MASS PRODUCTION OF ROTIFER USING SELF-PRODUCED CONCENTRATED *NANNOCHLOROPSIS* SP.

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BORNEO MARINE RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH

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MASS PRODUCTION OF ROTIFER USING SELF-PRODUCED CONCENTRATED *NANNOCHLOROPSIS* SP.

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

Mass Production of Rotifer Using Self-produced Concentrated *Nannochloropsis* sp.

Rotifer is the most important live feed in aquaculture, but attempts to produce sufficient quantities continuously with low cost have been unsuccessful. In this study, rotifer culture was tested to be improved by using self-produced concentrated *Nannochloropsis* sp. (SC-Nanno). SC-Nanno was prepared by concentrating *Nannochloropsis* sp. cells from an outdoor culture of *Nannochloropsis* sp. grown in a 20-tonne tank using a water purifier filter Sterapore-PKO. The estimated cost of SC-Nanno was RM 4.51/L. The optimum salinity for rotifer culture was 10 ppt. The effectiveness of using SC-Nanno for rotifer culture was tested by comparing the rotifer production fed with different feed, which were SC-Nanno, commercial concentrated *Nannochloropsis* sp. (CC-Nanno) and baker's yeast. Both frozen SC-Nanno and CC-Nanno were thawed while baker's yeast was mixed with seawater before feeding to the rotifer. The mean rotifer individual density fed with SC-Nanno was higher (578±14 individuals/mL) than those fed with CC-Nanno (520±124 individuals/mL) and baker's yeast (243±21 individuals/mL). Rotifer culture fed with SC-Nanno in different storage conditions namely fresh, chilled and frozen was also studied. The mean rotifer individual density fed with fresh SC-Nanno was higher (796±9 individuals/mL) than those fed with chilled SC-Nanno (663±7 individuals/mL) and frozen SC-Nanno (578±14 individuals/mL). From the results, mass production of rotifer is feasible since SC-Nanno can be produced in hatcheries instead of relying on expensive imported concentrated *Nannochloropsis* sp. Moreover, SC-Nanno can be stored in either chilled or frozen condition.

ABSTRAK

Pengkulturan Rotifer Menggunakan Nannochloropsis sp. Pekat yang Dihasilkan Sendiri

Rotifer adalah sejenis makanan hidup yang penting dalam bidang akuakultur. Namun, usaha untuk menghasilkannya dalam kuantiti yang cukup pada kos yang rendah masih belum berjaya. Dalam kajian ini, pengkulturan rotifer telah diuji dengan menggunakan Nannochloropsis sp. pekat yang dihasilkan sendiri (SC-Nanno). SC-Nanno ini disediakan dengan cara menapis sel-sel Nannochloropsis sp. yang dikultur dalam tangki pengkulturan Nannochloropsis sp. berkapasiti 20 ton dengan menggunakan penapis air Sterapore-PKO. Anggaran kos pengeluaran SC-Nanno adalah sebanyak RM4.51/L. Saliniti yang paling sesuai untuk mengkultur rotifer adalah 10 ppt. Keberkesanan penggunaan SC-Nanno untuk pengkulturan rotifer telah diuji dengan membandingkan pengeluaran rotifer yang diberi makanan yang berbeza iaitu, SC-Nanno, komersial Nannochloropsis sp. pekat (CC-Nanno) dan yis. SC-Nanno dan CC-Nanno yang didingin-bekukan telah dicairkan manakala yis pula dicampur dengan air masin sebelum diberi makan kepada rotifer. Min kepadatan individu rotifer yang dikultur dengan SC-Nanno adalah tinggi (578±14 individu/mL) berbanding dengan CC-Nanno (520±124 individu/mL) dan yis (243±21 individu/mL). Pengkulturan rotifer menggunakan SC-Nanno yang disimpan dalam keadaan berlainan seperti segar, disejukkan dan dingin-beku turut dikaji. Min kepadatan individu rotifer yang dikultur dengan SC-Nanno memberi jumlah tertinggi (796±9 individu/mL) berbanding dengan SC-Nanno yang disejukkan (663±7 individu/mL) dan SC-Nanno yang didingin-bekukan (578±14 individu/mL). Berdasarkan keputusan kajian ini, pengkulturan rotifer secara komersial boleh dijalankan kerana SC-Nanno boleh diperolehi dengan mudah dan berupaya menggantikan penggunaan CC-Nanno yang mahal. Selain daripada itu, SC-Nanno juga boleh disimpan dalam keadaan sejuk atau dingin-beku.

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LIST OF ABBREVIATIONS

%	percentage
am	morning
pm	evening
&	and
ºC	degree Celsius
CC-Nanno	commercial concentrated *Nannochloropsis* sp.
cells/mL	cells per millilitre
cm	centimetre
/day	Per day
DHA	docosahexaenoic acid
DO	dissolved oxygen
eds.	edited, edition, editors
Eggs/mL	eggs per millilitre
EPA	eicosapentaenoic acid
et al.	and others, and the rest
g	gram
HDPE	high density polyethylene
Individuals/mL	individuals per millilitre
kg	kilogram
L	litre
m	metre
m^3	cubic metre
mL	millilitre
mm	millimetre
NPK	Nitrogen: Phosphate: Potassium
pH	hydrogen ion concentration
ppt	part per thousand
RM	Ringgit Malaysia
SD	Standard deviation

SC-Nanno	self-produced concentrated *Nannochloropsis* sp.
SL	salinity levels
sp.	species
µm	Micrometre
UMS	Universiti Malaysia Sabah
UMS Hatchery	Marine Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah

KEY WORDS

Baker's yeast, commercial concentrated *Nannochloropsis* sp., *Nannochloropsis* sp., rotifer, salinity levels, self-produced concentrated *Nannochloropsis* sp., water purifier filter.

CHAPTER 1

INTRODUCTION

1.1 Introduction of Rotifer

Rotifer is the most important live feed for fish larvae in aquaculture (Korstad *et al.*, 1989; Lavens & Sorgeloos, 1996; Hagiwara *et al.*, 2001; Suantika *et al.*, 2001; Cheng *et al.*, 2004). Mass production of rotifer was first developed in Japan in the 1960s (Lavens & Sorgeloos, 1996). Twenty-five years after the first production of rotifer, several culture systems were being used worldwide (Lavens & Sorgeloos, 1996). Rotifer is used as live feed for more than 60 species of fish larvae such as yellowtail, *Seriola quinqueradiata*; red sea bream, *Pagrus major*; Asian sea bass, *Lates calcarifer*; turbot, *Scophthalmus maximus*; mullet, *Mugil cephalus*; pufferfish, *Fugu rubripes*; gilthead sea bream, *Sparus aurata* and the European sea bass, *Dicentrarchus labrax* (Lubzens & Zmora, 2003).

Rotifer is small and slow swimming, which is a suitable feed to the fish larvae (Liu, 1996). Two types of rotifer are being cultured, *Brachionus pllicatilis* (130–340 µm in lorica length) and *B. rotundiformis* (100–210 µm) (Hagiwara *et al.*, 2001; Lubzens & Zmora, 2003; Kotani *et al.*, 2005). These are small enough to be fed to the fish larvae. Their swimming behaviour enables the fish larvae to hunt them (Lubzens & Zmora, 2003). Rotifer is a filter feeder that acts as a nutritional transferor and its nutritional value depends on the intake of feed (Lavens & Sorgeloos, 1996; Lubzens & Zmora, 2003).

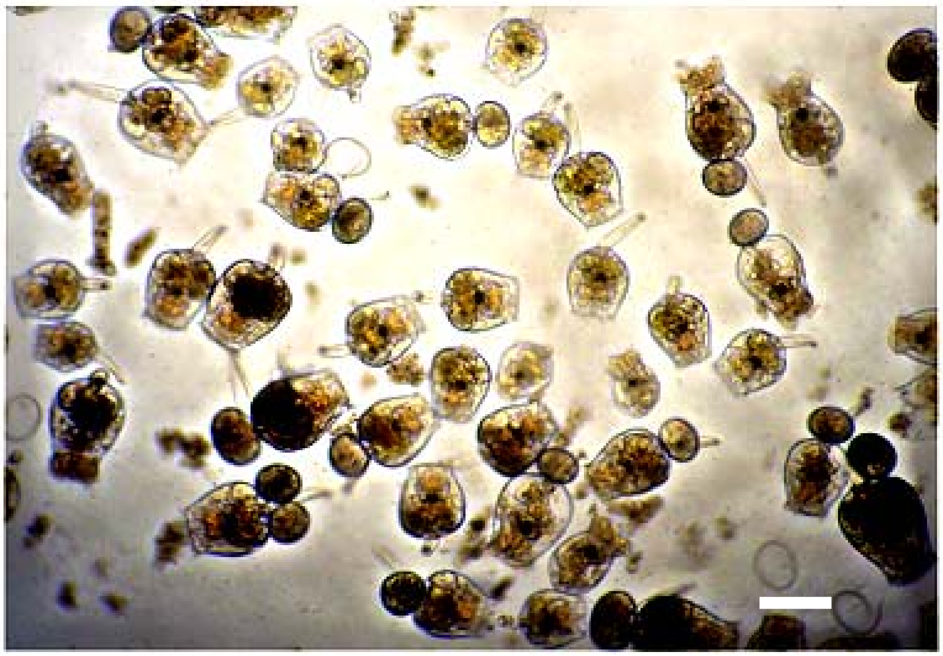

Photo 1: Rotifer cultured in the Marine Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS Hatchery). Scale bar, 100 µm.

1.2. Rotifer culture

Rotifer is well suited to mass culture because it is prolific and tolerates a wide variety of environmental conditions (Liu, 1996). The abiotic factors affecting rotifer culture are high densities, types of food, water temperature, salinity, light penetration and water quality (Minkoff *et al.*, 1983; McVey, 1993; Cheng *et al.*, 2004). The optimum temperature and salinity levels for rotifer growth are between 22-30 ºC and 10-35 ppt (McVey, 1993; Lubzens & Zmora, 2003). Rotifer prefers pH of 7.5-8.0, but both the pH value and the light penetration are normally not controlled in mass production (McVey, 1993; Lavens & Sorgeloos, 1996; Lubzens & Zmora, 2003).

The common rotifer culture systems used in hatcheries are batch, semi-continuous, continuous and high-density cultures. In batch culture, a total harvest of rotifer is conducted with part being used as feed for fish larvae and part as inoculums of the next culture (Lavens & Sorgeloos, 1996; Dhert, *et al*., 2001). In semi-continuous culture, however, a certain volume of rotifer is harvested daily from the culture tank and is replaced with feed suspension medium daily (Lavens & Sorgeloos, 1996; Dhert, *et al*., 2001). In continuous culture, rotifer can be harvested continuously; their nutritional quality is maintained by providing adequate feed (Lavens & Sorgeloos, 1996; Lubzens & Zmora, 2003). High-density culture which was first developed in Japan uses concentrated freshwater *Chlorella vulgaris* as feed and the maximum rotifer density can reach around 20-35x10^3 per ml (Hagiwara, 2001).

Rotifer is fed with microalgae, baker's yeast or both; and bacteria. Microalgae such as *Nannochloropsis* sp., *Nannochloris* sp., *Dunaliella* sp., *Tetraselmis* sp. and freshwater *Chlorella* sp. have been introduced in rotifer culture (McVey, 1993; Lubzens & Zmora, 2003). Among these species, *Nannochloropsis oculata*, *Tetraselmis tetrathele* and *Chlorella vulgaris* are the most popular feeds for rotifer culture (McVey, 1993; Dhert, 2001; Hagiwara, 2001). Baker's yeast is the common microorganism used for rotifer feed because it is cheap (McVey, 1993; Dhert, *et al*., 2001; Hagiwara, 2001). Bacteria are also used in small-scale rotifer culture that is carried out in laboratories (McVey, 1993). However, rotifer communities fed with bacteria have not been investigated thoroughly (Tucker, 2000).

1.3. Problems of rotifer culture

The main feeds fed to rotifer are *Nannochloropsis* sp., baker's yeast and commercial concentrated *Nannochloropsis* sp. (CC-Nanno). However, these feeds are insufficient for and incompatible with rotifer for several reasons. Several preliminary experiments were done to overcome this problem in the Marine Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS Hatchery) (Appendix A).

1.3.1. *Nannochloropsis* sp.

Nannochloropsis sp. is the most important microalgae that is cultured to feed rotifer (Lubzens & Zmora, 2003). *Nannochloropsis* sp. is a free-living or aggregated alga with ovoid cell walls (Graham, 2003; Muller-Feuga *et al.*, 2003a). The cells are 2-4 µm in diameter, which classifies them as nannoplankton (Graham, 2003; Muller-Feuga *et al.*, 2003a). *Nannochloropsis* sp. has been selected for mass culture based on its rapid growth (0.5/day), culture stability and range of nutrients (McVey, 1993).

The importance of microalgae in live feed chains lies in the transfer of the essential fatty acids and other dietary components through rotifer to fish larvae (Muller-Feuga *et al.*, 2003a). The nutritional value of *Nannochloropsis* sp. is varying according to the growth phase and culture conditions in mass culture (Muller-Feuga *et al.*, 2003a). The nutritional value of *Nannochloropsis* sp. is affected by abiotic factors such as light intensity, salinity, dissolved oxygen (DO), pH and fertilizers (Muller-Feuga *et al.*, 2003a).

There are three kinds of culture methods, namely batch, continuous and semi continuous. Batch culture begins from a small stock culture and proceeds by dilution into larger culture volumes until the final volume is achieved (McVey, 1993; Muller-Feuga *et al.*, 2003a). In continuous culture, nutrients and filtered seawater are continuously added into a tank for a long period of time (McVey, 1993; Muller-Feuga *et al.*, 2003a). Semi-continuous culture uses techniques from both batch and continuous culture methods (McVey, 1993; Muller-Feuga *et al.*, 2003a).

Rotifer production rate and density depend on the quantity of feed delivered (Duncan, 1989; Yúfera & Pascual, 1985; Muller-Feuga *et al*, 2003b). Generally, *Nannochloropsis* sp. culture ($2x10^7$ cells/mL) is used to feed rotifer in most hatcheries in the past. However, it is insufficient for rotifer production in high quantity with small areas (Hirayama *et al.*, 1989; Muller-Feuga *et al.*, 2003b). It means that *Nannochloropsis* sp. and rotifer culture require large space (Borowitzka, 1997; Ogle *et. al.,* 2005) in order to produce sufficient rotifer.

Nannochloropsis sp. culture requires labour and expertise (Borowitzka, 1997, Ogle *et al.,* 2005). Since *Nannochloropsis* sp. culture requires a large space, it requires more labour to maintain the culture. Zooplankton and microorganisms that contaminate the culture easily can also deplete its growth. Due to these reasons, *Nannochloropsis* sp. culture requires expertise (Borowitzka, 1997). The problems faced in *Nannochloropsis* sp. culture indicate that rotifer culture is indirectly affected.

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