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ORIGINAL ARTICLE

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Reproductive performance of fairy shrimp *Branchinecta orientalis* (G. O. Sars 1901) (Crustacea: Anostraca), fed with effluent of rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) ponds

N. Pormehr[1] | N. Agh[2] | L. Beladjal[3] | B. Atashbar[2] | G. Van Stappen[1]

[1]Faculty of Bioscience Engineering, Laboratory of Aquaculture & Artemia Reference Center, Department of Animal Production, Ghent University, Ghent, Belgium

[2]Artemia and Aquaculture Research Institute, Department of Biology and Aquaculture, Urmia University, Urmia, Iran

[3]Faculty of Science, Terrestrial Ecology Unit, Department of Biology, Ghent University, Ghent, Belgium

Correspondence
N. Pormehr, Faculty of Bioscience Engineering, Laboratory of Aquaculture & *Artemia* Reference Center, Department of Animal Production, Ghent University, Ghent, Belgium.
Email: Navid.Pormehryabandeh@UGent.be

Abstract

Aquaculture production is predicted to increase sharply. In this regard, live feed plays a crucial role in the larval phase of many aquaculture organisms. Hence, a persistent concern in aquaculture is to find low-cost and eco-friendly feed sources to culture live feed organisms. *Branchinecta orientalis* (G. O. Sars 1901), a fresh/brackish water fairy shrimp, was reared using effluent from rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) ponds, either fresh but supplemented with two species of microalgae, *Scenedesmus* sp. and *Haematococcus* sp., or non-supplemented but after "ageing" of the culture medium. The feeding experiment was designed at a density of 100 individuals L^{-1} in 2-L vessels. The results indicated that differences between final length, survival and most reproductive parameters of the treatment with aged medium and the treatment using fresh medium supplemented with *Scenedesmus* sp. were non-significant ($p > .05$). Better results were obtained for a number of reproductive parameters in the treatment supplemented with *Haematococcus* sp. Thus, for intensive resting egg production of *B. orientalis*, microalgae can be replaced by aged non-supplemented effluent from trout ponds as a nutrient-rich feed source. This consequently can reduce drainage of nutrients into the environment and thus decrease aquatic pollution.

KEYWORDS

Anostraca, *Branchinecta orientalis*, fairy shrimp, large Branchiopoda, resting egg, reused waste water

1 | INTRODUCTION

The fairy shrimp *Branchinecta orientalis* (G. O. Sars 1901) is a non-selective filter feeder of plankton and suspended material like other fairy shrimps (Beladjal, Peiren, Dierckens, & Mertens, 1997), which inhabits vernal pools. These pools periodically dry, particularly during summer, and are very important habitats for large branchiopods (Atashbar, Agh, Van Stappen, Mertens, & Beladjal, 2014), many of which are endangered (Kneitel, Samiylenko, Rosas-Saenz, & Nerida, 2017).

Most Anostracans are characterized by obligate sexual reproduction (Reniers, Vanschoenwinkel, Rabet, & Brendonck, 2013). They reproduce only by producing resting eggs (Reniers et al., 2013), unlike *Artemia* (Leach 1819) which can produce both nauplii and resting eggs based on the environmental conditions. The elongated shape of the brood pouch enables the females to inject the resting eggs into the soil to a depth of almost 10 mm (Kraus, Eder, Møller, & Werding, 2004). The resting eggs are capable of tolerating harsh conditions during drought or freezing, which in most cases is a must before they can hatch (Kraus et al., 2004). When the pools are filled

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with water again, they hatch and initiate a new generation of fairy shrimps (Beladjal & Mertens, 2017). Anostracan resting eggs are dispersed by several vectors, such as wind (depending on the season), water (Beladjal & Mertens, 2009), insects, amphibians, fish, birds, mammals and unintentionally by humans (Boix et al., 2016).

Branchinecta orientalis is a broadly distributed species as it is reported in Europe from Spain (Manca & Mura, 1997), Austria, Hungary and Serbia where it appears in huge numbers during the period of water bird migration (Horváth, Vad, Vörös, & Boros, 2013). Additionally, resting eggs have been collected from two north-western provinces in Iran, West Azerbaijan (Atashbar, Agh, Beladjal, Jalili, & Mertens, 2012) and East Azerbaijan (Mura & Azari Takami, 2000). The species is commonly observed in low salinity pools (<5 g/L), often coexisting with other fairy shrimps, such as *Chirocephalus skorikowi* Daday, 1912 (Atashbar et al., 2014) or *Phallocryptus spinosa* (M. Milne-Edwards, 1840) (Mura & Azari Takami, 2000).

Considering the present human population of around 7.5 billion people in the world, higher production of aquatic food is necessary. In this regard, live feed is a very important element in the larval rearing stage of many aquaculture organisms. In this larval stage, generally live feed is preferred over formulated diets because of its favourable nutritional composition, high digestibility, and ready acceptance by the fish and shellfish larvae (Velu & Munuswamy, 2008). Many studies have shown the application of fairy shrimps and their high nutritional value as a potential and new food source in aquaculture (Dumont & Munuswamy, 1997; Velu & Munuswamy, 2007, 2008; Sornsupharp, Dahms, & Sanoamuang, 2013; Sornsupharp, Lomthaisong, Dahms, & Sanoamuang, 2015). Especially for fresh and brackish water species, fairy shrimp may be a valuable alternative for the use of the widely used larvae (nauplii) of the brine shrimp *Artemia*, but relatively little is known about their life cycle, except for a few studies, for example Beladjal, Khattabi, and Mertens (2003), Beladjal, Peiren, Vandekerckhove, and Mertens (2003), Dararat, Starkweather, and Sanoamuang (2011), and Atashbar et al. (2014).

To reduce nutrient inputs through the feed and its effects on the environment and to optimize recycling of nutrients in effluents, several aquaculture techniques and rearing systems have been developed (Nevejan et al., 2016). In Iran, trout *Oncorhynchus mykiss* is one of the most common cold water fish produced, with a production of 127,000 tons in 2014 (FAO 2015). Hence massive amounts of trout farm effluents are readily available as a potential culture medium for fairy shrimp. In a first study (Pormehr Yabandeh, Beladjal, Agh, Atashbar, & Van Stappen, 2017), initial data were obtained demonstrating that *B. orientalis* can survive and grow in trout pond effluents. However, their reproductive performance in this environment was not studied. Therefore, the purpose of this study was assessing the potential of using trout pond effluents as an eco-friendly, low-cost, easily accessible and nutritious culture medium by studying the reproductive characteristics of *B. orientalis* when cultured in trout pond effluents. As culture medium either fresh effluent was used supplemented with microalgae or non-supplemented effluent, but after an "ageing" process of the culture medium. We wanted to test whether "ageing" of the effluent would compensate for the supplementation with microalgae, which are the most common feed source when culturing fairy shrimp.

2 | MATERIAL AND METHODS

2.1 | Resting egg hatching

Resting eggs of *B. orientalis* provided by the *Artemia* and Aquaculture Research Institute (AARI), Urmia University, Iran, were hatched using tap water (EC: 265 µS/cm, pH 8.2) at 21°C under constant white fluorescent light (Atashbar et al., 2012).

These resting eggs had been stored at AARI since their collection in the summer of 2015 from Khaselou, a small pool (120 m^2, 40 cm depth, 1297 m a.s.l, 37°49′50″N-45°50′03″E) located north east of Urmia Lake in the East Azerbaijan region, Iran.

2.2 | Feed preparation

2.2.1 | Pond effluent

Wastewater from trout ponds was transferred into two 1000-L concrete ponds and left for 3 weeks at natural photoperiod to allow the growth of unicellular algae, heterotrophic bacteria and the development of the microbial community which consequently can form flocs. To oxygenate this so-called aged water, to keep its particles in suspension and to provide optimal conditions for aerobic bacteria, an aeration system was fixed at the bottom of the ponds. After this period, to eliminate large aquatic organisms, the collected aged water was passed through a 50-µm sieve (estimated as maximum uptake size of *B. orientalis*) before transferring it into the growth test vessels. This aged water was used as culture medium as such and as the only feed source in treatment 1 (T1). In two other treatments, fresh non-aged effluent was used as culture medium, supplemented with either the microalgae *Scenedesmus* sp. (T2) or *Haematococcus* sp. (T3).

2.2.2 | Microalgae

Algae (*Scenedesmus* sp. and *Haematococcus* sp.), being the most prevalent freshwater microalgae for fairy shrimp culture (Atashbar et al., 2012), were cultured under standard laboratory conditions, as used at the Terrestrial Ecology Unit (TEREC) (Ghent University, Belgium), by diluting 20 ml of both a standard mineral and a nutrient solution (modified from Guillard & Lorenzen, 1972) in a 2-L bottle filled with tap water together with 50 ml of *Scenedesmus* sp. and *Haematococcus* sp. originating from a cultured stock as inoculum from TEREC and AARI, respectively. Intense aeration, 24-h photoperiod of constant light (1,500 lux of white fluorescent light) and heating (22–25°C) were provided to stimulate photosynthesis. After 5–7 days, the algae were counted with a haemocytometer and in case of low cell density, they were centrifuged to increase the concentration to 18×10^6

TABLE 1 Coefficient of increase in feed quantity as compared to the previous day, as a function of growth, based on Coutteau et al. (1992)

Day	2-3-4	5-6	7	8	9	10-11	12-13	14-15	16-17	18-19	20 and following
Coefficient of increase	2	1.5	1.33	1.25	1.62	1.18	1.25	1.2	1.17	1.21	1.18

cells/ml as target. This concentrated algae suspension was used for maximally 5 days while kept in the refrigerator at 4°C.

2.3 | Growth and survival test

Feeding was started in 2-L cylindrical culture vessels, containing 200 *B. orientalis* individuals each, and continued until the end of the experiment after 21 days, in all the treatments with *B. orientalis* cultured with effluent of rainbow trout ponds, either aged non-supplemented (T1) or fresh supplemented with *Scenedesmus* sp. (T2) and with *Haematococcus* sp. (T3). Each treatment was conducted in three replicates. Feed quantity used on day 1 for T2 and T3 was 0.8 ml of algae suspension, based on an *Artemia* feeding regime as described by Coutteau, Brendonck, Lavens, and Sorgeloos (1992). But compared to *Artemia*, twice the amount of feed was applied because of the bigger size and higher weight of adult *B. orientalis*. The daily feeding ration was adjusted to compensate for growth, according to the coefficient of daily increase (Coutteau et al., 1992). It was also adjusted according to the number of surviving animals (Table 1). From day two of the experiment onward, 30% of the culture medium was renewed every day in all treatments. Mild aeration was provided in the vessels. The ambient temperature for the culture system was 18 ± 1°C with a natural photoperiod of approximately 12:12 L:D. Abiotic factors (temperature, oxygen: 6.9–7.9 mg L, and pH: 7.5–8.0) and total dissolved solids (TDS: 310–550 mg/L) were measured daily using sensors (CRISON MM40, Spain). The levels of NH_4^+, NO_2^- and NO_3^- were recorded every week using a photometer (Palintest 7500, UK) and remained within the ranges 0.5–0.9, 0.07–1.0 and 0.5–1.7 mg/L, respectively.

For biometrical analysis, 50 fairy shrimps were taken randomly from the hatching container at day 1, and 10 fairy shrimps from each replicate of the treatments at days 7, 15 and 21 of the growth test. The animals were measured from the anterior part of the head to the posterior margin of the telson using a stereomicroscope (Stemi SV 11, Zeiss, Germany) equipped with a drawing tube. The drawings were digitized using a digitizer (Graphtec Corporation KD 3320) connected to a computer (see Beladjal, Vandekerckhove et al., 2003; Atashbar et al., 2012 for details). Also the survival in each replicate was checked on days 7, 15 and 21 by counting the animals individually using a counter.

2.4 | Reproduction test

Upon reaching maturity, 15 pairs from each treatment were randomly taken; each pair was placed separately in a 70-ml cylindrical cup, containing 50 ml of medium from the growth test vessels at 18 ± 1°C. The amount of feed given to each couple per day was based on the feeding regime used in the growth test (so taking into account the age of the animals), but sometimes feeding was adjusted depending on the transparency of the culture medium, based on visual inspection. The cups were monitored daily, the produced resting eggs were counted and removed from the cups, and a number of reproductive characteristics were calculated (see Table 4). The reproductive test lasted as long as the females were alive. In case of male death, the male was replaced by another male from the corresponding growth test vessels (Beladjal, Khattabi et al., 2003; Beladjal, Vandekerckhove et al., 2003; Atashbar et al., 2012). No aeration was used in this test.

During the reproductive test, the diameter of 100 resting eggs from each brood in all the treatments was measured using a light microscope with an eyepiece equipped with a graticule (Sorgeloos, 1997).

2.5 | Statistical analysis

The results were statistically analysed using SPSS (version 16) through an analysis of variance (one-way ANOVA). For all dependent variables expressed as percentages, the assumption of normal distribution within groups was fulfilled (Kolmogorov–Smirnov test) and Levene's test was used to assess of the homogeneity of variances. The averages were compared using the post hoc Tukey test. $p < .05$ was chosen as significance level. The variance of the data is reported as standard deviation (*SD*) of the mean of three replicates for growth and survival of *B. orientalis*, of 15 replicates for the reproductive test, and of 100 resting eggs for the resting egg diameter.

3 | RESULTS

At day 7, the non-supplemented treatment (T1) was performed significantly ($p < .05$) lower in terms of survival than the other two treatments (T2 and T3). By the end of the growth test (day 21), survival percentage in the treatment supplemented with *Haematococcus* sp. (T3: 53.0%) was significantly higher than in the non-supplemented treatment (T1: 40.8%), with the treatment supplemented with *Scenedesmus* sp. taking an intermediate position (T2: 45.3%) (Table 2).

The total length at day 21 was significantly higher in the treatment supplemented with *Haematococcus* sp. (T3: 11.6 mm) in comparison with the other two treatments that showed no significant difference between them (T1: 9.3, T2: 9.0 mm) (Table 3).

First, sexually mature individuals were observed at days 18, 22 and 16 in T1, T2 and T3, respectively. Females in the treatment supplemented with *Haematococcus* sp. had significantly higher values for resting eggs per brood (T3: 133) than the non-supplemented treatment (T1: 105) and than the one supplemented with *Scenedesmus* sp. (T2: 107). In addition, the number of broods per female in T3 (7.7) was significantly higher than in T1 (6.5). Consequently, the total number of resting eggs in the treatment supplemented with *Haematococcus* sp. (T3: 1014) was significantly higher than the non-supplemented treatment and also than the treatment supplemented with *Scenedesmus* sp. (688 and 750 resting eggs, respectively). In the mentioned reproductive parameters, no significant differences were observed between T1 and T2. The highest value for total number of resting eggs, observed in a single female, was 1380. On the other hand, feeding *B. orientalis* with fresh effluent supplemented with *Scenedesmus* sp. (T2) resulted in a significantly longer reproductive period (29.7 days) compared to the non-supplemented treatment (T1: 24.7 days), whereas the differences between T2 and T3 (28.7 days) were not significant (Table 4).

In all broods (except brood 3), the mean resting egg diameter from the treatment supplemented with *Haematococcus* sp. (T3) was significantly lower compared to the non-supplemented treatment (T1) and/or the one supplemented with *Scenedesmus* sp. (T2). The mean resting egg diameter was the highest (297.7 μm) in brood 6 of the treatment supplemented with *Scenedesmus* sp. while it was the lowest (249.8 μm) in brood 7 (the last brood) of the treatment supplemented with *Haematococcus* sp. (Table 5). Individual resting egg diameters ranged between 189.4 and 357.1 μm.

TABLE 2 Survival (%) (mean ± *SD*, *n* = 3) of *Branchinecta orientalis*, cultured with effluent of rainbow trout ponds, either aged non-supplemented (T1) or fresh supplemented with *Scenedesmus* sp. (T2) and with *Haematococcus* sp. (T3)

Treatments	Day 7	Day 15	Day 21
1	64.3 ± 0.8^{a}	49.7 ± 3.2^{a}	40.8 ± 4.2^{a}
2	90.5 ± 1.3^{b}	58.0 ± 7.2^{ab}	45.3 ± 2.4^{ab}
3	92.7 ± 2.6^{b}	70.3 ± 3.5^{b}	53.0 ± 4.1^{b}

Values in the same column followed by different letters are significantly different ($p < .05$).

4 | DISCUSSION

Undoubtedly live feed has a vital position in larviculture of many aquatic organisms. The exploration of the potential of production and use of fairy shrimps as an alternative live feed for *Artemia*, chiefly for fresh and brackish water species, is an important issue. To provide more information about this issue, studies are needed not only on growth and survival of fairy shrimps but also on their reproductive characteristics.

TABLE 3 Growth (mm) (mean ± *SD*, *n* = 3) of *B. orientalis*, cultured using effluent of rainbow trout ponds, either aged non-supplemented (T1) or fresh supplemented with *Scenedesmus* sp. (T2) and with *Haematococcus* sp. (T3)

Treatments	Day 1	Day 7	Day 15	Day 21
1	0.54 ± 0.07	2.59 ± 0.49^{a}	7.61 ± 1.41^{a}	9.31 ± 1.28^{a}
2	0.54 ± 0.07	3.44 ± 0.61^{b}	7.99 ± 0.82^{a}	9.04 ± 0.99^{a}
3	0.54 ± 0.07	4.02 ± 0.69^{c}	10.22 ± 0.94^{b}	11.61 ± 0.83^{b}

Values in the same column followed by different letters are significantly different ($p < .05$).

TABLE 4 Reproductive characteristics of *B. orientalis* (mean (*SD*), *n* = 15 replicates, one couple in each) cultured using effluent of rainbow trout ponds, either aged non-supplemented (T1) or fresh supplemented with *Scenedesmus* sp. (T2) and with *Haematococcus* sp. (T3)

Treatments	1	2	3
Total number of resting eggs	688.13 (179.12)^{a}	749.90 (195.50)^{a}	1013.93 (164.56)^{b}
Number of broods per female	6.53 (1.13)^{a}	7.07 (0.96)^{ab}	7.67 (1.11)^{b}
Intervals between broods (days)	4.07 (0.81)^{a}	4.39 (0.67)^{a}	3.97 (0.46)^{a}
Resting eggs per brood	105.48 (22.64)^{a}	106.78 (27.43)^{a}	133.05 (17.32)^{b}
Prereproductive period (days)	18.40 (0.63)^{b}	22.27 (0.46)^{c}	15.60 (0.74)^{a}
Reproductive period (days)	24.73 (5.44)^{a}	29.73 (4.80)^{b}	28.67 (5.60)^{ab}
Postreproductive period (days)	1.13 (1.51)^{a}	0.60 (0.91)^{a}	0.47 (0.64)^{a}
Days without resting egg production (%)	40.07 (6.24)^{a}	41.08 (12.47)^{a}	41.23 (5.71)^{a}
Lifespan (days)	44.60 (5.50)^{a}	51.27 (4.91)^{b}	42.60 (5.94)^{a}

Values in the same row followed by different letters are significantly different ($p < .05$).

TABLE 5 Resting egg diameter (μm) in all broods of *B. orientalis* (mean (*SD*), *n* = 100 resting eggs), cultured using effluent of rainbow trout ponds, either aged non-supplemented (T1) or fresh supplemented with *Scenedesmus* sp. (T2) and with *Haematococcus* sp. (T3)

	Broods						
Treatments	**1**	**2**	**3**	**4**	**5**	**6**	**7**
T1	264.8 (24.5)^{a}	269.2 (21.6)^{b}	281.7 (28.9)^{a}	278.6 (23.0)^{b}	281.6 (25.6)^{b}	295.2 (20.7)^{b}	263.8 (24.4)^{b}
T2	276.3 (21.7)^{b}	280.8 (21.1)^{c}	283.7 (31.8)^{a}	258.2 (19.6)^{a}	271.6 (16.7)^{a}	297.7 (25.7)^{b}	251.4 (24.5)^{a}
T3	265.6 (24.1)^{a}	257.4 (25.3)^{a}	281.4 (20.8)^{a}	262.4 (25.5)^{a}	274.2 (20.0)^{a}	274.5 (20.9)^{a}	249.8 (21.3)^{a}

Values in the same column followed by different letters are significantly different ($p < .05$).

The present study demonstrates that the growth and survival of *B. orientalis* were not significantly affected using either aged non-supplemented effluent or fresh effluent supplemented with *Scenedesmus* sp. The highest length values obtained after 21 days of culture in our study (11.6 mm) are almost equal as in Atashbar et al. (2012), who obtained 11.7 mm in 1-L vessels with the same species, culture density and temperature using only *Scenedesmus* sp. as feed. However, the maximal final survival at day 21 obtained in the present study (53.0%) was substantially lower than in Atashbar et al. (2012) (81.7%).

Our results also indicated that most reproductive characteristics were significantly correlated with the applied feed. The ranges of mean total number of resting eggs (688–1014) and mean resting eggs per brood (105–133) in our study are in accordance with Atashbar et al. (2012) who obtained similar results at 18°C for total number of resting eggs (1094) and resting eggs per brood (109) over a total lifespan of 51 days, when *B. orientalis* was fed only *Scenedesmus* sp. On the contrary, the values of total number of resting eggs in three other species of fairy shrimps, *Streptocephalus sirindhornae* (Sanoamuang, Murugan, Weekers, and Dumont 2000), *Streptocephalus siamensis* (Sanoamuang & Saengphan 2006) and *Branchinecta thailandesis* (Sanoamuang, Saengphan and Murugan 2002) fed *Chlorella* sp. were considerably higher (17865, 7634 and 6365 resting eggs, respectively) (Dararat et al., 2011) than in our study. The objective of the study of Dararat et al. (2011) was to define the biological characteristics of the three mentioned species of fairy shrimps and to assess their suitability for aquaculture and other commercial purposes. *B. thailandensis* showed rapid growth and high hatching percentage, which is suitable for mass production, while *S. sirindhornae* had high fecundity which is appropriate for the commercial production of eggs (Dararat et al., 2011). In addition, the number of broods per female in Atashbar et al. (2012) (11.4) was higher than in the present work (6.5–7.7). The prereproductive period of *B. orientalis* in this study (15.6–18.4 days) was lower than in *B. orientalis* in Atashbar et al. (2012) (22.4 days), while the reproductive period in the present study (24.7–29.7 days) was in line with Atashbar et al. (2012) (28.4 days), when *B. orientalis* was fed *Scenedesmus* sp. Despite the similarities between our study and that of Atashbar et al. (2012) in terms of *Branchinecta* species, culture density and temperature used, the differences in survival and a number of reproductive parameters between the two studies might be related to differences in other culture conditions, such as the properties of the culture medium (tap water) in Atashbar et al. (2012).

The values for resting egg size, found in our study (250–298 μm), were to a large extent in the same range as those found in literature: 263 μm in Petkovski (1991) and 277–280 μm in Thiéry, Brtek, and Gasc (1995), both under natural conditions; 275–292 μm in Atashbar et al. (2012) under laboratory conditions. However, Alonso and Alcaraz (1984) reported a smaller size (219 μm), whereas Atashbar et al. (2012) found a higher value in the natural biotope (Khaselou pool: 311 ± 23 μm). In our study, perhaps the higher resting egg production in the treatment with *Haematococcus* sp. may be linked to the smaller resting egg size, although in Atashbar et al. (2012), both the highest resting egg production and biggest resting egg size were observed in the same treatment at 18°C. In addition to the present study which demonstrates significant effects of the type of feed on resting egg diameter of *B. orientalis*, Atashbar et al. (2012) reported significant effects of temperature on resting egg diameter: significantly larger resting eggs were produced at 18°C (optimal temperature to culture *B. orientalis*), than at higher (21 and 27°C) and lower (at 12 and 15°C) temperatures. In our study, the resting egg diameter seemed not to follow a specific pattern (e.g., increasing or decreasing resting egg size) with the ranking number of the brood.

Overall, the treatment fed fresh effluent supplemented with *Haematococcus* sp. produced better results. *Haematococcus* sp. is a unicellular biflagellate green microalgae species. Its cell morphology has been described by Yamagishi and Akiyama (1998). *Scenedesmus* sp. can make colonies of two, four, eight and 16 cells attached side by side, arranged linearly or zigzag, as described in detail by Akiyama (1977). *Haematococcus* sp. has been reported to contain more astaxanthin than *Scenedesmus* sp. (Orosa, Valero, Herrero, & Abalde, 2001), but it is not clear what of its features contribute to the better performance of *Branchinecta* in our study.

Culturing algae requires considerable expenses and skilled labour, while preparation of the fish effluent as an easy to use, available and cheap nutrient source does not need very specific knowledge nor equipment. Such advantages can improve the cost/benefit ratio and facilitate the use of this potential source of feed by farmers. Additionally, as part of the suspended materials is recycled as a feed for *B. orientalis*, using the effluent can decrease the nutrient loads discharged into the environment (Pormehr Yabandeh et al., 2017).

The presence of bioflocs in the treatment fed aged non-supplemented effluent might be the reason of similar performance in terms of reproductive characteristics of *B. orientalis* compared to the treatment fed fresh effluent supplemented with *Scenedesmus* sp. Although no proper quantification of bioflocs was performed, they may have contributed to *Branchinecta* nutrition (Pormehr Yabandeh et al., 2017). Flocculation in biological systems is a complex process. In the effluent, the combination of environmental (physical, chemical and biological factors) aspects influences the production and characteristics of the flocs (Salehizadeh & Shojaosadati, 2001): organic loading rate, dissolved oxygen levels, temperature, pH, salinity and especially mixing intensity affect the quantitative and/or qualitative aspects of floc formation (De Schryver, Crab, Defoirdt, Boon, & Verstraete, 2008). Thus, a correct steering of these parameters might optimize the use of aged non-supplemented effluent as a promising feed.

As a conclusion, additional feeding of algae generally did not lead to significant improvement of reproductive performance of *B. orientalis* in comparison with the aged non-supplemented effluent. Thus, in this regard, aged effluent can be used as a reliable feed to substitute microalgae. The outcomes of this study can be useful for future research, either indoor or outdoor, to optimize using effluents from culture systems of aquatic organisms, such as freshwater fish, for mass culture of *B. orientalis*. Also, investigating the hatching characteristics of resting eggs of fairy shrimps, produced in these conditions, is highly recommended, especially when aiming at their commercial production. Finally, the characteristics of the planktonic community (phytoplankton and zooplankton) present in the residual water of the trout crop should be documented to understand how it contributes to the culture success of *B. orientalis*.

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ORCID

N. Pormehr http://orcid.org/0000-0002-1158-7279
L. Beladjal http://orcid.org/0000-0002-5755-7235

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