Multi-effects of temperature and particle size on the filter-feeding rate of brine shrimp Artemia at different growth stages and densities

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Abstract

Brine shrimp *Artemia* is able to filter particulate substances non-selectively and continuously, which make it an useful experimental animal in aquatic toxicological study. In this study, the filter-feeding rate (FFR) of *Artemia franciscana* at different temperatures (20°C/25°C/30°C) and densities (20/40/75/100 ind./100 mL at two earlier growth stages; 5/10/20 ind./100 mL at two later growth stages) on three unicellular algae (*Chlorella vulgaris*, *Porphyridium purpureum*, *Phaeodactylum tricornutum*) and two sizes of polyethylene balls (30 µm and 50 µm) was determined at *Artemia* four growth stages. The results showed that the FFR was positively correlated with the ambient temperature and *Artemia* body length, while it was negatively correlated with the *Artemia* density and particle size, and one way ANOVA analysis showed that the above factors mostly had significant effects on FFR (P < 0.05). And the favorable filtration particle size of *Artemia* increased with its body length. The equation of FFR in function of temperature, *Artemia* body length and density, and particle size was obtained using multiple linear regression analysis: FFR = 0.487*BL + 0.067*T-0.01D-0.064PS-1.508 (R^2 = 0.513). Of these four variables, body length had the greatest effect on FFR, followed by ambient temperature and particle size, and *Artemia* density. The results of this study provide a valuable guidance for proper feeding in the controlled *Artemia* production and standardization of experimental protocol of ecotoxicity and fundamental *Artemia* research, as well as facilities the application of *Artemia* in aquaculture wastewater treatment.

1. Introduction

Brine shrimp *Artemia* is extensively dispersed in salt lakes and coastal saltponds, with a wide range of tolerance and adaptation to the water temperature, salinity, ionic composition and pH, etc. (Gajardo and Beardmore, 2012; Libralato et al., 2016). The zooplanktonic *Artemia* is an important item of the food chain in hypersaline waterbody, serving as the main consumer of phytoplankton (Lenormand et al., 2018), the host of various parasites (Redón et al., 2011), as well as the prey of aquatic birds (Green et al., 2005; Muñoz et al., 2013). Apart from being a crucial live feed of marine fish and shrimp larvae (Sorgeloos et al., 2001), the biological characteristics of *Artemia* such as non-selective filter-feeding behavior, ease of cultivation, short life cycle, and high number of offspring per brood, make it a widely used experimental organism in aquatic ecotoxicology study (Jeyavani et al., 2022; Tzima et al., 2022). Moreover, *Artemia* has been shown to be effective in removing particulate nitrogen (i.e. undigested feed and manure) from aquaculture system (Marinho-Soriano et al., 2011), and thus can be applied in bioremediation of aquaculture effluent.

*Artemia* is a continuous and non-selective filter feeder that can take up particles smaller than 50 µm (Nevejan et al., 2018). As a primitive crustacean, the nauplius of *Artemia* grows and differentiates via about 15 molts before reaching adult. The newly hatched instar I nauplius is unable to eat which survives on their yolk reserves. But 8 h after hatching when molting into the instar II stage, *Artemia* nauplius starts filtering the food particles through flapping the bristles on their appendages (Lavens and Sorgeloos, 1996). *Artemia* can feed on a wide range of particulate substances, including microalgae (Gui et al.,
bacteria and archaea (Lopes-dos-Santos et al., 2019a; Lopes-dos-Santos et al., 2019b), as well as organic debris (Ogburn et al., 2023).

Artemia exhibits different FFR with favorable food size options while growing. Makridis (1999) demonstrated that Artemia franciscana preferred particle size of 3–8 µm at 2-, 4- and 7- days old and its maximum FFR increased with Artemia growth. Through determining the remaining particles in Artemia gut, Fernández et al. (2001) reported the majority of the particles filtered by Artemia (body length of 1–9 mm) ranged of 6.8–27.5 µm, with an ideal particle size of approximately 16.0 µm. Riisgard et al. (2015) reported that FFR of Artemia (body length of 0.95-10 mm) is positively correlated with the ambient temperature in range of 19.9–26.9℃. Nevertheless, the FFR of Artemia is affected by various factors, which is influenced not only by the particle size and growth stage of Artemia, but also by the density of Artemia and ambient temperature, etc.

In this study, the FFR of Artemia towards five sizes of particulate substances at three ambient temperatures were investigated with four Artemia densities and at four growth stages, and trends in FFR were plotted in correlation with the above parameters. The outcome of the study will provide a guidance for the standardization of experimental feeding protocol in ecotoxicity and fundamental Artemia research, and proper feeding strategy in the controlled Artemia production, as well as facilities the application of Artemia in aquaculture wastewater treatment.

2. Materials and methods

2.1 Experimental design

The cysts of Artemia franciscana originated from Great Salt Lake, Utah, USA (INVE Aquaculture, Belgium) were hatched at 28℃ for 24 h. The newly hatched nauplii were grown at 25℃ and fed with microalgae Chlorella vulgaris until reaching the desired body lengths. Artemia at four growth stages were chosen for filtration tests, i.e., three days after hatching (day 3, average body length of 0.91 ± 0.05 mm), five days after hatching (day 5, average body length of 1.53 ± 0.22 mm), fourteen days after hatching (day 14, average body length of 4.72 ± 0.51 mm) and twenty-five days after hatching (day 25, average body length of 10.26 ± 0.46 mm).

To study the FFR of various particle size, three species of microalgae (i.e. green algae Chlorella vulgaris (average size of 2.50 ± 0.34 µm), red algae Porphyridium purpureum (average size of 7.30 ± 1.59 µm) and diatom Phaeodactylum tricornutum (average size of 26.87 ± 3.57µm × 12.24 ± 2.63 µm), and two sizes of polyethylene balls (30 µm and 50 µm, respectively) were chosen. C. vulgaris was provided by SDIC Biotechnology Investment Co., Ltd. P purpureum and Ph. tricornutum were provided by Tianjin Fisheries Research Institute. The polyethylene balls were purchased from Beijing Biotyscience Technology, Co., Ltd. A hemocytometer was used to quantify the algal cell density, and a microscope was used to determine the size of algal cells.
To study the influence of *Artemia* density on the FFR, four *Artemia* density gradients (20 ind./100 mL, 40 ind./100 mL, 75 ind./100 mL and 100 ind./100 mL, respectively) were set for *Artemia* at two earlier growth stage (day 3 and day 5), while three *Artemia* density gradients (5 ind./100 mL, 10 ind./100 mL and 20 ind./100 mL, respectively) were set for *Artemia* at two later growth stage (day 14 and day 25).

All experiments were conducted at three water temperatures (20°C, 25°C and 30°C) and in triplicates. A total of 144 combinations of factors and levels were measured (Table 1).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Average <em>Artemia</em> body length (mm)</th>
<th><em>Artemia</em> density (ind./100 mL)</th>
<th>Particle concentration (cells or particles/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. vulgaris: 1×10⁶</td>
</tr>
<tr>
<td>20/25/30</td>
<td>0.91 ± 0.05</td>
<td>20/40/75/100</td>
<td>√</td>
</tr>
<tr>
<td>20/25/30</td>
<td>1.53 ± 0.22</td>
<td>20/40/75/100</td>
<td>√</td>
</tr>
<tr>
<td>20/25/30</td>
<td>4.72 ± 0.51</td>
<td>5/10/20</td>
<td>√</td>
</tr>
<tr>
<td>20/25/30</td>
<td>10.26 ± 0.46</td>
<td>5/10/20</td>
<td>√</td>
</tr>
</tbody>
</table>

### 2.2 Filter-feeding experiments

The brine water (salinity 150) obtained from local artisanal saltworks was diluted with tap water. After being autoclaved and removed the debris, the diluted brine (salinity 30) was filtered through 0.45 µm cellulose acetate membrane. Prior to the experiments, *Artemia* were starved for 24 h to empty the gut and to adapt the setting temperature. *Artemia* with desired numbers were quickly transferred to the 200 mL glass cones containing 100 mL diluted brine and target particles with desired concentrations. Gentle aeration was given to ensure a proper dissolved oxygen level (5–6 mg/L) and the suspension of particles. Exact one hour later, the particle density in each cone were examined and the FFR was calculated as follows:

\[
FFR = \frac{c_0 - c_t}{t \times N} \times 100\%
\]

*C₀*: initial particle density before filtration; *Cₜ*: final particle density after filtration; *t*: time in hour; *N*: number of *Artemia*.

### 2.3 Data analysis
Data were expressed as mean ± standard deviation. The significant difference of FFR among different groups were analyzed by One-way analysis of variance (P < 0.05, IBM SPSS Statistics 26). The multiple linear regression analysis was conducted by IBM SPSS Statistics 26. Origin (2021) was used to fit on the data. Graphpad Prism 8 was used to plot the graph.

3. Results

3.1 Effect of temperature on FFR

The combination of temperature levels and other factors mostly had significant effect on FFR (P < 0.05, Fig. 1, Tab. S1). The FFR of *Artemia* increased with rising temperature, and the highest FFR for all size of particulate substances were observed at 30°C. In addition, *Artemia* had their favorable filtration particle size at different growth stages and temperatures. On day 3 and day 5, *Artemia* had higher FFR for smaller-size *C. vulgaris*. At later growth stages, a higher FFR were observed for medium-size *P. purpureum* on day 14, and for bigger-size of *Ph. tricornutum* on day 25, respectively; while the FFR for polyethylene balls (30 µm and 50 µm) on day 25 was lower than that of microalgae. The fitting equations indicated that most of the FFR positively related to the ambient temperature level, with R² ranging from 0.84 to 0.99 (Table 2).
Table 2
Fitting equation of temperature to the FFR given in Fig. 1.

<table>
<thead>
<tr>
<th>Growth stages of Artemia</th>
<th>Equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 3●</td>
<td>FFR = 49.50T−515.69</td>
<td>0.99</td>
</tr>
<tr>
<td>day 3■</td>
<td>FFR = 45.95T−506.34</td>
<td>0.99</td>
</tr>
<tr>
<td>day 3▲</td>
<td>FFR = 17.74T−110.31</td>
<td>0.99</td>
</tr>
<tr>
<td>day 5●</td>
<td>FFR = 171.16T−479.57</td>
<td>0.99</td>
</tr>
<tr>
<td>day 5■</td>
<td>FFR = 117.03T−54.71</td>
<td>0.99</td>
</tr>
<tr>
<td>day 5▲</td>
<td>FFR = 75.65T + 503.7059</td>
<td>0.95</td>
</tr>
<tr>
<td>day 14●</td>
<td>FFR = 637.87T−4559.82</td>
<td>0.94</td>
</tr>
<tr>
<td>day 14■</td>
<td>FFR = 466.38T + 1727.79</td>
<td>0.99</td>
</tr>
<tr>
<td>day 14▲</td>
<td>FFR = 632.44T−7948.86</td>
<td>0.99</td>
</tr>
<tr>
<td>day 25●</td>
<td>FFR = 1830.40T−19716.46</td>
<td>0.84</td>
</tr>
<tr>
<td>day 25■</td>
<td>FFR = 1350.71T−2588.88</td>
<td>0.99</td>
</tr>
<tr>
<td>day 25▲</td>
<td>FFR = 1337.82T + 31257.29</td>
<td>0.99</td>
</tr>
<tr>
<td>day 25□</td>
<td>FFR = 437.32T−6557.41</td>
<td>0.96</td>
</tr>
<tr>
<td>day 25△</td>
<td>FFR = 216.81T−1870.06</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Note: ● indicate Artemia filter-feeding on C. vulgaris; ■ indicate Artemia filter-feeding on P. purpureum; ▲ indicate Artemia filter-feeding on Ph. tricornutum; □ indicate Artemia filter-feeding on 30 µm polyethylene balls; △ indicate Artemia filter-feeding on 50 µm polyethylene balls.

### 3.2 Effect of Artemia density on FFR

The combination of Artemia density levels and other factors mostly had significant effect on FFR (P < 0.05, Fig. 2, Tab S2). The FFR decreased with the increasing Artemia density. Artemia had the highest FFR for all particle sizes at the lowest density, and the lowest value was obtained at the highest Artemia density. Artemia had a higher FFR for C. vulgaris on day 3 and day 5, while a higher FFR for P. purpureum were obtained on day 14, and a higher FFR for Ph. tricornutum were obtained on day 25. Similarly, Artemia had a lower FFR for polyethylene balls (30 µm and 50 µm) than microalgae. The fitting equations indicated that most of the FFR negatively related to the Artemia density level except day 5▲ and day 25△ (Table 3).
<table>
<thead>
<tr>
<th>Growth stages of <em>Artemia</em></th>
<th>Equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 3●</td>
<td>FFR = -3.17D + 828.34</td>
<td>0.88</td>
</tr>
<tr>
<td>day 3■</td>
<td>FFR = -4.62D + 834.03</td>
<td>0.87</td>
</tr>
<tr>
<td>day 3▲</td>
<td>FFR = -2.61D + 384.80</td>
<td>0.91</td>
</tr>
<tr>
<td>day 5●</td>
<td>FFR = -19.79D + 4503.61</td>
<td>0.99</td>
</tr>
<tr>
<td>day 5■</td>
<td>FFR = -14.77D + 3494.86</td>
<td>0.91</td>
</tr>
<tr>
<td>day 5▲</td>
<td>FFR = -7.32D + 2220.54</td>
<td>0.25</td>
</tr>
<tr>
<td>day 14●</td>
<td>FFR = -443.25D + 22265.63</td>
<td>0.98</td>
</tr>
<tr>
<td>day 14■</td>
<td>FFR = -936.18D + 34091.59</td>
<td>0.98</td>
</tr>
<tr>
<td>day 14▲</td>
<td>FFR = -367.62D + 18477.82</td>
<td>0.99</td>
</tr>
<tr>
<td>day 25●</td>
<td>FFR = -1543.89D + 59314.87</td>
<td>0.99</td>
</tr>
<tr>
<td>day 25■</td>
<td>FFR = -2799.65D + 93720.24</td>
<td>0.98</td>
</tr>
<tr>
<td>day 25▲</td>
<td>FFR = -3831.13D + 164122.65</td>
<td>1</td>
</tr>
<tr>
<td>day 25□</td>
<td>FFR = -1221.49D + 30679.73</td>
<td>0.97</td>
</tr>
<tr>
<td>day 25△</td>
<td>FFR = -270.48D + 9371.82</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Note: ● indicate *Artemia* filter-feeding *C. vulgaris*; ■ indicate *Artemia* filter-feeding *P. purpureum*; ▲ indicate *Artemia* filter-feeding *Ph. tricornutum*; □ indicate *Artemia* filter-feeding 30 µm polyethylene balls; △ indicate *Artemia* filter-feeding 50 µm polyethylene balls.

### 3.3 Effect of particle size on FFR

The combination of particle size levels and other factors mostly had significant effect on FFR ($P < 0.05$, Fig. 3, Tab S3). The FFR of *Artemia* gradually decreased with the increase of particle size on day 3 and day 5, but the FFR did not show a similar trend at two later growth stages. On day 14, FFR peaked at medium particle size (*P. purpureum* with particle size range of 5.79–8.89 µm), but abruptly decreased for polyethylene balls (Fig.B). On day 25, FFR peaked at larger particle size (*Ph. tricornutum* with average size of 26.87 ± 3.57µm × 12.24 ± 2.63 µm), and then abruptly decreased for polyethylene balls (Fig. S1).

### 3.4 Effect of *Artemia* body length on FFR

At the same temperature and density, the FFR of *Artemia* increased with the increase of body length, the combination of body length levels and other factors mostly had significant effect on FFR ($P < 0.05$, Fig. 4, Tab S4). Similar to the experimental results of temperature, density and particle size, *Artemia* had higher FFR for *C. vulgaris* on day 3 and day 5 (Fig. S2), and they had higher FFR for *P. purpureum* on day 14. On
day 25, the FFR for *Ph. tricornutum* was higher. In addition, from day 14 to day 25, FFR increased rapidly with the increase of body length.

### 3.5 Combined effect on FFR

According to 144 combinations of factors and levels (Tab. S5), the multiple linear regression fitting equation was obtained: \( \text{FFR} = 0.487 \times \text{body length} + 0.067 \times \text{temperature} - 0.01 \times \text{density} - 0.064 \times \text{particle size} - 1.508 \), with explained variance \( R^2 = 0.513 \), indicating that the four factors had 51.3% effect on the FFR of *Artemia*. Of these four variables, body length of *Artemia* had the greatest effect on FFR, followed by ambient temperature and particle size, and the least effect was *Artemia* density.

### 4. Discussion

Understanding the FFR of *Artemia* helps to apply better feeding strategy in *Artemia* culture and formulate practical experiment protocol in ecotoxicity research. As early as 1960s, Reeve (1963) studied the FFR of *Artemia* on three microalgae (*Ph. tricornutum*, *Dunaliella tertiolecta* and *Chlorella stigmatophor*) at six growth stages (body length of 0.5 mm, 1.0 mm, 2.0 mm, 5.0 mm, 7.5 mm and 10 mm, respectively). The results showed that *Artemia* could regulate the FFR according to the food size and growth stage, which tended to feed on bigger microalgae *D. tertiolecta* cells at the same food concentration, and the FFR increased with the body length of *Artemia*. Makridis et al. (1999) also showed that *Artemia* within 7 days after hatching preferred particle size of 4–8 µm. Fernández et al. (2001) studied the filter feeding ability of *Artemia* for different particle size, revealing that *Artemia* at different growth stages were selective for particle size. Recently, Riisgård et al (2015) studied the effects of body length and temperature on FFR, and the results showed that as body length and temperature rise, FFR also increased. Overall, the previous studies indicate the food preference in the early growth stages of *Artemia*, there was a greater preference for foods with small particle sizes, but this preference will change. In this study, the FFR of *Artemia* towards five sizes of particulate substances at three ambient temperatures were investigated with four *Artemia* densities and at four growth stages, these factors had a significant multi-effect on FFR.

In this study, *Artemia*'s FFR increased with temperature increase. Similar results were observed other filter feeding clam species *Ruditapes philippinarum* that the FFR increased with increased experimental temperature range (5°C-25°C) (Han et al., 2008). In general, higher ambient temperature generally leads to a higher metabolic rate, and therefore animal needs to take up more food and produce more energy (Clarke and Fraser, 2004). This is more obvious for the poikilotherms. On the other hand, when the temperature is out of tolerance, metabolic compensation may occur, resulting in an overall decrease in metabolism (Yampolsky et al., 2014). For example, the FFR of medium-sized oysters *Crassostrea nippona* reached the highest at 28°C in temperature range of 16°C-32°C. (Wang and Li, 2020). Similarly, the FFR of oysters *C. corteziensis* reached the highest at 29°C when being exposed to the temperature range of 23°C-32°C (Guzmán-Agüero et al., 2013). *Artemia* survive with appropriate temperature range of 15°C-30°C, but when the temperature reaches 34°C, survival rate decreases rapidly (Lavens and Sorgeloos, 1996; Pinto et al., 2013; Riisgård et al., 2015). In this study, three temperatures (30°C, 25°C and 20°C, respectively) were
set according to aquaculture practice, therefore we did not obtain the optimal temperature, which maybe observed at the temperature above 30.

Our results showed that FFR decreased with density increase. This is because the increase in density leads to greater competitive pressure under the limited food sources (Tantanasarit et al., 2013). There are relatively few studies on the relationship between the population density and FFR, with some studies in bivalves. Our results also showed that the FFR increases substantially with the increase of body length, and the favorable particle size gradually increased as well. Because the bristles are increasingly spaced when Artemia grows (Makridis, 1999), and larger particles can be filtered. Our results also proved that the FFR of Artemia decreased with the increase of the particle size.

It should be mentioned that we found a great difference in FFR between the flat and triangle-shaped diatom Ph. tricornutum (26.87±3.57 µm long and 12.24±2.63 µm wide) and polyethylene balls (30 µm diameter). The reasons for this may be the initial particle concentration of Ph. tricornutum was 10-folds of polyethylene balls (1×10^6 cells/mL for Ph. tricornutum and 1×10^5/ mL for polyethylene balls) on the one hand, and the shape preference of Artemia on the other (Evjemo and Olsen, 1999). We hypothesized that Artemia would prefer to filter from the side with smaller width when filtering Ph. tricornutum, while 30 µm polyethylene microplastics are regular and uniform spherical, much larger than the width of Ph. tricornutum, resulting in a higher FFR for Ph. tricornutum.

In this study, the levels of each factor were set according to the practical aquaculture conditions. However, there are other factors that may affect Artemia FFR, such as particle concentration (Kundu et al., 2021), salinity (El-Bermawi et al., 2004; El-Gamal, 2011), light (Asil et al., 2013), pH (Ben Naceur et al., 2012; Sui et al., 2014), etc.

## 5. Conclusion

The FFR of Artemia can be affected by various factors. It positively correlated with the increased temperature and body length, but negatively correlated with the increased density and particle size. As Artemia grows, its favorable particle changes from smaller size to bigger size. We also obtain the multiple linear regression fitting equation for Artemia, FFR = 0.487*BL + 0.067*T-0.01D-0.064PS-1.508, R^2 = 0.513.

## Declarations

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National Key Research and Development Plan Blue Granary Technology Innovation Project (2020YFD0900705): Demonstration of the Integration of three industries and three industries based on the purification and utilization of water resources in Bohai Bay tidal flat (2020YFD0900705-2).

### Competing Interests
The authors have declared that no competing interest exists.

**Author contributions**

Ke Li: Conducting the experiment, data analysis and manuscript preparation. Yudie Wang: Conducting the experiment and data analysis. Guoru Du and Xueliang Yao: microalga preparation. Hanyan Bao and Xuekai Han: providing the constructive discussions. Liying Sui: Experimental design, data analysis and manuscript revision.

**Data availability statement**

All relevant data are within the paper

**References**


**Figures**
Figure 1

Effect of temperature on FFR of *Artemia* at different growth stages (taking *Artemia* density of 20 ind./100 mL as an example).
Figure 2

Effect of *Artemia* density on FFR of *Artemia* at different growth stages (taking water temperature of 30°C as an example).

Figure 3

Effects of particle size on FFR of *Artemia* at different growth stage (taking *Artemia* density of 20 ind./100 mL as an example).
Figure 4

Selection of particulate substances and FFR of *Artemia* with different body lengths at temperature (30°C) and density (20 ind./100 mL).

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterialfigure.docx
- Supplementarymaterialtable.xlsx