Evaluation of an intensive culture system for the culture of the rotifer, *Brachionus plicatilis*using ammonia removers

Soliman Abdel Rahman[*1]; Mahmoud Attallah[1]; Mohammed Abu Zeid[2]; Fatma Abdel Razek[1] and Ahmed Hellal[2]

1- National Institute of Oceanography & Fisheries, Alexandria, Egypt

2- Department of Marine Science, Faculty of Science, Al-Azhar University, Cairo, Egypt

*Corresponding author: soliman2410@gmail.com

ABSTRACT

Rotifers are an essential starting live food for many fish and crustacean larvae in marine hatcheries. Recently, high-density intensive systems for the culture of the rotifer *Brachionus plicatilis* were initiated as an alternative for the traditional batch culture systems. Among the most common problems facing intensive culture systems, is the accumulation of dissociated ammonia (NH_3) which hinders the development of this technique. This study was conducted to test the effect of different ammonia removal compounds on the population growth of the rotifer, *Brachionus plicatilis* in an intensive system. The experimental closed-system design is composed of a rearing tank (100l capacity), a protein skimmer and a settling tank. Rotifers were fed with concentrated microalgae cells, *Nannochloropsis oculata*(3 x 10^9 cell/ml) and each experiment lasted for 10 days at $24C^0$. To investigate the effect of ammonia removers on population growth of rotifers, two commercial water conditioners were used (ChlorAm-X®, sodium hydroxymethanesulfonate, *Aqua Science Research Group*, Inc., MO, USA., added daily at a rate of 0.25 gm/10^6 rotifers) and (Ammo-Lock*, a registered trademark of *Mars Fishcare*, North America, Inc. added once at one gm/1l of culture water). At the end of each experiment, rotifer population numbers ranged from 300 – 1800 individual/ml. The best treatment method to get rid of ammonia and attain high population growth rates of rotifers was the use Ammo-Lock* followed by ChlorAm-X®.

Keywords: *Rotifer intensive culture, Brachionus plicatilis, ammonia removers.*

INTRODUCTION

Feeding of the early larval stages of fish and shrimp is one of the major obstacles for the industrial expansion of their culture. Evolutionary, larvae of most fish and crustaceans are adapted to prey on motile organisms and they encounter problems to accept inert dry diets. The marine rotifer, *Brachionus plicatilis*, is the most important and widely used live feed for larviculture of fish and other aquatic animals at their early stages. Rotifers are cultured in fish hatcheries using a wide variety of culture systems, including batch, semi-continuous, and intensive culture methods. Although some studies show that high-density rotifer cultivation can be accomplished, critical aspects of production, such as accumulation of very high levels of solids and unionized ammonia (NH_3-N), excessive foam formation, and the complicated nature of the systems, remain major problems that need to be solved. Very few modifications or improvements on the culture techniques of rotifers have been reported, including use of concentrated algae suspensions, dry micro-capsulated diets and intensive culture systems. In recent years, the aquaculture production in the world has increased. To produce fish and crustacean fry for aquaculture, rotifers play an indispensable role as an initial food item. The culture technology of rotifers still

dependent on the empirical skills of technicians and the productivity of rotifers was comparatively low (Kitajima, 1983; Yoshida, 1989). About 10 years ago, Japanese and European scientists demonstrated that the major factors that inhibit or limit the propagation of rotifers were mainly the lack of feed concentration, shortage of dissolved oxygen and toxicity of undissociated ammonia accumulated in the culture medium (Yoshimura, 1995; Yoshimura *et al.,* 1996 and Varghese and Krishnan, 2010). Several culture methods and devices were developed to eliminate these inhibitory factors from the rotifer culture medium. The deficiency of feed was resolved by the use of condensed micro-algal paste, such as freshwater Chlorella (Yoshimura *et al*., 1992). The shortage of dissolved oxygen was also resolved by supplying high purity oxygen gas instead of ambient air to the culture seawater (Yoshimura, 1995; and Dhert P., *et al.,* 2001). The toxicity of undissociated ammonia was controlled by maintaining culture pH at 7.0 with automatic addition of HCl solution to promote the dissociation of NH_3. This technique, however, require special care and continuous monitoring of the culture water. To achieve further improvement of the high-density culture method, it is necessary to suppress the inhibitory effects of ammonia coexisting with rotifers more completely.

The aim of this study is to investigate the effect of different ammonia removing methods on the performance of a small-scale intensive rotifer culture unit. Performance parameters included rotifer total number and fecundity as indicators of population growth, and changes in NH_3-N as a major indicator of water quality.

MATERIALS AND METHODS

The rotifer, *Brachionus plicatilis* (L-type) ranging in size from 180 – 280 µm was used in this study and obtained from the batch culture maintained in our laboratory. The experimental system is shown in Fig. 1 and composed of a rotifer rearing tank (100l), settlement sump, protein skimmer and a pump. The experiments were carried out using the facilities of marine invertebrate hatchery (LARVA-NU) located in the National Institute of Oceanography & Fisheries, Anfoushy, Alexandria, Egypt.

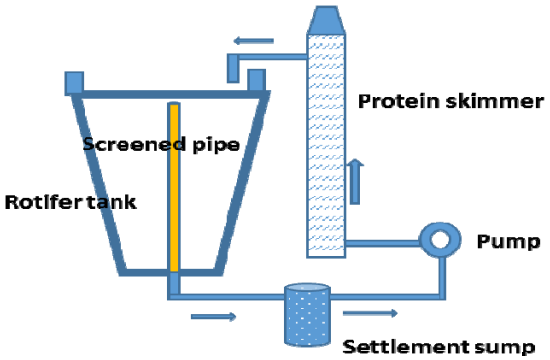

Fig. 1: Design of experimental rotifer intensive culture system

Physico-chemical properties of the rearing water were as follows: water temperature maintained at 24.5 ± 1 °C using submersed heaters, salinity was ambient and ranged between 35 and 36 ppt, dissolved oxygen was kept over 6 mg/l by moderate aeration and infrequent use of pure oxygen, and pH at start of the experiment ranged between 7.2 – 7.8. Temperature, pH and dissolved oxygen were monitored during the experimental period using a YSI-556 multi-parameter water

quality meter (YSI instruments, USA). Each tank was equipped with a central nylon screen (50 µm) to retain rotifers in the tank and facilitate water changing. De-ionized ammonia nitrogen (NH_3-N) quantity were performed with water samples (20-100ml) that were filtered through a GF/Ffilter (0.45µm) and NH_3-N concentrations were measured using a Hach DR890 colorimeter (Hach Co., CO, USA). Aeration in the culture tanks was provided through an aeration collar located at the bottom of the central screen to maintain homogeneous distribution of rotifers and algae in the water column and postponed screen blockage.

To investigate the effect of ammonia removers on population growth of rotifers, two commercial water conditioners were used; (ChlorAm-X®, sodium hydroxymethanesulfonate, *Aqua Science Research Group*, Inc., MO, USA., was added daily at a rate of 0.25 gm/10^6rotifers, T1) and (Ammo-Lock*, a registered trademark of *Mars Fishcare*, North America, Inc. added once at one gm/1l of culture water, T2) both products were used according to the manufacturer instruction andthey are safe enough and does not harm saltwater invertebrates. The ex periment was performed in parallel with another ammonia treatment method, through strong aeration, in the protein skimmer (T3), in addition to a control without any treatment (T4). The rotifers in the four treatments were fed with concentrated cells of microalgae, *Nannochloropsis oculata* produced from a bioreactor established in LARVA-NU and concentrated by centrifugation using cream separator. The concentrated cell paste contained 18 x 10^9 cell/ml. One ml of this emulsion was fed and maintained almost fixed to one million rotifers. Rotifers in culture was counted daily by sampling ten 1-mL samples from different areas of the tank and diluting the combined samples to 100 mL with seawater. Lugol's solution was added before counting to immobilize and stain the rotifers. Rotifers were counted using a Sedgewick rafter cell counter with a stereo microscope. The specific growth rate (SGR) was calculated using the equation given by Øie *et al*. (1994):

$$\text{Specific growth rate} = (\ln N_t - \ln N_0)/t$$

where N_0 is the initial rotifer concentration, N_t is the rotifer concentration at time t, and t is the culture period (days). Rotifer females carrying eggs were also counted and recorded as "No. Females with Eggs".

RESULTS

The physico chemical parameters of the experiment are shown in Table 1. Initially the experiment was started in indoor controlled room with the same water conditions to avoid great fluctuations in these parameters. Temperatures were almost constant ranged from 23.5 C^0 to a maximum of 25.5 C^0 as it was regulated by thermostat heaters. Dissolved oxygen ranged between 7.1 – 8.3 mg/l, and values showed limited variation with the exception in treatment T3 that showed slightly high values at the end of the experiment due to the use of strong aeration in the protein skimmer. Values of deionized ammonia NH_3-N were greatly influenced by the ammonia treatment procedures. In T1 (treated with " ChlorAm-X®") NH_3-N fluctuated from 0.7 – 3.5 mg/l, and in T2 (treated with "Ammo-lock*), NH_3-N concentrations ranged between 0.6 – 2.8 mg/l showing the significant effect of ammonia removers on water quality irrespective of the increase in rotifer population numbers. In T3 (no ammonia treatment + strong aeration), NH_3-N increased from 0.6 to 7.2 mg/l and in T4 (without any treatment for deionized ammonia), the values increased from 0.6 to 9.3 mg/l reaching high levels that could affect the reproduction and survival of rotifers.

Table1: Physico-chemicalparametersduringthe experiments.

	Physico-chemical parameters			
Treatment No.	Temperature (C^0)	NH_3-N (mg/l)	pH	Dissolved Oxygen (mg/l)
T1	23.5 – 24.6	0.7 – 3.5	7.2 – 7.9	7.0 – 8.2
T2	23.8 – 25.0	0.6 – 2.8	7.8 – 8.2	6.9 – 8.2
T3	24.1 – 25.5	0.6 – 7.2	7.8 – 8.1	7.1 – 8.3
T4	23.8 – 24.9	0.6 – 9.3	7.5 – 9.2	7.3 – 8.3

Rotifer numbers and fecundity (number of rotifers carrying eggs/total population number) were determined based on the average of five samples (1 ml each) taken from different locations of the culture tank. The total number of rotifers, rotifers carrying eggs, fecundity, specific growth rate and general observation for rotifer conditions are shown in Table 2.

Table 2: Population growth and fecundity of rotifers during the experimental period

Day	Treatment (T1)				Treatment (T2)				Treatment (T3)				Treatmen (T4)			
	T.N.	RCE	F (%)	SGR	T.N.	RCE	F (%)	SGR	T.N.	RCE	F (%)	SGR	T.N.	RCE	F (%)	SGR
1	300	42	14	0.43	300	39	13	0.65	300	53	18	0.28	300	42	14	0.35
2	420	98	23	0.51	425	156	37	0.26	330	119	36	0.44	330	63	19	0.51
3	760	274	36	0.19	720	295	41	0.12	456	97	21	0.13	380	45	12	0.07
4	1110	155	33	0.31	890	463	52	0.37	467	76	16	0.14	410	32	8	0.11
5	1125	123	39	0.11	1136	772	68	0.14	498	89	18	0.08	533	54	10	0.05
6	1250	144	44	0.18	1421	767	54	0.06	510	102	20	0.03	602	67	11	0.02
7	1389	160	51	0.2	1367	793	58	0.36	578	89	15	0.05	452	50	11	0.06
8	1580	163	38	0.11	1830	769	42	0.62	654	64	10	0.6	325	32	10	0.03

* T.N.= Total number of rotifers; RCE=Rotifers carrying eggs; F= fecundity, RCE/TN*100; SGR=specific growth rate.

Number of female carrying eggs were higher in groups treated with ammonia removers (T1 & T2) than the other two groups. This was reflected also for the increase of population number in the treated groups (Table 2). Changes in the number of rotifers and ammonia concentrations in the tanks are shown in Figure 2 (a & b). The maximum number of rotifers (1830 individual/ml) was obtained after 8 days of culture in treatment T2 using Ammo-lock* as ammonia remover followed by treatment T2 (1580 individual/ml) using ChlorAm*.

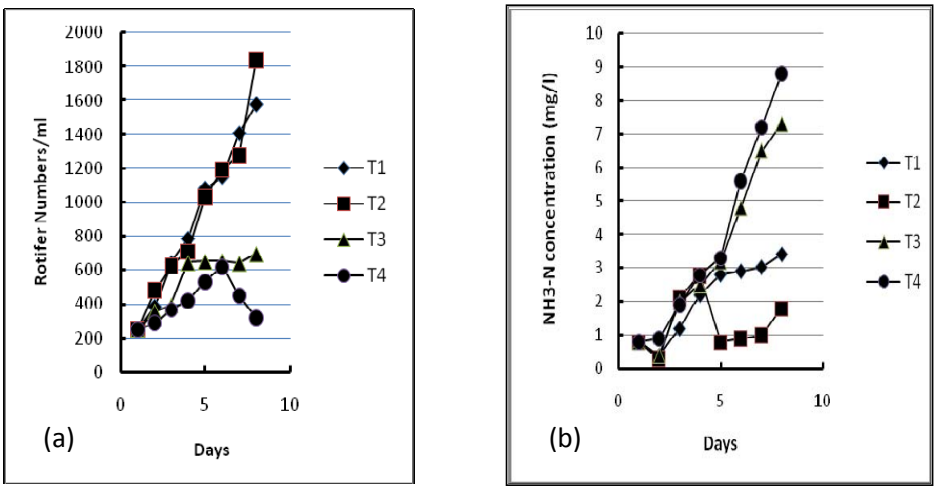

Fig. 2: Daily changes in rotifer population number (a) with different ammonia treatments, and ammonia level concentrations (b) during the experimental period

DISCUSSION

In intensive rotifer cultures, significant concentrations of NH_3-N have been reported when rotifer densities reach 500 individuals/ml (Yoshimura *et al*., 2003). In the present study we used several methods to get rid of harmful dissociated ammonia. Chemical compounds used to remove ammonia included; sodium hydroxymethanesulfonate (product of Sigma Chemicals), Ammonex* commercial product of Argent laboratories, USA, (composed of Zeolite and ion exchange resin). In addition, we used other physical methods such as strong aeration and water exchange in the protein skimmer. As the rotifer population increased, NH_3-N levels increased to a maximum of 9.2 mg/L, but increasing the amount of ammonia remover and the water exchange rate (up to 50%) prevented higher concentrations and possible negative effects on population growth. Sodium hydroxymethane-sulfonate and Ammonex* neutralizes NH_3-N, creating a non-toxic molecule; its potentials have been evaluated for use in shipping fish, crustaceans, and mollusks, as well as in rotifer culturing (Bentley *et al*., 2008). In batch-cultures NH_3-N concentrations were reported to be the major limiting factor when reaching 10 mg/L, which necessitated rinsing and restocking (Fu *et al*., 1997). The toxic LC50 concentration of unionized ammonia for marine Brachionus was reported to be 17.0 mg/L at 23 °C during a period of 24 h, which resulted in 50% lower growth and fecundity, as compared to treatments that had 7.8 and 13.2 mg/L of NH3-N (14). On the other hand, up to 22-34 × 103 rotifers/mL were cultured at total ammonia nitrogen (TAN) levels of 1000 mg/mL at pH 7.0 (12). However, in contrast to the NH3-N concentrations reported in the present study, recently reported values for NH3-N ranged between 0.06 and 0.1 mg/L in a high-density rotifer system in which sodium hydroxymethanesulfonate (ClorAm-X®) was used to control TAN concentration. Despite higher concentrations of ammonia remover in the present study, the differences between the two ammonia removal compounds are not significant; therefore, further research is required to determine the optimal dose of ammonia remover and to determine its effects on NH3-N concentrations.

Rotifer densities up to 800 individuals/mL in the recirculating systems with water exchange rates ranging between 20% and 50% of total culture volume per day have been found to be effective in decreasing ammonia level (Hirayama *et al*., 1973). The continuous replenishment of culture water with fresh seawater reduces the accumulation of toxic substances and stabilizes the culture medium but with high effort. However, increased rates of water flow into rotifer culture tanks may also reduce the algae concentration due to dilution. We observed that feed loss may be occurred due to foam fractionation in a recirculating rotifer culture system. On the other hand, feed loss can be minimized by adjusting the feeding rate and water flow into the culture tank. Our observations indicated that when rotifer density reached 1000 individuals/mL, the rotifers consumed concentrated algae within 20-30 min, as indicated by the color of the culture water. Hence, the rate of feed loss can be minimized by feeding rotifers multiple times a day, i.e. every hour, and by turning off the water flow into the rotifer tank during feeding. This may result in effective use of concentrated algae, as it was reported that feed cost was the most expensive component of a continuous system, which is 45% higher than that of a batch system. The use of nonviable condensed *N. oculata* for intensive rotifer culture has a number of advantages. It is commercially available and itcan be stored long term (>4 weeks) at -20 C without detrimental effects on nutritional quality (Lubzens *et al*. 1995). It also eliminates the labor and large amount of hatchery space required for.

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ARABIC SUMMARY

تقييم نظام استزراع مكثف لإنتاج الروتفيرا من نوع برا كيونس بليكاتيليس باستخدام نوعان من مزيلات الأمونيا

سليمان عبد الرحمن*[١] - محمود عطا الله[١] - محمد أبوزيد[٢] - فاطمة عبد الرازق[١] - أحمد متولي[٢]

١- المعهد القومي لعلوم البحار والمصايد ، الإسكندرية

٢- قسم علوم البحار ، كلية العلوم، جامعة الأزهر، القاهرة

الروتفيرا هي الغذاء الطبيعي الرئيسي ليرقات الأسماك البحرية والقشريات الاقتصادية في كل المفرخات. وفي الآونة الأخيرة فقد بدأ تطوير أنظمة مكثفة لإنتاج الروتفيرا بديلا عن الأنظمة القديمة التقليدية والتي تعاني من كثير من العيوب. ومن أهم المشاكل التي تواجه تطوير الأنظمة الحديثة هو تراكم مستويات عالية من الأمونيا المتأينة التي تسبب انخفاض الإنتاجية وتنتهي بنفوق هذه الحيوانات. وقد تمت هذه الدراسة لتقييم استخدام وسائل متعددة للتخلص من الأمونيا المتأينة ومدي تأثيرها علي الزيادة العددية للروتفيرا من نوع برا كيونس بليكاتيليس. وقد تم تصميم نظام مغلق للتربية المكثفة للروتفيرا مكون من حوض للتربية سعة ١٠٠ لتر، بروتين سكيمر، مضخة، حوض ترسيب. تمت تغذية المعاملات بمعلق مركز من الطحالب الميكروسكوبية الحية من نوع نانوكلوروبسيسأوكيولاتا (٣ مليار خلية / المليلتر) واستمرت التجربة لمدة ١٠ أيام عند درجة حرارة متوسطة ٢٤ درجة مئوية. تم استخدام نوعان من مزيلات الأمونيا المتاينة التجارية هما كلورأمواكس* بتركيز ٢٥٠ ملجم لكل مليون روتيفيرا، و أمولوك* بتركيز جرام واحد لكل لتر مياه تربية. كما تم تجربة استخدام هواء مندفع قوي في البروتين سكيمر كمحاولة للتخلص من الأمونيا. استخدام أمولوك* أعطي أفضل النتائج من حيث عدد الروتفيرا (١٨٣٠ حيوان/مل) تبعه استخدام كلورأموكس*(١٥٨٠ حيوان/مل) بينما كان استخدام الهواء المندفع محدودا في التخلص من الأمونيا المتاينة (٥٦٤).