter 3

Methods and Materials

This experiment was conducted at Borneo Marine Research Institute of Universiti Malaysia Sabah. The experiment was repeated 2 times within 9 to 14 days at the hatchery of Borneo Marine Research Institute. Green water was produced by monoculture and mix culture of 3 freshwater species. These species are Red Tilapia, *Oreochromis niloticus*, African Catfish, *Clarias gariepinus*, and Common Carp, *Cyprinus carpio* in outdoor culture system. Every morning and evening, the algae counting and observation on the culture system was done.

3.1 Tank and green water preparation

Twelve tanks of 700L was used for reared the fishes and to produce the green water for this experiment. The tanks was washed and dried for 2 days before start to fill with stocking freshwater. After 2 days, freshwater from stocking tanks filled to the tanks and checked the chlorine concentration to ensure no chlorine contamination. The chlorine will influence the green water growth and development. Each tank is connected to the aeration tube with one aeration stone with control for enough dissolved oxygen supplied. Tanks was be labeled randomly with R1 to R3 for Red

Tilapia, A1 to A3 for African Catfish, C1 to C3 for Common Carp and M1 to M3 for mix culture of three of the species above. Each treatment (represented R, A, C and M) have three replicates (represented as 1,2 and 3).

Photo 3.1: An opaque 700L tank for more surface area.

The green water resource is from tank of UMS hatchery freshwater tank known as D6. Below is the calculation that used for pumped the amount of green water into the 12 tanks. The green water was filtered by 40μm net for prevent contaminated by zooplankton such as rotifer and ciliates. Each tank starts with 50,000 ± 10,000 cells mil/ml of green water and sediment. The starting cells density was calculated by using equation below:

Green water cell density determination:

$$D_e(V_e) = D_{gw}(V_{gw})$$

With;

D_e = Wanted cells density (50,000 cells/ml)

V_e = Total volume for experiment (400L)

D_{gw} = Density of green water from D6 tank

V_{gw} = Volume of green water needed

After pumped in the green water from D6 tank, phytoplankton sp. was counted to ensure the amount in each tank are in the range of 50,000 ± 10,000 cells/ml as starting point. Fishes were stocked into the tanks after ensure that each tanks cells/ml are in the range of 50,000 ± 10,000 cells/ml.

REFERENCES

Becker, W., 2004. Microalgae for aquaculture; nutritional values of microalgae for aquaculture. In :(editor) Amos Richmond. *Hand book of micro algae; biotechnology and applied phycology*. Black Well Publishing, Australia.

Boyd, C. E., Tucker C. S., 1998. Pages 1-371 in C.E. Boyd and C.S. Tucker: Water Quality Requirements. *Pond Aquaculture Water Quality Management.* Kluwer Acedemic Publisher, Norwell, Massachusetts, USA.

Dadzie, S., 1982. Species combination in tilapia culture. *Aquaculture*, **27**, 295-299.

Dawes, C. J., 1981. *Marine botany*. John Wiley & SonInc, United States.

Grobbelaar, J. U., 2004. Mineral nutrition. In :(editor) Amos Richmond. *Hand book of micro algae; biotechnology and applied phycology*. Black Well Publishing, Australia.

Gulf States Marine Fisheries Commission, 2003. http//www.gsmfc.org/.

Gulf States Marine Fisheries Commission, 2004. http//www.gsmfc.org/.

Jakobsen, S. T., 1994. Aerobic decomposition of organic wastes I. Stoichiometric calculation of air change. *Resources, Conservation and Recycling*, **12**, 165-175.

Frei, M., Razzak M.A., Hossain M.M., Oehme M., Dewan S. and Becker K., 2007. Performance of common carp, *Cyprinus carpio* L. and Nile tilapia, *Oreochromis niloticus* (L.) in integrated rice–fish culture in Bangladesh. *Aquaculture*. **262**, 250-259.

Masojidek, J., Koblizek M. and Torzillo G., 2004. Photosynthesis in microalgae. In :(editor) Amos Richmond. *Hand book of micro algae; biotechnology and applied phycology*. Black Well Publishing, Australia.

McConnaughey, T. A., 1994. Calcification, photosynthesis and global carbon cycles. *Bulletin de l'Institut Oceanographique*, Monaco 13: 137 – 161.

Michaels, V.K., 1988. Carp farming. Fishing News Book Ltd, Surrey, England.

Moriarty, D. J. W., 1997. The role of microorganisms in aquaculture ponds. Aquaculture. **151**, 333-349.

Naegel, L. C. A., 1977. Combined production of fish and plants in recirculating water. *Aquaculture*. **10**, 17-24.

Parker, R. 2002. *Aquaculture science. Second Edition.* Demler Thomsom Learning, new York.

Ritvo, G., Kochba M. and Avnimelech Y., 2004. The effects of common carp bioturbation on fishpond bottom soil. *Aquaculture*. **242**, 345-356.

Sanders, R. W., Williamson C. E., Stutzman P. L., Moeller R. E., Goulden C. E., and Aoki-Goldsmith T.. 1996. Reproductive success of"herbivore" zooplankton fed algae and nonalgal food resource. *Limnology and Oceanography*. **41**, 1295-1305.

Sommer, U. 1985. Comparison between steady state and non-steady state competition: Experiments with natural phytoplankton. *Limnology and Oceanography*. 30, 335-346.

Stickney, R.R., 2000. *Encyclopedia of Aquaculture*. John W. & Sons. Canada.

Toko, I., Fiogbe E. D., Koukpode B. and Kestemont P.. 2007. Rearing of African catfish (*Clarias gariepinus*) and vundu catfish (*Heterobranchus longifilis*) in traditional fish ponds (whedos): Effect of stocking density on growth, production and body composition. *Aquaculture*. **262**, 65-72.

Tomaselli, L., 2004. The Microalgal cell. In :(editor) Amos Richmond. *Hand book of micro algae; biotechnology and applied phycology*. Black Well Publishing, Australia.

Turker, H., Eversole A. G. and Brune D. E., 2003. Comparative Nile tilapia and silver carp filtration rates of Partitioned Aquaculture System Phytoplankton, *Aquaculture*. **220**, 449-457

Zmora, O. and Ricmond, A., 2004. Microalgae for aquaculture: microalgae production for aquaculture. In :(editor) Amos Richmond. *Hand book of micro algae; biotechnology and applied phycology*. Black Well Publishing, Australia.