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ROTIFERA XIV

Culturing *Brachionus rotundiformis* Tschugunoff (Rotifera) using dried foods: application of gamma-aminobutyric acid (GABA)

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Abstract This study investigated the population growth of the rotifer *Brachionus rotundiformis* (S-type) in batch cultures using dried *Nannochloropsis oculata* and *Chlorella vulgaris* with gamma-aminobutyric acid (GABA) supplementation. The effectiveness of GABA (50 mg l^{-1}) was tested during the lag phase of rotifer growth and every 2 days in small cultures, at an initial density of 50 ind ml^{-1}. In large cultures, 200 rotifers ml^{-1} were exposed to GABA for 24 and 48 h before upscaling to 20 l cultures. GABA enhanced rotifer population density and egg/female ratio in both foods compared to the control. Pre-GABA exposure for 48 h caused higher rotifer population densities on days 5 and 6 (with both foods) and 8 and 10 (with *C. vulgaris*), than their respective controls. Ammonia concentration increased equally in all of the treatments. Production of *B. rotundiformis* using dried *N. oculata* and *C. vulgaris* can be significantly enhanced by 48 h of pre-GABA exposure to reach maximum density within 6 and 8 days, respectively, despite high ammonia concentrations. These findings provide insight into mechanisms for maintaining continuous reproduction of rotifers in natural habitats by using GABA as a chemical signal to regulate their life cycle and overcome the potential effects of physiological stresses such as high ammonia.

Keywords *Chlorella vulgaris* · GABA · *Nannochloropsis oculata* · Live food · Microalgae · Physiological stress

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Introduction

Live microalgal diets are commonly used to culture live foods, such as rotifers, for aquaculture. However, year round cultivation of sufficient live microalgae, especially *Nannochloropsis oculata* (Droop) Hibberd, 1955 (Maruyama et al., 1997), is a heavy burden on many hatcheries (Duerr et al., 1998). Also, the live microalgae have a short shelf life (Maruyama et al., 1997), which limits pre-planning of fish larval production in microalgae-based hatcheries (Lubzens et al., 1995). As an alternative, frozen microalgal diets can be used to culture rotifers (Hamada & Hagiwara, 1993; Lubzens et al., 1995; Yufera & Navarro, 1998), but high storage costs are incurred and

a stable supply of energy cannot be guaranteed everywhere to maintain freezer facilities. Dried *N. oculata* and *Chlorella vulgaris* Beijerinck, 1890 can be stored at room temperature for up to 2 years and can support population growth of rotifers (Hirayama & Nakamura, 1976; Dobberfuhl & Elser, 1999). However, culture instabilities due to poor ambient conditions have been reported (Yufera & Navarro, 1995), hence the requirements for techniques to stabilize these cultures.

Various chemicals have been used to stabilize rotifer cultures (Gallardo et al., 1997, 1999). For example, growth hormone (GH) promotes rotifer reproduction under optimal conditions (Gallardo et al., 1999), while gamma-aminobutyric acid (GABA), an amino acid derivative that regulates physiological processes in animals (Nogrady & Alai, 1983), enhances amictic reproduction in the progeny of the rotifer *Brachionus plicatilis* Müller, 1786 during high ammonia and food scarcity (Gallardo et al., 1997, 1999, 2000a). Marine aquatic habitats that rotifers inhabit are characterized by a combination of physiological stresses such as fluctuating salinity, high ammonia, food scarcity, protozoa (*Euplotes* sp.) contamination, and high water viscosity. These stresses affect the reproduction, swimming activity, and ingestion rate of rotifers (Hagiwara et al., 1998). Moreover, during rotifer resting egg formation, these physiological stresses can influence the induction of mixis, egg hatchability, fecundity, and lifespan of rotifers, resulting in different sexual and asexual populations (Snell & Hoff, 1985; Snell, 1986; Hagiwara et al., 2005). GABA can be employed to mitigate the effects of these stressors (Gallardo et al., 1997, 1999), thus ensuring uninterrupted rotifer reproduction in natural habitats. For example, GABA has been found to neutralize the combined effects of high ammonia, viscosity, food scarcity, and *Euplotes* sp. contamination on rotifer reproductive characteristics and enzyme (glucosidase) activities (de Araujo et al., 2001; Araujo & Hagiwara, 2005). Gamma-aminobutyric acid has also been used to maintain the viability of the physiological condition of rotifers at low temperature and during population growth after preservation at low temperature (Assavaaree & Hagiwara, 2011). Supplementation with 50 μg ml^{-1} of GABA and 0.025 IU ml^{-1} of porcine GH in the culture media can also enhance L-type rotifer population growth in individual cultures after 48 h of exposure to these chemicals prior to their transfer to new media (Assavaaree & Hagiwara, 2011).

In both natural and mesocosm environments, GABA has been found to induce the settlement and metamorphosis in the larvae of many species of marine invertebrates, e.g., the abalone *Haliotis rufescens* Swainson, 1822 (Morse et al., 1979; Gapasin & Polohan, 2004), the mussels *Mytilus galloprovincialis* Lamarck, 1819, the clams *Venerupis pullastra* Montagu, 1803, the oysters *Ostrea edulis* Linnaeus, 1758 (Doroudi & Southgate, 2002), the black chiton *Katharina tunicate* Wood, 1815 (Rumrill & Cameron, 1983), and the sea urchins (Naidenko, 1996). It has been hypothesized that rotifers can be used as conduits to transfer GABA to target species, thus improving their reproduction in habitats where combined environmental stressors occur. In this study, we explored whether GABA would reduce physiological stress in *B. rotundiformis* Tschugunoff caused by deteriorating culture conditions (e.g., high ammonia) as reported in other rotifer cultures with dried microalgal diets (Yufera & Navarro, 1995). Most previous studies have focused on the biological manipulations of the rotifer *B. plicatilis* using a live *N. oculata* diet with GABA supplementation in the culture media (Gallardo et al., 2000a; Araujo & Hagiwara, 2005; Assavaaree & Hagiwara, 2011). However, the rotifer *B. rotundiformis* is also an indispensable larval fish food, whose stable production using dried algae with GABA supplementation could provide a positive step toward achieving pre-planned hatchery fish larval production and improving the reproduction of target species. In the literature, the population growth response of the rotifer *B. rotundiformis* to dried *N. oculata* and *C. vulgaris* with GABA supplementation is unclear. To advance knowledge in this area, we hypothesized that (1) GABA supplementation does not enhance the population growth of *B. rotundiformis* with dried *N. oculata* and *C. vulgaris*; (2) effectiveness of GABA supplementation on rotifer reproduction does not depend on the rotifer growth phase stage or GABA exposure time before mass cultures; and (3) GABA application does not mitigate effects of high ammonia in rotifer mass cultures. To test these hypotheses, we determined the effectiveness of GABA supplementation during lag phase growth stage of the rotifers and every 2 days on the population density and egg/female ratio of this rotifer species in small batches. Then, we exposed concentrated rotifers

to GABA for 24 and 48 h in small culture vessels before upscaling to larger mass cultures, where ammonia production was monitored.

Materials and methods

Algae and rotifers

Firstly, we established a sufficiently high rotifer population by providing cultures with 7.0×10^6 cells ml^{-1} of either live *C. vulgaris* or *N. oculata* (Araujo & Hagiwara, 2005) daily for 7 days at 25 ± 1°C. *C. vulgaris* was supplied as a live liquid paste by a Chlorella Industrial Company Ltd. in Japan. Stock cultures of *N. oculata* and the rotifer *B. rotundiformis* (S-type, Perth strain) were available in the laboratory of Aquaculture Biology, Nagasaki University, Japan. We cultured *N. oculata* using modified Erd Schreiber medium (Hagiwara et al., 1994) at 25°C under constant illumination (115.5 μmol^{-1} m^{-2}) with gentle aeration. The alga was harvested during the log phase of growth by centrifugation (2100×*g* for 8 min) and the pellet was re-suspended in sterilized seawater (22 ppt.). We determined the algal density of the microalgae in diluted aliquots of suspended concentrate using a hemocytometer. Dried *N. oculata* (cell diameter 2.5 µm) and *C. vulgaris* (cell diameter 3.0 µm) were obtained from AlgaSpring (The Netherlands) and Daesang EMERALD (South Korea), respectively. The dried microalgae were stored at room temperature in the laboratory. The nutritional composition of the dried algae is shown in Table 1.

Table 1 Nutritional composition of the dried microalgae (based on dry weight) used in the experiment

Nutrients	*Nannochloropsis oculata*	*Chlorella vulgaris*
Proteins	45%	60.6%
Fats	22%	12.8%
Ash	11%	4.5%
Moisture	–	5.4%
Carbohydrates	7%	15.7%
Vitamin B_{12}	–	1000 µg/100 g
Vitamin C	744 mg/kg	74 mg/100 g
Vitamin D	–	277.6 µg/100 g
Vitamin E	289 mg/kg	22.8 mg/100 g
Phosphorus	–	1060 µg/100 g
EPA	5%	–

Source N. oculata, AlgaSpring, the Netherlands; *C. vulgaris*, EMERALD, South Korea

Experimental design

Before starting the experiments, the rotifers were preconditioned to the respective dried foods at 800 mg dry weight 10^{-6} rotifers day^{-1} for 2 days. In the small trials, we cultured the rotifers in 1 l jars containing 300 ml of seawater at an initial density of 50 ind ml^{-1}. These were gently shaken in a triple shaker machine (NR-80, Taitec Co. Japan) at 61 rpm to keep the dried microalgae suspended. The rotifers were divided into three treatment groups with three replicates each: (1) no GABA addition (control), (2) GABA (50 mg l^{-1}) addition once at lag phase, and (3) GABA addition every 2 days. The rotifers were incubated at 25 ± 1°C in total darkness and fed daily with either dried *N. oculata* or *C. vulgaris* at the rate of 800 mg dry weight 10^{-6} rotifers day^{-1} for 5 days. The dried algae were first re-suspended in 3–5 ml of seawater and then subjected to ultrasonic agitation for 5–10 min to break down the aggregates of cells before adding to the rotifer cultures. The diet quantities were adjusted daily depending on the rotifer population density, which was estimated by counting the rotifers in a 1 ml subsample from each replicate. In the same samples, the number of amictic females and the number of amictic eggs (attached or detached) were counted and recorded.

In the mass cultures, we employed the optimal results of the small scale trials. We first concentrated the rotifers in 1 l culture medium at 200 ind ml^{-1} and applied 50 mg l^{-1} of GABA once at lag phase 24 and 48 h (Gallardo et al., 2000a) before upscaling to 20 l cultures with aeration at room temperature. For each treatment, a corresponding control was prepared. The rotifer population density monitoring and feeding was done daily as explained above. The specific growth rate (SGR) was calculated during the exponential growth phase using the formula: $r = [\ln N_t - \ln N_o]/t$ (Krebs, 1985), where N_o = initial population density, N_t = population density after the time (t); and $t = 6$ days. Every 2 days, 30 ml of water samples were obtained from each replicate tank of the 48 h of GABA experiment for ammonia (NH_3–N mg l^{-1}) determination (Palintest® 8000 Ltd, USA).

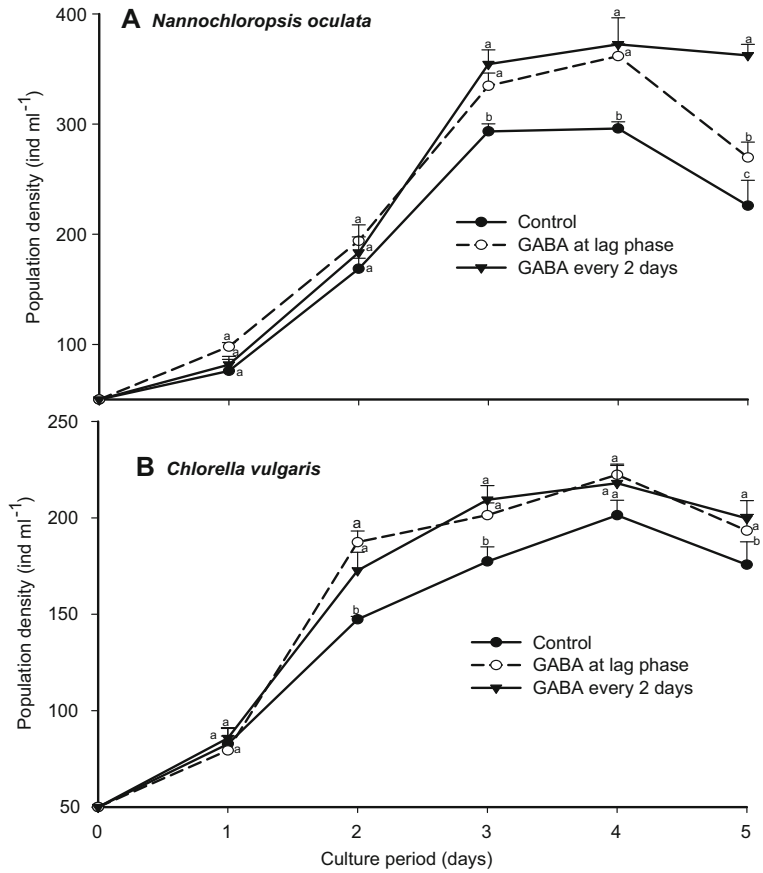

Fig. 1 The population growth curves of the rotifers fed with dried *N. oculata* (**A**) and *C. vulgaris* (**B**) with GABA supplementation at lag phase and every 2 days, and without GABA addition (control). Each plot represents mean value of three replicates ± SD. Two-way ANOVA, Tukey HSD test, $n = 54$; different *letters* in each day represent significant differences at $P < 0.02$, a > b > c

Statistical analysis

The data were analyzed using R statistical software (version 3.2.1 of the R Foundation for Statistical Computing Platform © 2015). The Bartlett test was used to test the homogeneity of variances. Two-way ANOVA was used to test the effects of culture period, food type, and GABA on rotifer population density, specific population growth rate, and ammonia levels. Where significant differences were detected, Tukey's HSD Post Hoc test was performed to locate them. Wilcoxon rank-sum test was used to compare the rotifer population densities between 24 h and 48 h GABA culture experiments. All statistical differences were accepted at $P < 0.05$.

Results

In the small cultures, the population density of the rotifers fed with *N. oculata* was significantly affected by culture period ($F = 988.05$; $P = 0.00$), GABA ($F = 77.82$; $P = 0.00$), and their interaction ($F = 16.91$; $P = 0.00$). GABA supplementation at lag phase and every 2 days resulted in significantly higher population densities on days 3–5 in the treatments than in the control (Fig. 1A). Similarly, with a *C. vulgaris* diet, there was a significant effect of culture period ($F = 810.34$; $P = 0.00$), GABA ($F = 33.20$; $P = 0.00$), and their interaction ($F = 4.96$; $P = 0.00$) on the rotifer population density. Here, GABA supplementation at lag phase and every 2 days caused significantly higher population densities on days 2, 3, and 5 than in the control (Fig. 1B).

With the *N. oculata* diet, the egg/female ratio was significantly affected by culture period ($F = 424.62$; $p = 0.00$), GABA ($F = 700.51$; $P = 0.00$), and their interaction ($F = 13.86$; $P = 0.00$). Higher egg/female ratio was observed on days 1–5 in the treatments than in the control ($P < 0.02$) (Fig. 2A). Similarly, with a *C. vulgaris* diet, the egg/female ratio was significantly affected by culture period ($F = 905.33$; $P = 0.00$),

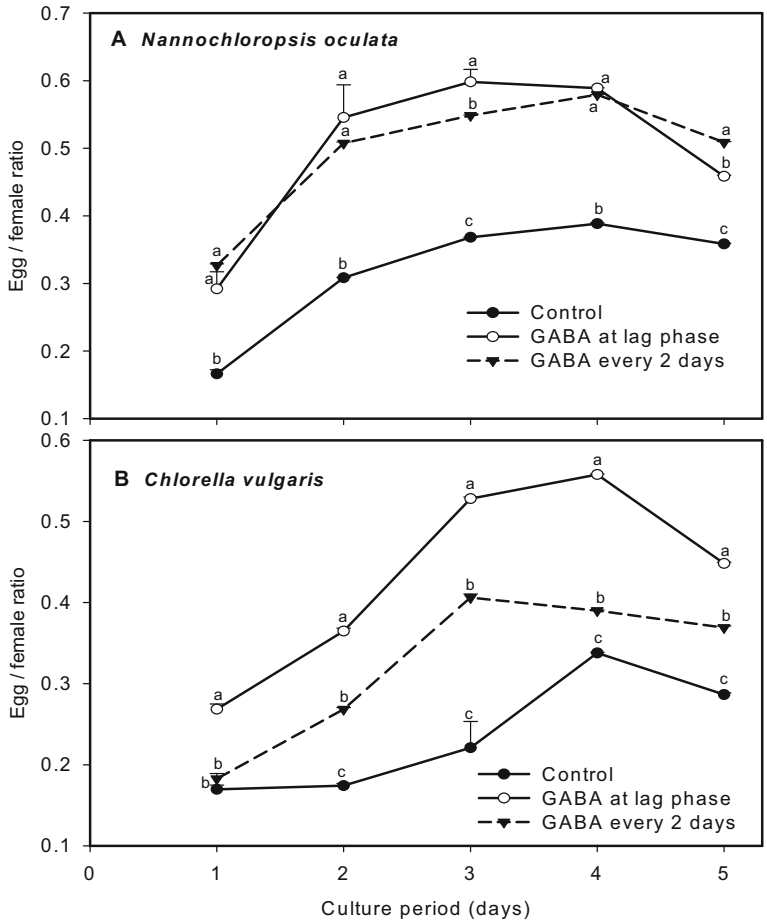

Fig. 2 Egg/female ratios of *B. rotundiformis* fed with dried *N. oculata* (**A**) and *C. vulgaris* (**B**) and supplemented with GABA at lag phase and every 2 days. Each plot represents the mean of three replicates ± SD. Two-way ANOVA, Tukey HSD test, $n = 90$; different *letters* in each day represent significant differences at $P < 0.001$, a > b > c

GABA ($F = 1760.08$; $P = 0.00$), and their interaction ($F = 68.81$; $P = 0.00$) with higher egg/female ratios in the treatments than in the control being observed on days 2–5 ($p < 0.02$) (Fig. 2B). The highest egg/female ratio (0.59 ± 0.02) was obtained in the *N. oculata* diet on day 3 with GABA supplementation at lag phase (Fig. 2A).

In the mass cultures, there was a significant effect of culture period, food type, and GABA supplementation on the rotifer population densities, but without significant interaction effects in the 24 h of pre-GABA supplementation (Table 2). No significant differences occurred in the daily rotifer population densities among the treatments in the 24 h GABA mass cultures (Fig. 3). However, 48 h of pre-GABA supplementation caused significantly higher rotifer population densities on days 5 and 6 (with both foods) and days 8 and 10 (with *C. vulgaris*) than their respective controls ($p < 0.01$) (Fig. 4). Highest rotifer population densities of 301.3 ± 22.2 and 246.3 ± 10.1 ind ml^{-1} were obtained with *N. oculata* +GABA and *C. vulgaris* +GABA on days 6 and 8, respectively (Fig. 4). GABA treatment for 48 h prior to mass culture produced significantly higher rotifer population densities than the 24 h of pre-GABA treatment (Wilcoxon rank-sum test; $W = 1725.5$, $P = 0.03$).

Specific growth rate was affected by food type only ($F = 8.34$; $P = 0.02$) in the 24 h GABA experiment where rotifers fed with *N. oculata* had significantly higher SGR more than those fed with *C. vulgaris* ($P = 0.03$) (Fig. 5A). On the other hand, only GABA affected the SGR after 48 h of prior GABA incubation ($F = 31.97$; $P = 0.00$) and the GABA-treated rotifers had higher SGR than those in non-GABA treatments ($P = 0.00$) (Fig. 5B). There were no significant difference in SGR between 24 and 48 h GABA mass cultures by day 6 (Student t test, $t = -1.71$; df = 11, $P = 0.11$). Highest SGR of 0.42 ± 0.03 day^{-1} was realized with *N. oculata* + GABA for the 48 h of pre-treatment with GABA. Ammonia concentration was

Table 2 Effects of culture period (days), feed type, and GABA on the population density of the rotifers cultured after GABA treatment for 24 and 48 h

	df	SS	MS	*F*	*P*
24 h cultures					
Day	10	425527	42553	181.04	0.000*
Food	1	7113	7113	30.26	0.000*
GABA	1	6708	6708	28.54	0.000*
Day × food	10	8169	817	3.47	0.000*
Day × GABA	10	6854	685	2.91	0.000*
Food × GABA	1	18	18	0.07	0.781
Day × food × GABA	10	1778	178	0.75	0.669
Residuals	88	20683	235		
48 h cultures					
Day	10	589404	58940	192.61	0.000*
Food	1	22569	22569	73.75	0.000*
GABA	1	45920	45920	150.06	0.000*
Day × food	10	18461	1846	6.03	0.000*
Day × GABA	10	24856	2486	8.12	0.000*
Food × GABA	1	189	189	0.61	0.433
Day × food × GABA	10	9246	925	3.02	0.002*
Residuals	88	26928	306		
Ammonia (NH_3–N) mg l^{-1}					
Day	5	3.930	0.786	318.84	0.000*
Food	1	0.002	0.002	0.813	0.371
GABA	1	0.005	0.005	2.165	0.147
Day × food	5	0.004	0.001	0.390	0.853
Day × GABA	5	0.014	0.002	1.169	0.338
Food × GABA	1	0.002	0.003	1.192	0.280
Day × food × GABA	5	0.007	0.001	0.641	0.668
Residuals	48	0.118	0.002		

Concentrations of ammonia were measured from the 48 h of GABA-treated culture tanks only. Two-way ANOVA

df degrees of freedom, *SS* sum of squares, *MS* mean square, *F* F-ratio, *P* level of significance

* $P < 0.05$

significantly affected by culture period, but not by food type or GABA (Table 2). Ammonia levels increased each day equally in all the treatments, reaching about 0.7 mg l^{-1} on day 10 (Fig. 6).

Discussion

The difficulties in maintaining artificial food chains of microalgae and rotifers in hatcheries often hinder the pre-planning of fish larval production programs (Lubzens et al., 1995; Hagiwara et al., 2001a, b). Our study has shown that production of *B. rotundiformis* using dried *N. oculata* and *C. vulgaris* can be significantly improved by GABA supplementation, thus providing an opportunity to eliminate direct dependence on freshly cultured live alga. GABA can be applied during the lag phase growth stage of the rotifers 48 h before upscaling to mass cultures. This can enhance the rotifer population density within 6 and 8 days of culture with dried *N. oculata* and *C. vulgaris*, respectively. In our small culture trials, GABA application at lag phase and every 2 days caused higher population growth but GABA application at lag phase was preferred during mass cultures due to cost implications. Our results are consistent with the findings of Gallardo et al. (2000a), who reported the effectiveness of GABA treatment during lag phase and every 2 days for the reproduction of the rotifer *B. plicatilis* supplied with a fresh *N. oculata* diet in small and mass batch cultures. Nonetheless, Gallardo et al. (2000a) reported that continuous application of GABA (every 2 days) causes rotifer culture crash after 8 days of culture. This observation provides an interesting

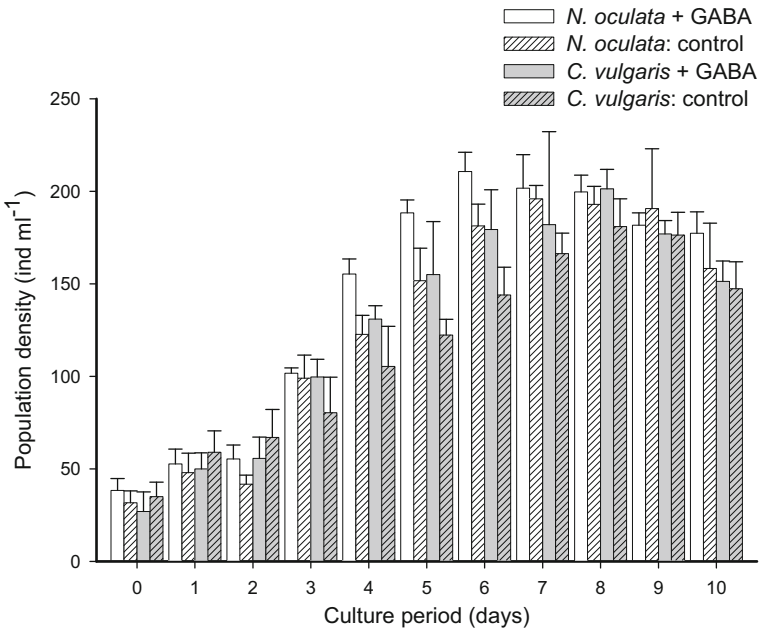

Fig. 3 Population density of the rotifers fed with dried *N. oculata* and *C. vulgaris* with GABA supplementation for 24 h before mass culture. The *bars* are mean ± SD of three replicates. Two-way ANOVA, Tukey HSD test, $n = 132$; No significant differences occurred on daily population densities among treatments $P > 0.05$

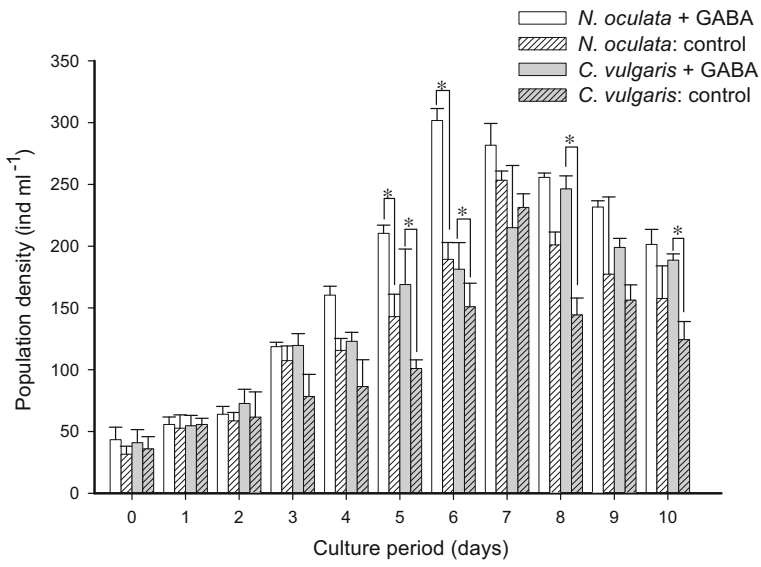

Fig. 4 Population density of the rotifers fed with dried *N. oculata* and *C. vulgaris* with GABA supplementation for 48 h before mass culture. The *bars* are mean ± SD of three replicates. Two-way ANOVA, Tukey HSD test, $n = 132$; *asterisk* in each day denotes significant differences for each feed at $P < 0.01$

insight into the potential ecotoxicological effects of unlimited GABA supply (e.g., from industrial spillage) in aquatic ecosystems. Indeed, rotifers are ubiquitous organisms and their ability to transport aquatic pollutants across the food web makes them special model organisms for studies in ecophysiology, ecotoxicology, and environmental genomics (Snell & Janssen, 1995; Snell, 1998; Snell & Joaquim-Justo, 2007; Dahms et al., 2011). Further studies are recommended to assess the potential toxicological effects of GABA in rotifer life cycle.

The higher egg/female ratio and population densities observed with GABA treatment in this study suggested a continued effect on rotifer reproduction beyond their exponential growth phases. This phenomenon is attributable to the influence of GABA on subsequent rotifer progenies. This observation corroborates previous studies that GABA causes even

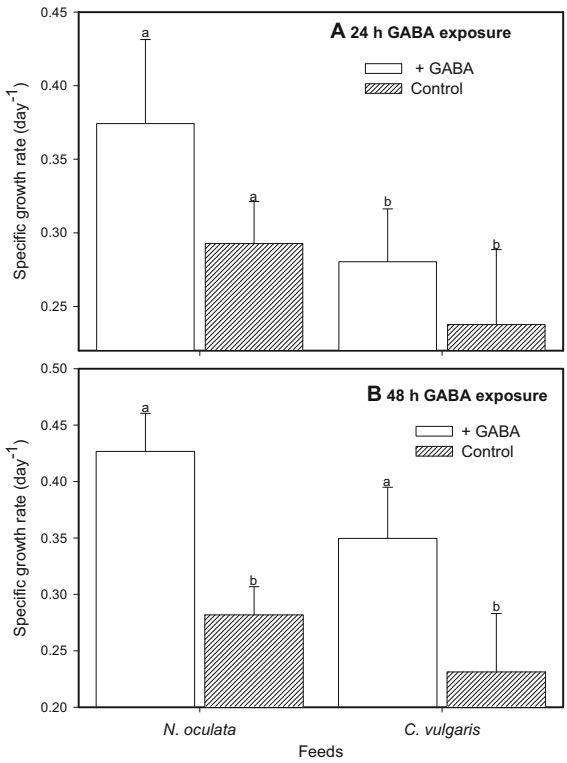

Fig. 5 The SGR of the rotifers fed on *N. oculata* and *C. vulgaris* with GABA supplementation for 24 h (**A**) and 48 h (**B**) before mass culture. Two-way ANOVA, Tukey HSD test, $n = 12$; different *letters* denote significant differences at $P < 0.05$; a > b

stronger growth effects on the F_1 and F_2 generations of *B. plicatilis*, which were not initially exposed to GABA (Gallardo et al., 1997, 1999). In our study, GABA exposure at lag phase for 48 h prior to mass culture was more effective than for 24 h. This observation suggests that a longer GABA absorption time is necessary to trigger higher rotifer population growth. Similar observations have been reported in the literature, although for the rotifer *B. plicatilis* fed with live *N. oculata* diet (Gallardo et al., 2000a; Assavaaree & Hagiwara, 2011). However, Gallardo et al. (2000a) cautioned that holding rotifers for longer than 48 h at high density may be counterproductive. According to Gallardo et al. (2000a), there is a time lag for GABA entry and utilization before its effectiveness against stress can be realized, and it takes about 4 days at 25°C before a positive effect on the rotifer mass cultures can be observed. In the current study, GABA produced better population growth results on days 6 and 8 with *N. oculata* and *C. vulgaris*, respectively. By using dried *C. vulgaris* in a 12 l batch culture, Hirayama & Nakamura (1976) achieved 434 ind ml^{-1} of *B. plicatilis* on day 16 from an initial inoculation of 13.2 ind ml^{-1}. The current study reported 301.3 ± 22.2 ind ml^{-1} with *N. oculata* on day 6 and 246.3 ± 10.1 ind ml^{-1} with *C. vulgaris* on day 8 from an initial inoculation of 10 ind ml^{-1}, demonstrating

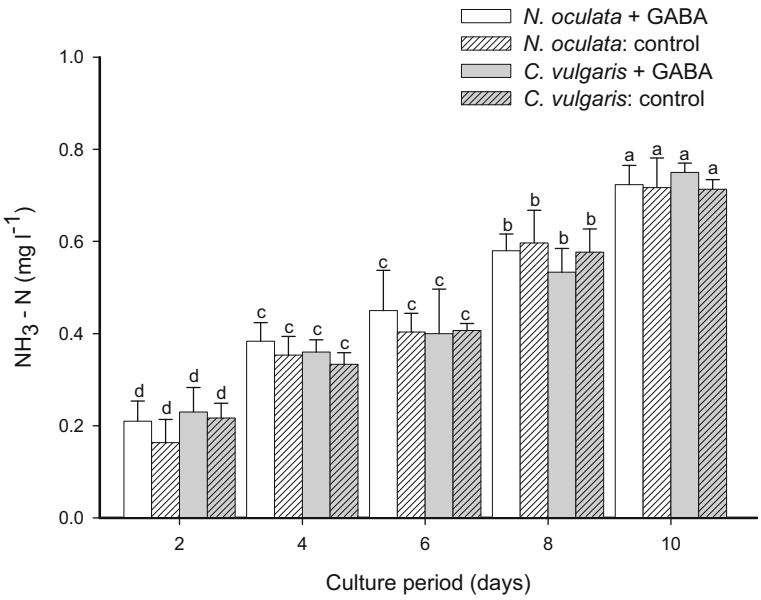

Fig. 6 Daily NH_3–N fluctuations in culture tanks for *B. rotundiformis* fed with *N. oculata* and *C. vulgaris* after 48 h of GABA supplementation before mass culture. The *bars* are mean ± SD of three replicates. Two-way ANOVA, Tukey HSD test, $n = 72$. Different *letters* on each day denote significant differences among the treatments at $P < 0.05$, a > b > c

the significance of GABA supplementation. Nonetheless, other studies have reported 600–1000 rotifers ml^{-1} using fresh microalgal diets in batch cultures after about 4 days starting with 200–250 rotifers ml^{-1} (Dhert, 1996). However, the problem with using fresh microalgal diets is instability and high cost of maintaining the cultures.

In this study, we explored whether GABA would be able to reduce physiological stress in *B. rotundiformis* caused by deteriorating culture conditions (e.g., high ammonia and rotifer density) as reported in other rotifer cultures with dried microalgal diets (Yufera & Navarro, 1995). Indeed, despite increasing NH_3–N concentration in all treatments (up to 0.7 mg^{-1} on day 10), the GABA-treated cultures produced higher rotifer densities and SGR than the controls. Even though food was regularly supplied in the cultures, it is possible that food limitations might have occurred at some points, as algae condensed on the tank sides while rotifer population density increased significantly, at least in the GABA-treated tanks. Therefore, it is reasonable to suggest that GABA enhanced the physiological condition of the rotifers against effects of high ammonia, food limitation, and high rotifer density in the cultures, confirming the results of previous studies of Gallardo et al. (1997, 1999, 2000b) in which GABA enhanced rotifer reproduction under sub-optimal conditions. This observation further compliments the conclusions of Araujo & Hagiwara (2005) that GABA mitigates the effects of the environmental stressors and stabilizes the quality of rotifer cultures.

During rotifer egg formation, physiological stressors such as food limitation may influence mictic induction, egg hatchability, lifespan, and fecundity of rotifers (Snell, 1986; Hagiwara & Hino, 1990; Hagiwara et al., 2005), resulting in different parthenogenetic populations and mixis investment (Snell & Hoff, 1985). Mixis investment causes short-term fitness of rotifer clones (Chen & Cuijuan, 2015) as more energy is invested in fertilization (Gilbert, 2010). This reduces life expectancy, fecundity, and rotifer population density (Snell & Hoff, 1985). GABA is a chemical signal that regulates the rotifer life cycle and reduces the effects of the physiological stressors (Gallardo et al., 1999) commonly found in natural habitats. Therefore, through GABA supplementation, rotifers can overcome the potential effects of these stressors to favor female parthenogenesis and facilitate colonization even during food scarcity (Gallardo et al., 1999). GABA has also been found to improve visual capacity in animals (Sandberg et al., 2014), which can be important in feeding, mating, and predator avoidance in rotifers. Rotifers play significant roles as top microbial predators in the aquatic food chains (Wallace et al., 2015); hence their populations should be maintained for effective ecological functions. GABA is an amino acid derivative that has a direct effect on rotifer growth when consumed directly as a nutrient (Morse, 1984) and it is also important for maintaining the physiological condition of rotifers under low temperatures (Assavaaree & Hagiwara, 2011). This can be useful, especially for increasing the fecundity of asexual females of the S- and SS-type rotifers, which are highly susceptible to low temperature stress (Araujo & hagiwara, 2005). GABA induces the hormonal production of endorphins, which have calming and stress-reducing effects in rotifers (Nogrady & Alai, 1983). Also, GABA may have induced the secretion of GH in *B. rotundiformis* (Gallardo et al., 1997, 1999), as has been demonstrated in higher animals, e.g., rats (Abe et al., 1977).

In other ecological studies, GABA has been found to induce settlement and metamorphosis of the larvae of many marine invertebrates (Rumrill & Cameron, 1983; Naidenko, 1996; Doroudi & Southgate, 2002; Gapasin & Polohan, 2004; Garcia-Lavandeira et al., 2005). A GABA dose between 0.5 and 10 μM for 48 h has been reported as the optimum for effective induction of settlement and metamorphosis in most larvae of marine invertebrates (Gapasin & Polohan, 2004; Garcia-Lavandeira et al., 2005). Even though the mechanism by which GABA induces metamorphosis is poorly understood, there is evidence suggesting that the algae-recognizing receptors are the same receptors that recognize GABA for settlement and metamorphosis (Morse et al., 1979). Settlement and metamorphosis are crucial stages in the commercial culture of bivalve molluscs. Therefore, the use of rotifers as conduits of GABA transfer could be important in promoting the culture of these valuable marine invertebrates.

There is strong evidence that GABA controls the level of the quorum-sensing signal in some pathogenic bacteria, e.g., *Agrobacterium tumefaciens* Smith & Townsend, 1907 (Chevrot et al., 2006) and *Pseudomonas aeruginosa* Migula, 1900 (Dagorn et al., 2013), in plants and animals (Park et al., 2010).

Quorum-sensing mechanisms are suspected to cause mixis induction in rotifers (Fussman et al., 2007). This provides opportunities to develop strategies for controlling the virulence of pathogenic bacteria in rotifer cultures. This observation partially complements the findings of de Araujo et al. (2001) and Araujo & Hagiwara (2005) that GABA can neutralize the effects of protozoa (*Euplotes* sp.) contamination in rotifer cultures. Even though we did not determine the bacterial loads in our cultures, it is speculated that GABA may have neutralized the effects of pathogenic bacteria on rotifer growth. More studies should focus on this aspect to provide more specific information. Based on the premise of Fait et al. (2008), it is probable to suggest that GABA is also important in tricarboxylic acid (TCA) cycle and carbon–nitrogen balance control in cellular plants under ecological duress. The cellular plants are the basis for aquatic food chains. GABA is biodegradable (Saskiawan, 2008), cheap (US$ 3.00 per gram; Sigma Chemical), and requires low doses (50 mg l^{-1}) making it economically attractive for a range of applications. It is speculated that GABA will be practically beneficial to fish larvae feeding on rotifers, but this speculation requires further scientific proof.

The production protocols for dried microalgal diets are already patented by different commercial companies. Dried *N. oculata* costs about US$ 2.0 per kg (AlgaSpring) and is readily available for export. Dried algae can be transported and stored for long periods, thus eliminating direct dependence on freshly cultured live alga. This is especially relevant where algal cultures are either restricted by seasonal conditions or lack infrastructure for high density algal production. Another advantage of dried algae is the possibility to pre-determine the nutritional content of this food by supplementing it with essential elements that are crucial for the proper development of the targeted fish larvae (Camacho-Rodriguez et al., 2013). Here, GABA can be useful during rotifer enrichment with marine oils to reduce physiological stresses caused by the oil additives and high population densities. Our study has demonstrated that mass production of the rotifer *B. rotundiformis* using dried *N. oculata* and *C. vulgaris* can be significantly enhanced by using GABA supplementation to reach maximum densities of 301.3 ± 22.2 and 246.3 ± 10.1 ind ml^{-1} within 6 and 8 days, respectively. GABA supplementation can be achieved by pre-incubating rotifers with 50 mg l^{-1} of GABA for 48 h before upscaling to 20 l mass cultures. The results of our study are relevant where mass culturing of rotifers is necessary for the pre-planning of fish larval production in aquaculture facilities. In addition to aquaculture, the larval fishes can be used to re-stock depleted natural stocks and GABA may cause ripple beneficial effects to other non-targeted marine biodiversity.

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