

Short Communication

Comparison of *Nannochloropsis oculata* Productions Cultivated in Two Different Systems: Outdoor Red Tilapia (*Oreochromis* sp.) Culture Tank and Indoor Pure Culture

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ABSTRACT

Production of *Nannochloropsis oculata* or a marine eukaryotic unicellular phytoplankton was the focus of this study. The cultivation of outdoor red tilapia (*Oreochromis* sp.) in the tank and indoor was compared in producing phytoplankton. Initial density of *N. oculata* for both culture systems was 0.5×10^6 cell/ml. Findings showed the highest density of *N. oculata* was attained from tilapia culture system at 9.6×10^6 cell/ml harvested at day 7 while in pure culture system was 8.5×10^6 cell/ml harvested at day 4. Contamination was dominated by protozoa (*Gymnodinium* sp.), range of $4.80-36.67 \times 10^3$ individual cells/ml and $0.00-41.10 \times 10^3$ individual cell/ml at both tilapia culture and pure culture systems respectively. Levels of ammonium, nitrite and nitrate in tilapia culture systems had significantly lower ($P < 0.05$) concentration. In contrast, total bacteria including vibrio yellow colonies showed higher concentration in tilapia culture system but remained insignificant ($P > 0.05$) for vibrio green colonies in both systems. This study concluded Tilapia culture system is as effective as pure culture system to produce *N. oculata* based on production and quality.

Keywords: *Nannochloropsis oculata*, pure culture system, tilapia culture system

ARTICLE INFO

Article history:

Received: 06 November 2017

Accepted: 25 April 2018

Published: 29 August 2018

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INTRODUCTION

Nannochloropsis oculata is a marine microalga from class Eustigmatophyceae (Hibberd, 1981) with diameter size of 2-4 µm (Rodolfi, Zittelli, Barsanti, Rosati, & Tredici, 2002). *N. oculata* has been long considered as one of the most important algae species in marine aquaculture industry particularly owing to its numerous nutritional values including eicosapentaenoic acid (EPA) (Sukenik, 1999), high lipid content (Fabregas, Maseda, Domínguez, & Otero, 2004) and some natural pigments (Lubián, Montero, Moreno-Garrido, Huertas, & Sobrino, 2000). Common cultivation of *N. oculata* in most of the marine fish and crustacean hatcheries is by initially culturing the seed in the laboratory through series of seed upscale from agar to flask, and subsequently mass production will be carried out outdoor. However, there are many constraints and challenges in mass production of *N. oculata* at the early stage (indoor laboratory phase) due to the requirement of specific culturing technique and often involving high production cost. Culture medium for *N. oculata* utilises natural or artificial seawater fertilised with macro and micronutrients based on formulation of F2 medium (Guillard, 1975) or Conway medium (Walne, 1979). Till data, an effective yet economical fertiliser medium is yet to be developed (Bae & Hur, 2011; El Nabris, 2012; Fabregas, Toribio, Abalde, Cabezas, & Herrero, 1987). However, undoubtedly pure culture of *N. oculata* is still significant as it serves as the

primary source of seed to initiate mass scale production through an outdoor culturing system.

This study proposed an alternative method of culturing *N. oculata* by using green water from red tilapia (*Oreochromis* sp.) culture system as a fertiliser instead of conventional mediums and fertiliser. Red tilapia was chosen because it is cheap, easy to be cultured and it available all year round. It also has been reported that red tilapia can promote the production of phytoplankton in the ponds or lake by recycling nutrient through excretion (Elser, Marzolf, & Goldman, 1990; McQueen, Post, & Mills, 1986). Nitrogenous organic waste comes from uneaten feeds and excretion of fishes are used by microalgae as nutrients for growth. In a previous study, *Chlorella* sp., a freshwater microalga has been successfully produced in red tilapia culture system (Matsubara, 2011). The objective of this study was to compare the production and quality of *N. oculata* cultured under two different systems (pure culture and tilapia culture system).

MATERIALS AND METHODS

This experiment was conducted at Marine fish Hatchery of PT Suri Tani Pemuka in Pemuteran, Bali, Indonesia in 2015. Two different culturing systems of *N. oculata* were prepared and compared, namely: (1) pure culture; and (2) red tilapia culture. *N. oculata* seed was obtained from Gondol Research Station for Coastal Fisheries Bali, Indonesia. In pure culture system, *N. oculata*

seed was initially cultured in laboratory and fertilised using f/2 medium (Guillard, 1975) and later upscaled from 100 ml flask into 18 gallons. Next, the seeds were gradually transferred into a 10 tonne tank made of high-density polyethylene (HDPE) placed at the outdoor area and fertilised with agricultural grade fertiliser. The water quality was maintained as follows: dissolved oxygen, DO (6.5 to 7.5 mgL⁻¹), pH (7.5 to 8.0) and temperature (28 to 32°C). Tanks were filled with 20 ppt saline water up to 800 litres and aerated evenly. Tanks were left uncovered to allow sunlight penetration for photosynthesis to take place. Light intensity ranged from 10,000 – 12,000 lux (light meter 840020, Sper Scientific, USA). The seeds were further fertilised using local improved formula of agricultural grade fertiliser (PT. Andalan Chemist, Kalimantan Timur, Indonesia). Three of one tonne tank were used as experimental tank and the initial seed of *N. oculata* was introduced at 1.0×10^6 cells/mL. Similar to pure culture system, three 1 tonne HDPE tank were used as experimental tank and the initial seed of *N. oculata* at 1.0×10^6 cells/m were introduced. Red tilapia. (Total length (TL): 10.00 ± 0.61 cm, body weight (BW): 18.0 ± 1.3 g) were stocked at 1.5 kg/tonne and fed commercial pellet at 1% of their body weight twice daily. The water quality was maintained in pure culture method. Analyses of *Ex-situ* parameters were performed on Nitrite (NO₂), Nitrate (NO₃) and Ammonium (NH₄) using Aquamerck water test kits respectively. A total of three samples of

1ml water were sampled for water quality analyses for 14 days. *N. oculata* density and contamination counts were performed by means of digital images obtained through an inverted microscope (40×) (Nikon, Eclipse E600, Japan). To calculate density of *N. oculata*, one ml of sample from both cultures were pipetted on to a Neubauer chamber (haemocytometer) placed under a light microscope, and cells were counted at 10x magnification and calculated by equation of density= count/4 x (dilution) x 10,000. Meanwhile, for contamination analysis, 5 ml of 10% formalin was added into 30 ml water sample taken from both systems in order to minimise protozoa movement for counting and identification purpose. Bacterial count was done using Tryptic Soy Agar (TSA) for total bacterial content and Thiosulfate Citrate Bile Sucrose Agar (TCBS) Water sample from both systems taken and cultured in 37°C for 24 hours in incubator and colonies that visible in the agar plate were counted (Kobayashi, Enomato, Sakazaki, & Kuwahara, 1963). All the quantitative data from this study were analysed using a non-parametric Mann-Whitney Test.

RESULTS

The findings revealed the highest density of *N. oculata* in red tilapia culture system and pure culture system were obtained at day 7 (9.6×10^6 cell/mL) and day 4 (8.5×10^6 cell/mL) respectively. From day 5 onwards, the density of *N. oculata* in red tilapia culture system remained significantly higher

($P < 0.05$) until the end of experiment. Contamination was dominated by protozoa (*Gymnodinium* sp.) in the range of $4.80 - 36.67 \times 10^3$ cell/mL and $0.02 - 41.10 \times 10^3$ cell/mL in both red tilapia culture and pure culture systems respectively. Contamination was first detected in red tilapia culture system and pure culture system at day 1 and 3 respectively, and their densities fluctuated until the end of experiment.

There was no significant difference in terms of water quality parameters between both systems. The DO and pH recorded in red tilapia culture system and pure culture system were $6.78 - 10.33$ mg/L and $7.80 - 10.37$ mg/L, and $7.9 - 8.2$ and $8.07 - 8.53$ respectively. A good control of water quality is a critical factor for higher yields of *N. oculata* in both systems. In Tilapia culture system, *N. oculata* culture did not crash and this revealed a balanced 'interaction' between nutrients, oxygen and pH that allow its growth. In contracts, *ex-situ* findings revealed the concentrations of ammonium in pure culture system ($1 - 10$ mg/L) was significantly higher ($P < 0.05$) compared with red tilapia culture system ($0.17 - 2$ mg/L). Ammonium concentration was extremely low in red tilapia culture system from day 4, but it took 12 days in pure culture system to reach lower concentration. Meanwhile, level of nitrate in pure culture system ($1.00 - 4.00$ mg/L) was significantly higher ($P < 0.05$) compared with red tilapia culture system ($2.67 - 15.00$ mg/L). The level of nitrite in pure culture system (0.12

$- 0.17$ mg/L) was significantly higher ($P < 0.05$) compared with red tilapia culture system ($0.01 - 0.33$ mg/L).

DISCUSSION

Red tilapia culture system has often been associated with the production of quality 'green water' for microalgae propagation in aquaculture pond (Carmen et al., 2007) and this is consistent with the findings of this study. Commercial feed given to red tilapia in this study was considered the main source of nutrients in the culture system as it provides additional nutrient for phytoplankton growth (Jin, Chang, Ji, & James, 2011). Although pure culture system had provided similar nutrient for *N. oculata* growth, microalga culture, balance abiotic and biotic elements such as water quality, nutrients, a light source, aeration and mixing are critical to ensure a satisfactory yield (Creswell, 2010), which was seen limited in pure culture system. Undoubtedly, nutrients in pure culture system were also considered sufficient in this study owing to the addition of nutrient rich of enrichment media, incorporating trace metals, vitamins and several organic and inorganic salt.

Contamination by various microorganisms, such as bacteria, fungi, algae, and protozoa, can affect the growth and quality, and sometimes leading to rapid collapse of the cultures (Ucko et al., 1989), therefore choosing the most ideal microalgae species that are able to tolerate wide range of contaminants and

environmental changes is vital. Although total bacteria count including vibrio yellow and green colonies was relatively higher in red tilapia culture system, there was no significant difference between both systems. A study conducted by Cremen, Martinez-Goss, Corre and Azanza (2017) showed the use of green-water derived from red tilapia culture system not only encouraged the growth of more favourable groups of algae, particularly *N. oculata*, but it also prevented algal collapses and vibriosis.

In the present study, the parameters that contributed to the stability of the *N. oculata* bloom were highly associated with the low N:P ratios and slightly high salinity. The biotic and abiotic factors in the red tilapia culture system were assumed to have provided optimum conditions that favoured the growth of *N. oculata*. The findings are consistent with those of Buttino (1994), affirming that commercial feeds given to red tilapia are the main source of nutrients where nitrogen and phosphorus provide optimum condition for *N. oculata* growth.

CONCLUSION

This study concluded red tilapia culture system can be an alternative means to produce *N. oculata* and it has a significant advantage over pure culture system.

ACKNOWLEDGEMENTS

The authors thank all the staff at the Fish Hatchery, Borneo Marine Research Institute, Universiti Malaysia Sabah and PT Suri

Tani Pemuka in Pemuteran, Bali, Indonesia for their full cooperation and technical assistance. This study was funded by the Exploratory Research Grant Scheme (ERGS0041-1/2013), Ministry of Higher Education Malaysia. **Comparison of *Nannochloropsis oculata* Productions Cultivated in Two Different Systems: Outdoor Red Tilapia (*Oreochromis* sp.) Culture Tank and Indoor Pure Culture.**

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APPENDIX

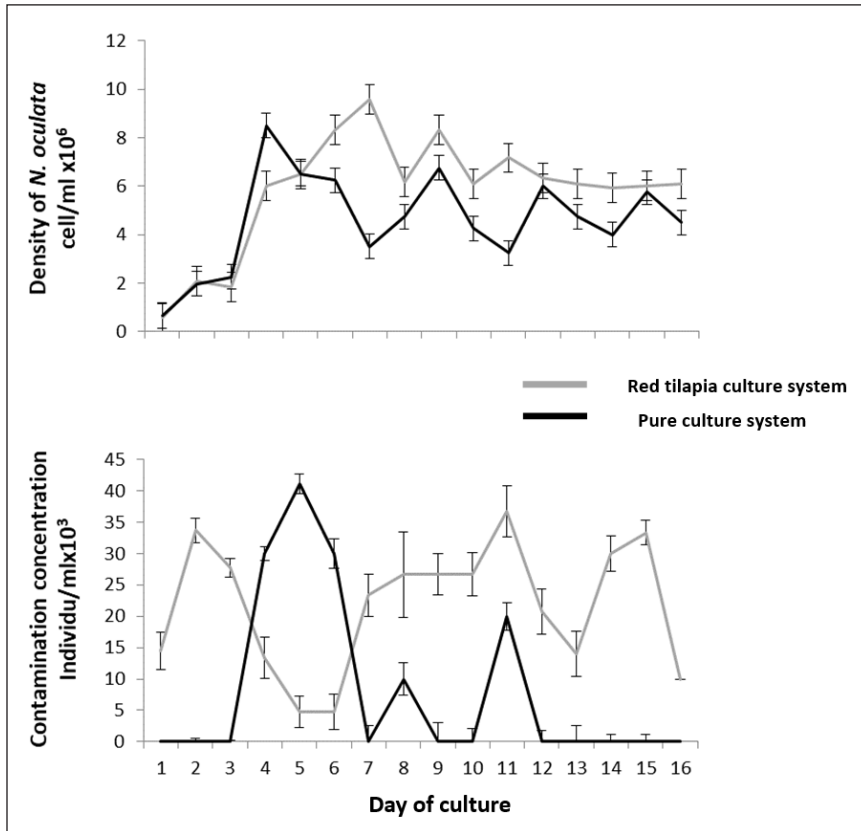


Figure 1. Changes of density and contamination concentration in *N. oculata* cultivated in two different systems. Black and grey lines represent tilapia culture and pure culture systems respectively.

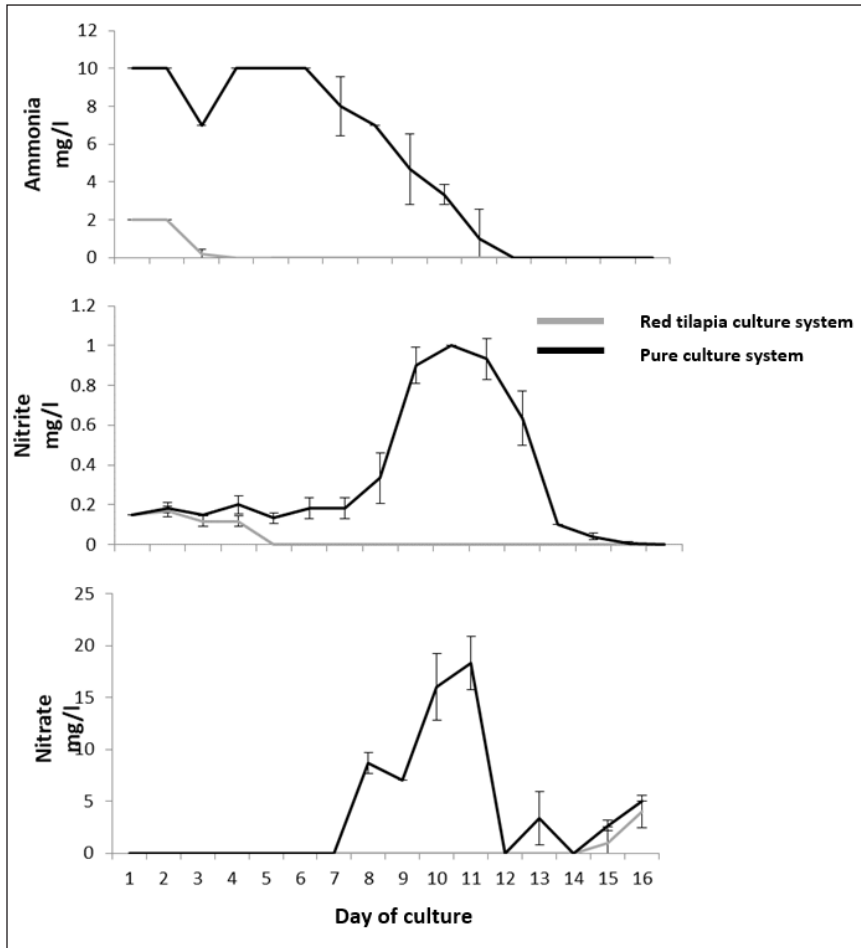


Figure 2 Changes of ammonium, nitrate and nitrite concentration in *N. oculata* cultivated in two different systems. Black and grey lines represent tilapia culture and pure culture systems respectively.

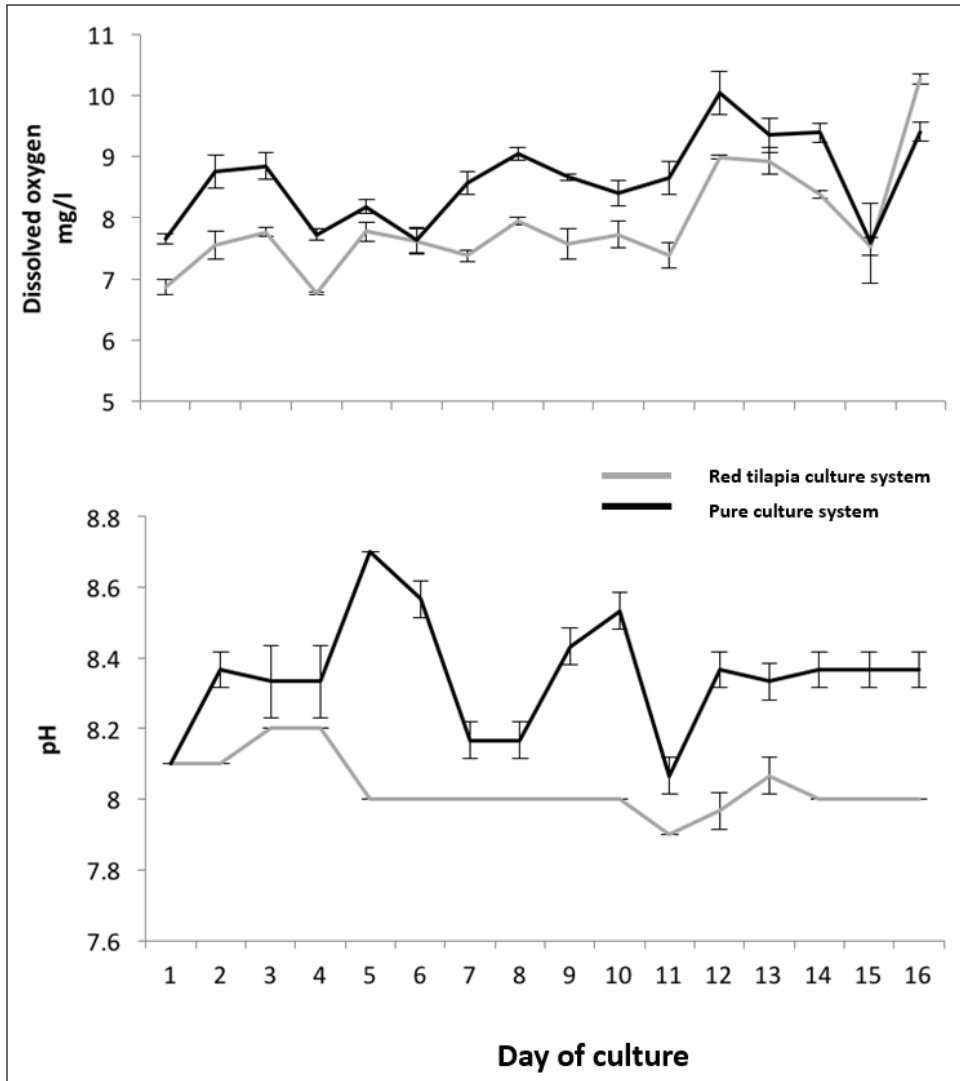


Figure 3 Changes of dissolved oxygen and pH level in *N. oculata* cultivated in two different systems. Black and grey lines represent tilapia culture and pure culture systems respectively.

