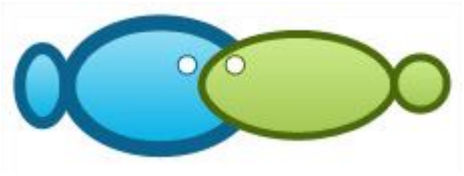

Black background improves the population growth of euryhaline rotifer, *Brachionus rotundiformis* reared in two photoperiod regimes

[1]Adrian Deil C. Manliclic, [1]Mark June S. Consigna,
[1]Marz Linnaeous L. Rabadon, [1,2]Mark Nell C. Corpuz

[1] Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, Bayan, Orani, Bataan 2112, Philippines; [2] School of Environmental Science and Management, University of the Philippines Los Baños, College, Los Baños, Laguna 4030, Philippines. Corresponding author: A. D. C. Manliclic, deilmanliclic@gmail.com

Abstract. This study evaluated the population growth of rotifer, *Brachionus rotundiformis* (Tschugunoff, 1921) cultured in different colour backgrounds at two photoperiods under non-axenic condition. Polyethylene tetrachloride bottles of different colours were used as experimental culture units with initial stocking density of 5 rotifers mL^{-1}. The cultures were fed daily with *Chlorella sorokiniana* Shihira and Krauss, 1965 at 1.0 x 10^6 cells mL^{-1} under ambient condition (28.2±0.6°C). Five colour backgrounds (red, black, white, blue, and yellow), and two photoperiod regimes (24-h illumination, and 1:1 light-dark photoperiod) were assigned as treatments; each replicated thrice and set in completely randomized design. Significant variation among colour treatments were observed ($F = 7.97$, $df = 4$, $p < 0.01$), with black background having the highest total mean population growth (4.32 x 10^5 ± 9.97 x 10^4) in 24-h illumination, and in 1:1 light-dark photoperiod (2.80 x 10^5 ± 5.30 x 10^4). Pooled rotifer densities of treatments cultured in 24-h illumination was higher compared to 1:1 light-dark photoperiod but not statistically significant ($t = 1.26$, $p > 0.05$). Specific growth rates were observed to be highly significantly different among treatments, with black having better specific growth rates (89-90% per day) in both photoperiods. This study demonstrated that *B. rotundiformis* reared in black background had better population growth performance compared to other environmental colorations, with no profound photoperiod effect.
Key Words: Bataan, natural food, rotifer density, zooplankton.

Introduction. Rotifers are an important group of live food organisms used in aquaculture. They are known as important source of nutrients and enzymes required for the normal growth and development of several commercially important fish species (Lubzens 1987). They are small type of zooplankton; however, in the world of aquaculture, they are very large object of attention by scientists and aquaculturists as they serve as 'living capsules', providing the nutrients required by the cultured fish larvae for proper development (Lubzens & Zamora 2003).

Brachionus rotundiformis (Tschugunoff, 1921), one of the known species of rotifer, is an important natural live food for the larval phase of many hatchery-reared fish and crustaceans. Despite of the nutritional benefits and food source potential of *B. rotundiformis* for many cultured fish larvae, their production in the Philippines remains very limited mainly due to non-adoption of low cost production system (De la Pena 2015). The supply of rotifers does not always meet the demand of the local aquaculture industry; hence it is necessary to apply necessary techniques (Kar et al 2017) that can be used to initiate rotifer culture systems on a large scale (Ortega-Salas et al 2013).

There are several studies conducted to determine the influence of light intensities (Yoshimatsu et al 2008; Kim et al 2014; Javaheri & Hosseini 2015) and light wavelength (Kim et al 2013) in the population density and behaviour of several species of rotifers. It is known that zooplanktons like rotifer, exhibit phototactic responses upon exposure to

light source (e.g. solar light) (Jékely et al 2008). Moreover, it was assumed that the manipulation of illumination in the culture can affect the rotifer's phototactic responses, which corresponds to energy consumption and reproduction of the cultured rotifer (Kim et al 2013, 2014). With such reference, the present study hypothesized that different colour backgrounds and length of light exposure can influence the population growth of *B. rotundiformis*.

To the best of our knowledge, there are no studies concerning the effect of different colour backgrounds, and photoperiod regimes in the population growth and density of *B. rotundiformis*. This study will fill-up a research gap in the hatchery management for improved production of this important natural food resource. Hence, the main goal of this study was to determine the population growth performance of *B. rotundiformis* under different colour backgrounds (blue, black, yellow, red, and white), and photoperiod regimes (24-h illumination, and normal 1:1 dark-light photoperiod) in non-axenic condition.

Material and Method

Microalgal culture. *Chlorella sorokiniana* Shihira & Krauss, 1965 were procured from Southeast Asian Fisheries Development Center (SEAFDEC) at Tigbauan Main Station, Iloilo City, Philippines. Preparation and upscaling of microalgae followed the methods of SEAFDEC with some modifications (De la Pena & Franco 2013). Three *Chlorella* media were prepared (1 inoculant: 4 distilled water ratio) in Erlenmeyer flasks with fertilizer, 14-14-14 NPK (14% NH_3PO_4, 14% P_2O_5, 14% K_2O) as source of nutrient. The fertilizers were dissolved in distilled water at a rate of 0.1 g L^{-1}. Subsequently, the culture was exposed continuously to 1,000-1,500 lux cool white fluorescent light (20 W). Salinity was maintained at 6-7 ‰ artificial seawater. The plankton densities were estimated using haemocytometer following the direct plankton enumeration technique (Martinez et al 1975).

Rotifer culture. Euryhaline *B. rotundiformis* (SS-type) was obtained from Bureau of Fisheries and Aquatic Resources-National Integrated Fisheries Technology Development Center (BFAR-NIFTDC) in Bonuan-Binloc, Dagupan City, Philippines. Concentrated densities of rotifers were initially diluted with *C. sorokiniana* in 15-L aquaria in triplicate samples. The culture was maintained at 28.3±0.7°C in Fisheries Laboratory Building, Bataan Peninsula State University (BPSU) for three days.

Experimental trial. Growth performance of rotifer was determined using two factors: photoperiod regime (1), and colour background (2). The experimental units were simultaneously subjected to five colour backgrounds (blue, black, yellow, red, and white) under two photoperiod regimens (24-h illumination, and 1:1 light and dark ratio). In 24-h photoperiod regime (hereafter denoted as 24 h), the treatments were illuminated with 1,000-1,500 lux cool white fluorescent lights (20 W), whilst the treatments exposed in natural diel periodicity (hereafter denoted as 1:1 light-dark) were set in indoor experiment utilizing the natural sunlight.

Three replicates were used in each treatment, and were assigned in completely randomized design. Thirty round polyethylene tetrachloride bottles (5 L capacity each) of different colours were used as culture units.

Culture experiment. The rotifers (ca. 0.1 mm) from the culture aquaria were randomly designated to the experimental culture units with an initial effective volume of 500 mL *C. sorokiniana*. The estimated density of the algal media was approximately 90 x 10^7 cells mL^{-1}. The initial culture stock was about 2,500 rotifers per culture unit or a stocking rate of five rotifers per mL. Salinity levels (6-7 ‰) were adjusted accordingly. The rotifer was fed daily with *C. sorokiniana* at a density of about 1.0 x 10^6 cells mL^{-1} (Hoff & Snell 2001). Feeding rate used in the study was 10% of the total culture volume for day 1 to 7, and 20% feeding rate at day 8 to 14 (Corpuz unplublished data). The study was conducted in BPSU-Orani Campus at Fisheries Laboratory Building on 04-18 of September 2017.

Daily monitoring of water quality parameters including dissolved oxygen (DO) and temperature (°C) (DO-temperature meter); levels of pH (handheld pH meter), and salinity (salinometer) were done in each experimental culture unit. The total ammonia-nitrogen (TAN) level was determined thrice a week using an ammonia test kit.

Sampling and growth rate. Sampling was done at around 16:00 h in the 7^{th} and 14^{th} day of culture to avoid possible contamination and stress to the rotifers. Three subsamples (1 mL each) per replicate were examined under a compound microscope (10 x magnification). The rotifer culture was first homogenized by mixing, and was sampled using a 1-mL syringe. The sampled rotifers were sacrificed to facilitate the counting. This was done by adding one drop of 3.5% formaldehyde into the Sedgewick rafter. Mean density (rotifer mL^{-1}) per replicate was estimated and the specific growth rate (SGR, % per day) was calculated as:

$$SGR = \frac{[Ln(N_f) - Ln(N_i)}{D} \times 100$$

where: N_f = final number of population;
N_i = initial number of population;
D = number of days of the culture period.

Statistical analysis. All data followed the normality assumptions (Shapiro-Wilk test), and with that, the data were analysed by t-test and analysis of variance (ANOVA), and followed by Tukey's post hoc test to assess statistically significant differences between treatments ($p < 0.05$). The arc sine-transformed SGR percentage in every treatment was also statistically compared ($p < 0.05$). Values are expressed as means±standard error of the mean for rotifer densities. All statistical analyses were performed using SPSS version 17.0, and graphs were produced using SigmaPlot version 10.0.

Results

Water quality parameters. Water quality variables did not vary much during the trial period. Water temperature levels and DO ranged between 27.6 and 29.2°C, and 4.12 and 6.35 mg L^{-1}, respectively. The TAN concentrations ranged from 0.0 to 0.13 mg L^{-1}, and pH ranged between 7.4 and 8.8 during the rearing period. Salinity concentration was maintained at 6.2 to 7.1 ‰ .

Population growth. Final volume of culture units was approximately 3.5 L. Changes in the population growth of rotifer in every treatment in two photoperiod treatments are presented in Figures 1A and 1B. After seven days of culture, population growth of rotifer in 24 h increased to approximately 6.86×10^4 to 1.99×10^5 rotifers per culture unit. In the 14^{th} day culture, population growth of rotifer increased to about 1.40×10^5 to 4.32×10^5 rotifers. In 1:1 light-dark, total densities reached 3.9×10^4 to 1.11×10^5 rotifers, and subsequently increased to about 9.30×10^4 to 2.80×10^5 rotifers at day 14.

Consistently, black treatments achieved the highest total rotifer densities in two photoperiod regimes. It is also discernible in the graphs that the exponential growth rates of several treatments (red in 24 h, blue in 1:1 light-dark, and black in both photoperiod regimes) were achieved between 7^{th} and 14^{th} days.

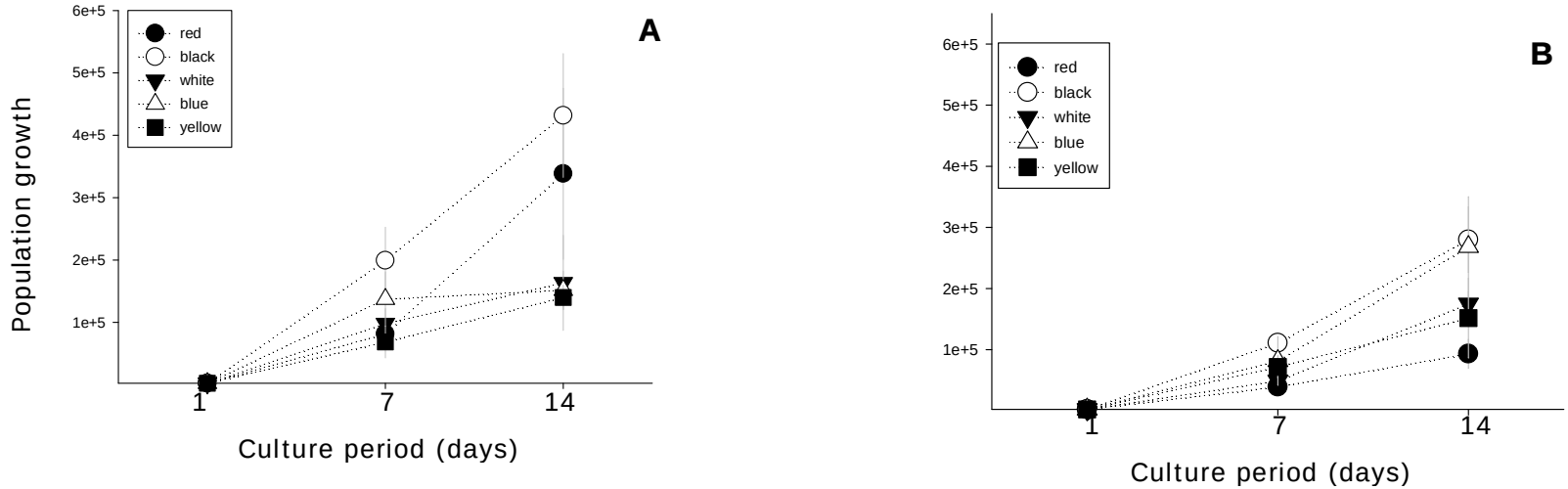

Figure 1. Changes in the mean population growth of *Brachionus rotundiformis* reared in five colour treatments. 24-h illumination (**A**); 1:1 light and dark photoperiod (**B**).

Variation on population growth. Variation in mean total population growth among and between treatments is presented in Figure 2. Although there was a variation in the pooled population growth of treatments between 24 h and 1:1 light-dark, the difference however, was not significant ($p > 0.05$).

After the final day of culture, significant variation was observed among the treatments cultured in 24 h ($F = 11.08$, $p < 0.01$). The black background had the highest estimated total population growth (mean = 4.31 x 10^5 rotifers), and followed by red (mean = 3.38 x 10^5 rotifers), albeit the two treatments were not significantly different ($Q = 2.35$, $p > 0.05$). The lowest rotifer densities were observed in yellow, blue, and white, each having a mean population growth of not more than 1.70 x 10^5 rotifers. For comparison, population growth under dark condition was 2.58 times higher than those under yellow, blue, and white backgrounds.

In 1:1 light-dark photoperiod, significant difference in population growth among treatments was also observed ($F = 5.36$, $p < 0.01$). Similar to the observation in 24 h, the highest estimated population growth was also recorded in black (mean = 2.80 x 10^5), and closely followed by blue (mean = 2.68 x 10^5), although there was no significant difference between the two treatments ($Q = 5.44$, $p > 0.05$). The lowest estimated total population growth was observed in red (mean = 9.30 x 10^4 rotifers); black was 3.01 times higher than red in 1:1 light-dark photoperiod condition.

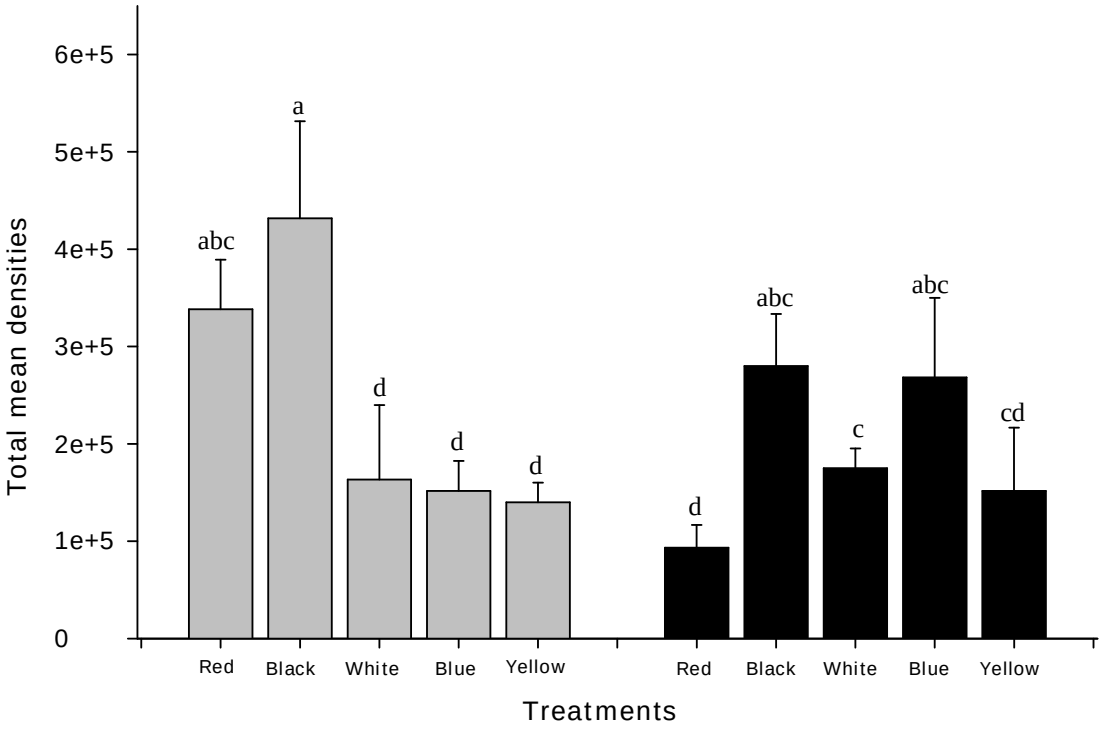

Figure 2. Estimated population growth (mean±SD) of *Brachionus rotundiformis* reared in different colour backgrounds and photoperiods. Gray bars represent 24-h illumination; black bars represent 1:1 light and dark photoperiod. Bars with same small letter are not significantly different ($p > 0.05$).

Specific growth rates. Mean SGR in each treatment exposed in two photoperiods is presented in Figure 3. Highest mean SGRs in 24 h were observed in black (92.29±2.68% per day) and red treatments (90.71±1.97% per day). These two treatments were statistically different to other treatment colours, with SGRs of no more than 85% per day. In 1:1 light-dark, highest SRGs were realized in black (89.28±2.27% per day), and blue treatments (88.51±3.97% per day), although these two treatments were not statistically different to white (86.03±1.47% per day), and yellow (83.79±5.19% per day).

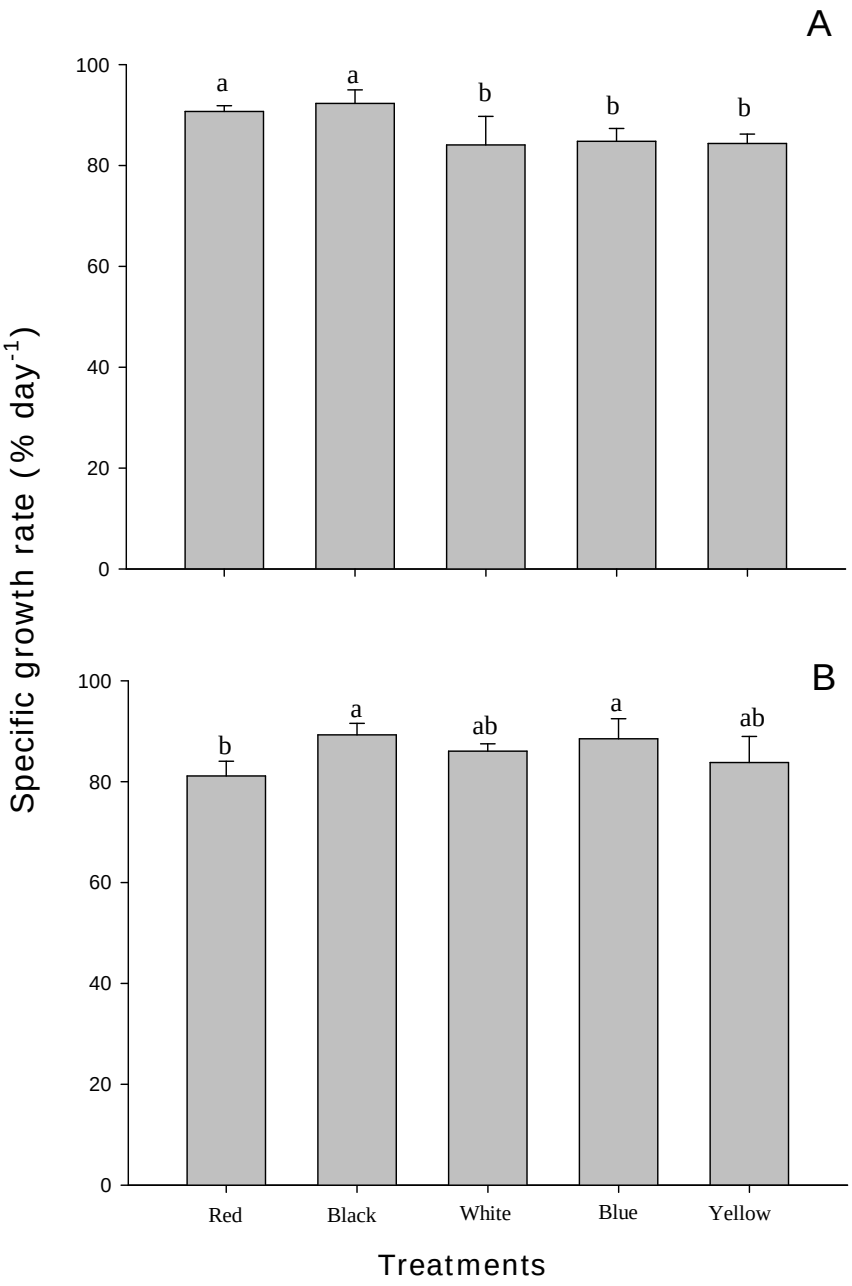

Figure 3. Specific growth rate (mean±SE) of *Brachionus rotundiformis* reared in five colour backgrounds at two photoperiods. Bars with same small letter are not significantly different ($p > 0.05$). 24-h illumination (**A**), 1:1 light and dark photoperiod (**B**).

Discussion. The manipulation of colour background has been widely known to influence the well-being of several important aquaculture species (Sierra-Flores et al 2015; Zhang et al 2015; Manliclic et al 2018) and cultured invertebrates (Aréchiga-Palomera et al 2018; Alimuddin et al 2019). In the present study, background colour of culture units considerably affects the population densities and growth rate of *B. rotundiformis* in non-axenic condition. Moreover, black colour background has positive effect on rotifer's population growth regardless whether the culture is set in 24-h illuminated condition or in normal diel light fluctuation. The present observation can be attributed to reported phototactic responses of congeneric *Brachionus* (Cornillac et al 1983), that is to say, photokinesis to light at different wavelengths (Kim et al 2013) and intensities (Mimouni et al 1993). Result of the present study conforms to the works of Kim et al (2014) in *B. plicatilis* and *B. manjavacas*, wherein, the highest population growth was observed in cultures reared in total darkness. It is hypothesized that high light intensities reduces the

population growth rate due to increased swimming speed and turning frequencies. Rotifer under this condition will spend more energy in movement resulting to decreased energy allotted for reproduction. This behaviour was also observed in freshwater rotifer, *B. calyciflorus* (Clément 1977 as reported by Kim et al 2013).

Although the present study did not expose the culture in total darkness, black container ensures that light intensity in the media was diminished. We posited that dark environment benefits the rotifer by inhibiting their accumulation in the illuminated side of the water medium. Their aggregation in a particular area in the culture medium may result to intensified competition in food resource, space, and oxygen, which in turn, can cause displacement or even physical damages to small rotifers. The dark environment also weakens the penetration of light in the culture medium, and since rotifer has a positive phototaxis in decreasing light intensity (Kim et al 2014), those in black containers will rather drift in the culture unit. This behaviour inhibits accumulation of *B. rotundifomis* and thus, effectively maximizing the water volume in the culture. This is the same practical reason why conditioning and hatching of brine shrimp (*Artemia salina*) in the Philippines is usually done using a covered dark container.

Apart from black background, it was observed that the red background resulted to high population growth rate at the 24-h illuminated set-up. It was documented that the eyespots of related species, *B. plicatilis* and *B. calicyflorus* efficiently absorbs wavelength ranging from 450 to 550 nm (Kim et al 2014), and 400 to 540 nm (Cornillac et al 1983), respectively. And the red colour in the visible spectrum ranges from 635 to 700 nm (Craig & Clothiaux 2006). However, the strong positive phototaxis of *B. plicatilis* (470 to 525 nm) became weak at 660 nm (Kim et al 2014). With this reference, red colour background reduces phototaxis in the 24-h illumination and thus, positively affects the population growth of *B. rotundiformis.*

In 1:1 normal light-dark environment, high population growth in blue and reduced growth in red was observed. Although it is not conclusive, the observation can be attributed to light source and intensity employed in the experiment. The influence of such factors to the population density and growth rate of *B. rotundiformis,* however is open to further investigations. Likewise, larger experimental units (e.g. aquaria or fibre glass tanks) of different colour background can be used for future researches to include socio-economic analysis in rotifer production.

Conclusions. The present study demonstrated that background colour influences the population growth of *B. rotundiformis*, with black being the most suitable colour background. Photoperiod regime did not significantly affect the rotifer's population growth performance. The use of dark containers in the culture of this important food resource is highly advisable for improved production.

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Authors:
Adrian Deil C. Manliclic, Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, 2112 Bayan, Orani, Bataan, Philippines, e-mail: deilmanliclic@gmail.com
Mark June S. Consigna, Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, 2112 Bayan, Orani, Bataan, Philippines, e-mail: mjconsigna@gmail.com
Marz Linnaeous L. Rabadon, Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, 2112 Bayan, Orani, Bataan, Philippines, e-mail: mrzrab@yahoo.com.ph
Mark Nell C. Corpuz, Institute of Fisheries and Aquatic Sciences, Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems, Bataan Peninsula State University, 2112 Bayan, Orani, Bataan, Philippines; School of Environmental Science and Management, 4030 University of the Philippines Los Baños, College, Los Baños, Laguna, Philippines, e-mail: marknellcorpuz@yahoo.com; mccorpuz@up.edu.ph

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