EFFECT OF BIOFLOC ON THE SURVIVAL AND GROWTH OF THE POSTLARVAE OF THREE PENAEIDS (LITOPENAEUS VANNAMEI, FENNEROPENAEUS CHINENSIS, AND MARSUPENAEUS JAPONICUS) AND THEIR BIOFLOC FEEDING EFFICIENCIES, AS RELATED TO THE MORPHOLOGICAL STRUCTURE OF THE THIRD MAXILLIPED

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ABSTRACT

Bioflocs contribute to the nutrition and growth of some penaeid shrimp, such as *L. vannamei*, by providing an additional food source in intensive, limited exchange production systems. However, it remains unclear whether other penaeids could use bioflocs as food sources. Furthermore, little is known concerning the feeding mechanism of shrimp for harvesting and consuming bioflocs. This study determines whether three penaeid species could use bioflocs as food sources and suggests mechanical functions of the third maxilliped to collect bioflocs in a water column. The postlarvae of *L. vannamei*, *F. chinensis*, and *M. japonicus* were assessed in 14-day culture trials with four treatments: biofloc water with feeding (BF-f), natural seawater with feeding (SW-f), biofloc water without feeding (BF-nf), and natural seawater without feeding (SW-nf). *Litopenaeus vannamei* exhibited a significantly higher specific growth rate (SGR) in the BF-f treatment than in the SW-f treatment, whereas the other two species did not demonstrate any differences in their SGR and survival rate between BF-f and SW-f treatments, suggesting that bioflocs might contribute to the growth of *L. vannamei* postlarvae but do not affect the survival and growth of *F. chinensis* or *M. japonicus*. The results obtained from the non-feeding experiments implied a filter feeding habit of *L. vannamei* and a cannibalistic habit of *F. chinensis*. Differences in the third maxillipeds are relevant in this regard. The feeding mechanism of *M. japonicus* with intermediate features of setae requires further study. The present study may contribute to understanding the different feeding abilities of bioflocs as food source in currently farmed penaeid shrimp in the Far East Asian countries.


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INTRODUCTION

In South Korea, two native penaeids, *Fenneropenaeus chinensis* (Osbeck, 1765) and *Marsupenaeus japonicus* (Bate, 1888), and an introduced species, *Litopenaeus vannamei* (Boone, 1931), are currently cultured, and the majority of the farmed production is derived from *L. vannamei* (Jang et al., 2009). Shrimp are one of the most economically important aquaculture species in Korea and worldwide. However, the farmed production of shrimp has been largely influenced by pathogens including white spot syndrome virus (WSSV) in Korea (Meng et al., 2010; Jang et al., 2011). For example, 32.9% of 468 shrimp farms in South Korea collapsed as a consequence of the WSSV outbreak in 2006, although production has recovered since then (Jang et al., 2009). Recently, culture methods using biofloc technology (BFT) have been proposed as a tool to reduce water exchange and to minimize the introduction of pathogens (Browdy et al., 2001; Burford et al., 2003; Samocha et al., 2007).

Biofloc is known to control pathogenic bacteria and enhance shrimp immunity (Kim et al., 2014), thereby reducing potential disease outbreaks (Horowitz and Horowitz, 2001). Biofloc is made up of a conglomeration of different organisms, including bacteria, algae, protozoa, and zooplankton (Avnimelech, 2011). Heterotrophic bacteria, dominant in biofloc, assimilate toxic ammonia into bacterial protein by consumption of organic carbon (Avnimelech, 2012), reducing the need for water exchange (Crab et al., 2009). Additionally, the biofloc consumed by cultured animals can increase feed utilization by the recovery of some fraction of excreted nutrients (Burford et al., 2004a, b; Wasielesky et al., 2006). Despite many studies on the effectiveness of bioflocs in shrimp aquaculture, little is known about the feeding mechanisms involved in harvesting bioflocs by cultured animals.

In decapod crustaceans, including penaeids, feeding is generally accomplished by mouthparts consisting of six pairs of appendages: mandibles, first and second maxillae, and three pairs of maxillipeds. The third maxilliped of decapods is known to perform multiple functions, including the manipulation of food particles, preening, and locomotion as a response to chemical stimuli (Alexander et al., 1980;
Lee and Meyers, 1995). The third maxilliped is the outermost and largest appendage of the mouthparts and consists of a large five-segmented endopod, a long exopod, and an epipod armed with various types of setae (Alexander et al., 1980; Suthers, 1984; Alexander and Hindley, 1985). Garm (2004a, b) has described the mechanical function and fine morphology of setae on the maxillipeds in seven decapod species, including Penaeus monodon Fabricius, 1798 and Palaemon adspersus Rathke, 1837. Of the two major functions of these setae, the sensory function plays chemosensory and mechanosensory roles (Voigt and Atema, 1992; Derby et al., 2001). Second, the mechanical function involves locomotion, digging, grooming, and feeding (Garm, 2004a). Kent et al. (2011) suggested that the third maxilliped plays a role in microalgae uptake by forming a ‘net’ structure of setae and filtering suspended particulates larger than 10 μm.

The filtering structure and function of feeding appendages of euphausiids have previously been described; herbivorous Euphausia Dana, 1852 have six pairs of thoracic legs that function as a feeding basket to filter food particles (Hamner, 1988). Medina et al. (1998) reported that Euphausia pacifica Hansen, 1911 and E. nana Brinton, 1962 are less than 5 μm, but it is 20 to 30 μm in E. recurva Hansen, 1905 (Suh and Choi, 1998). Bacteria are very small, typically less than 1 μm in size, but in dense microbial biomass conditions, they tend to congregate and create flocs with a diameter of 0.1 to several mm (Avnimelech, 2012).

It remains unclear whether all penaeid shrimp can use or harvest minute particles in water columns, although it has been observed in L. vannamei and P. monodon. Intake of particles such as phytoplankton and other detritus-associated particulates may largely contribute to the nutrition of penaeids (Dall et al., 1990; Cuzon et al., 2004; Kent et al., 2011). The present study aimed to determine whether three penaeid species could use bioflocs during postlarval stages and to understand how these species can capture suspended food particles.

**Materials and Methods**

**Experimental Animals**

The postlarvae of the three species used in the present study were obtained from local hatcheries during different seasons due to the different spawning times of these species. The postlarvae of L. vannamei and F. chinensis were obtained from Cheongsu Aquafarm (Chungnam, South Korea) in May and July 2010, while the postlarvae of M. japonicus were obtained from the Breeding Center of National Fisheries Research and Development Institute (NFRDI) in September 2011. The postlarvae of L. vannamei were produced from wild females caught from the western coast of South Korea in May 2010. The postlarvae of M. japonicus were produced from wild females caught from the southern coast in August 2011.

Postlarvae in the PL19-PL20 stage produced at hatchery facilities were transferred to the experimental station at the Crustacean Research Center of NFRDI, Taean, Chungnam Province. Before being transferred, the postlarvae were packed in plastic bags filled with filtered seawater by 50% in volume and fully saturated with pure oxygen. The plastic bags were moved into iceboxes, and the temperature inside the boxes was maintained at approximately 20°C using ice packs. After transportation for two (L. vannamei and F. chinensis) or eight hours (M. japonicus), no mortality of the postlarvae was observed. When the postlarvae arrived at the experimental station, they were moved into fiber reinforced plastic tanks (2 m³ in volume) filled with filtered seawater, provided with commercial larval feed (PL+300, EG, INVE, Belgium), and gradually acclimated to 26-28°C until culture trials began.

**Experimental Design and Culture Trial**

After acclimation for 5-7 days, the healthy postlarvae were placed in circular plastic tanks (34 cm diameter × 34 cm depth) filled with 20 l of culture water in a culture room. The photoperiod of the culture room was maintained with a regime of 14 hours of light and 10 hours of darkness, and the room temperature was maintained at 26-29°C with an air-conditioning system. Based on the culture medium and feeding conditions, four experimental treatments were prepared: biofloc water with feeding (BF-f), biofloc water without feeding (BF-nf), natural seawater with feeding (SW-f) and natural seawater without feeding (SW-nf). Before stock, 90 individuals of each species were separately measured for mean body weight (MBW). To equally stock total animal biomass per water volume, each tank contained 400 postlarvae for L. vannamei (MBW 14.12 mg) and F. chinensis (MBW 29.41 mg) and 220 postlarvae for M. japonicus (MBW 52.99 mg). Four experimental treatments were prepared in triplicate and cultured for two weeks.

The culture medium for seawater treatments (SW) was exchanged daily at a rate of 50% with seawater that was ozone-sterilized and passed through a 5-μm filter. For the biofloc treatments (BF), the culture medium was renewed daily with biofloc seawater at a rate of 50% to maintain fully matured biofloc or microbial communities conditions during this study. The biofloc water source renewed for BF treatments was provided daily from a greenhouse-enclosed intensive shrimp production raceway tank (14 m width × 22 m length × 1.2 m depth) with zero exchange at the experimental station. During the experiments, L. vannamei of approximately 5-10 g were grown with a stocking density of 400-450 shrimp/m² in the greenhouse raceway tank. The biofloc water of the greenhouse raceway was removed into reservoir tanks (200 l in volume) using a water pump, filtered with a 100-μm mesh and provided with strong aeration to keep particulates suspended until moved to the experimental tanks of this study. The tops of the experimental tanks were covered with plastic caps to prevent the animals from escaping. Each tank was provided with an air stone at the center of the bottom to keep particles gently suspended and to maintain dissolved oxygen above 4 mg l⁻¹. Uneaten feed and shrimp feces were removed daily from the tank bottoms before culture medium exchange using a newly designed net (100-1000 μm in mesh size) and siphon to prevent the postlarvae from escaping. Feeding groups were provided with a larval diet (45% crude protein, CJ Feed, Seoul, South Korea) at three equal daily portions (09:00 h, 17:00 h and 22:00 h).

**Analysis of Water Quality Parameters**

Water temperature, salinity, pH, and dissolved oxygen (DO) were measured in all culture tanks using a YSI 85 meter (YSI, Yellow Springs, OH, USA). For the measurement of ammonia-nitrogen (TA-N), nitrite-nitrogen (NO₂-N), nitrate-nitrogen (NO₃-N), chlorophyll-a (Chl-a), total suspended solids (TSS) and volatile suspended solids (VSS), 1 l water samples were taken from all reservoir tanks every 3 days. For the measurement of nitrogen compounds, 200-ml subsamples that were filtered through a 1.2-μm GF/C glass microfiber filter (Whatman, Piscataway, NJ, USA) were kept in a refrigerator and analyzed within 24 hours of collection. For TSS analysis, a well-mixed 50-ml sample was filtered through a 0.7-μm GF/F glass microfiber filter and dried for at least one hour at 103-105°C before weighing. After measuring TSS, this filtered sample was ignited at 550 ± 50°C for 15 minutes in a muffle furnace for weighing VSS. Another 50-ml subsample was filtered through a GF/C fiber filter and stored in a refrigerator for 24 hours until use for Chl-a measurement. All analyses of water quality were performed in accordance with the procedures outlined by Clesceri et al. (2005).

**Observation of Third Maxilliped Structure**

All specimens examined for the observation were at the postlarval stage. Five individuals of each species were fixed in 70% ethanol for a 24-hour period. The specimens were then placed on glass slides with an ethyl alcohol + glycerin solution (1:1, v/v). The specimens were examined and dissected under a stereomicroscope (SZX-7, Olympus, Tokyo, Japan).
using fine needles (12.7 × 0.33 mm, BD Ultra-Fine®, Becton Dickinson, Franklin Lakes, NJ, USA). Drawings of dissected appendages, particularly the third maxilliped, were made using a drawing tube connected to a light microscope (DM 2500, Leica, Wetzlar, Germany). The setal definition and classification generally followed that of Garm (2004a, b). The body length (BL) referred to the distance from the postorbital margin to the tip of the telson. The BL was measured to 0.01 mm using Vernier calipers (CD-20CPX, Mitutoyo, Japan). Measurements of the third maxilliped length, the number of seta (at 40× magnification), seta length and distance (at 400×) were performed under a light microscope (Z1 fluorescence microscope, Carl Zeiss, Oberkochen, Germany). Images were captured using an AxioCam MRm camera and the AxioVision Release 4.2 software (Carl Zeiss, Thornwood, NY, USA) to measure these parameters. Filtering areas (FA) of the third maxilliped endopods were calculated using an equation suggested by Suh and Nemoto (1987), which was modified as follows:

\[
FA = 2 \sum_{i=1}^{n}(p' \times I) + (p'' \times M) + (p''' \times C) + (p'''' \times P) + (p''''' \times D),
\]

where \(I, M, P \) and \(D \) represent the lengths of the ischium, merus, carpus, propodus and dactylus of the third maxilliped endopod, respectively; \(p', p'', p''', p'''' \) and \(p''''' \) are the average lengths of setae on the ischium, merus, carpus, propodus, and dactylus, respectively. For the average length of the setae, five to 10 setae were randomly measured from each endopod segment. All measurements were conducted five times for each specimen.

**Growth and Survival Rate of Shrimp**

At the end of the study, animals were harvested and the survival rate was calculated. To calculate the specific growth rate (SGR), the wet weights of 90 animals from each feeding treatment and all survivors from non-feeding treatments were individually measured to 0.01 mg using an electronic balance. The SGR was calculated using the following formula:

\[
SGR (%) = 100 \times \frac{\ln W_f - \ln W_0}{t} / W_0,
\]

where \(W_f, W_0 \) and \(t \) represent the mean final body weight, mean initial body weight and the duration of the experiment (days), respectively.

**Statistical Analysis**

Water quality parameters were determined by Student Newman Keuls (SNK) test following a one-way analysis of variance (ANOVA). Significant differences were analyzed between biofloc or seawater treatments of each species, or among the same treatments of the three different species. The body lengths, endopod lengths, setal parameters, survival rates and SGRs of the animals were compared using repeated-measures ANOVAs using species as a factor. The assumptions of normal distributions and the homogeneity of variance were verified before the analyses. All ANOVAs were tested using SPSS (Statistical Package for Social Science) version 13 software.

**RESULTS**

**Water Quality**

A summary of the water quality changes in the treatment tanks is presented in Table 1. The water temperature was maintained at a mean of 26.4-28.8°C and showed no significant differences between all treatments except for the seawater treatment for *F. chinensis*. The salinity was slightly lower for *F. chinensis* than for the other two species because of the rainy season during the culture study of *F. chinensis*. However, the salinity was maintained over 29.2 ppt in all treatments. DO concentrations were significantly different between biofloc treatments of the three species but were higher than 4.5 mg l⁻¹ in all treatments, ranging from a mean of 4.8 to 6.4 mg l⁻¹. The pH was significantly lower in all biofloc treatments (mean 7.4-7.8) than in the seawater treatments (mean 8.0-8.7). Nevertheless, the pH did not decrease below 7.3 in any of the treatments. Compared with the biofloc treatments, the concentrations of TA-N, NO₂⁻N and NO₃⁻N in all seawater treatments were almost negligible. The highest TA-N concentration was found in the biofloc treatment (1.25 mg l⁻¹) of *L. vannamei*. The mean concentration of NO₂⁻N was highest in the biofloc treatment of *F.*
with a range of 1.38 to 2.30 mg l$^{-1}$, followed by the concentrations in L. vannamei and M. japonicus. The mean concentration of NO$_3$-N was the highest (mean 151.40 mg l$^{-1}$) in the biofloc treatment of F. chinensis, which was almost 1.5 times higher than that of L. vannamei. TSS concentrations in all biofloc treatments ranged from 486.1 to 577.6 mg l$^{-1}$; however, there were no significant differences between species. The VSS to TSS ratios in the biofloc treatments were 60.5, 80.5 and 56.5% in L. vannamei, F. chinensis and M. japonicus, respectively, and were not significantly different between species. The TSS and VSS concentrations in all seawater treatments were negligible, compared with biofloc treatments. Concentrations of chlorophyll-a, inorganic nitrogen (TA-N, NO$_2$-N, and NO$_3$-N), TSS and VSS were significantly higher in biofloc treatments than in seawater in all species.

In contrast, mean TSS concentrations of the source biofloc-water, which was provided daily from the greenhouse-enclosed super-intensive shrimp production system, were approximately 402, 418 and 470 mg l$^{-1}$ during the culture study of L. vannamei, F. chinensis and M. japonicus, respectively.

Observation of Third Maxilliped Structures

The microscopic drawings and setal description of the endopod segments of the third maxilliped, hereafter called ‘maxilliped’ or ‘endopod,’ in the postlarvae of the three species are provided in Fig. 1 and Table 2, respectively. In general appearance, the maxilliped endopods of L. vannamei are covered with longer and more abundant setae than F. chinensis and M. japonicus. Furthermore, the setal features in the basal three segments, namely the ischium, merus, and carpus, were notably different between L. vannamei and the other two species: L. vannamei has mainly plumose setae compared with mainly simple setae in the other two species. Cuspidate setae were observed on the distal region of the propodus only, with three, five, and seven setae in L. vannamei, F. chinensis, and M. japonicus, respectively. The propodus of L. vannamei had additional plumose setae, was opposed to simple and serrulate setae in F. chinensis and long simple setae in M. japonicus. The dactylus of L. vannamei is the longest (approximately 0.7 times the length of the propodus) among the three species and is covered with rows of mainly plumose setae, whereas the other two species have simple and serrulate setae.

Detailed measurements of the setae and setules on the maxilliped endopod are presented in Table 3. The size of the specimens examined in this study were showing no significant difference in the body lengths (21.18~23.23 mm) of the three species. The total endopod length of L. vannamei was 1.18 mm, which is longer than the other two species. The total number of setae in L. vannamei was 46.8% and 20.9% more than F. chinensis and M. japonicus, respectively. The mean seta and setule lengths in L. vannamei were longer compared to the other two species. The setule lengths of the ischium was approximately 4- and 2-times longer than F. chinensis and M. japonicus, respectively. The seta distance, which determines the mesh size of the filtering area in the maxilliped, was significantly narrower in L. vannamei than in the other two species. The seta distance was the shortest in F. chinensis, approximately one-third and one-fourth of the L. vannamei and M. japonicus, respectively. The filtering area on the maxilliped endopod was the widest in L. vannamei, approximately two and three times wider than that in F. chinensis and M. japonicus, respectively. Figure 2 shows the measurements of the seta and setule on each segment of the maxilliped endopod. Although similar to the total segments of the endopod length (Table 3), most measurements of seta and setule parameters in each segment showed significant differences between L. vannamei and the other two species, except for the distance of the setae on the ischium. The setule lengths of the ischium
and the merus in *F. chinensis* and that of the ischium in *M. japonicus* were not measured because only simple setae were observed, as shown in Table 2.

**Growth and Survival Rates**

The results of the SGRs and survival rates in the four experimental groups of the three species are presented in Table 4. The SGR of *L. vannamei* was significantly higher than that of *F. chinensis* and *M. japonicus* in the feeding treatments (BF-f and SW-f). Compared with the latter two species, the SGR of *L. vannamei* was approximately three times higher in the BF-treatment and two times higher in the SW-treatment. *Litopenaeus vannamei* showed a significant difference in the SGR between BF-f and SW-f treatments but no significant difference in the survival rate. Although the survival rate in the BF-treatment of *F. chinensis* was as low as 32%, this value was not significantly different from that of the same species cultured in the SW-f treatment. Additionally, the SGRs of this species were not significantly different between BF-f and SW-f treatments. Identical to *F. chinensis*, *M. japonicus* showed no significant differences in the SGRs and in the survival rates between BF-f and SW-f treatments. The non-feeding treatments (BF-nf and SW-nf) of *F. chinensis* had the lowest survival rate of only 1%, with only 14 and 11 survivors, respectively, at the end of the study. However, the SGRs in BF-nf and SW-nf treatments for this species were 10.55 and 10.88% bw day⁻¹, respectively, which were significantly higher than those values of *L. vannamei* and *M. japonicus* under the same treatments. The SGRs of *F. chinensis* were also significantly higher than those values in BF-f and SW-f treatments of the same species. In the non-feeding treatments, the highest survival rate (33%) was found in the BF-nf treatment of *L. vannamei*.

**DISCUSSION**

**Water Quality and Growth and Survival of Shrimp**

Despite some differences in water quality parameters, these values were generally within the acceptable ranges for the survival and growth of the three species used in the present study (Chen and Tu, 1990; Hu and Lu, 1990; Davis and Arnold, 1998; Cohen et al., 2005; Zhao et al., 2012), with the exception of TSS and VSS between the treatments. The pH levels were significantly lower in biofloc treatments than in seawater treatments for all species, which may be due to higher respiration activity by bacteria and by other microorganisms in the biofloc medium, thus increasing the CO₂ (Tacon et al., 2002; Wasielsky et al., 2006). As expected, the other parameters, including Chl-a, nitrogen compounds, TSS and VSS, were significantly higher in all biofloc treatments compared to seawater because the water source for biofloc treatment during this study was provided daily from an intensive shrimp production system with zero exchange.

Among the nitrogen compounds, the concentration of the un-ionized ammonia, which is highly toxic to shrimp (Hargreaves, 1998), briefly increased up to 0.083 mg l⁻¹ (converted concentration of 1.25 mg l⁻¹ TA-N at the temperature and pH given in Table 1) in the biofloc treatment of *L. vannamei* at the end of the study. However, this value is far below the acceptable level of 0.1-0.5 mg l⁻¹ for nursery and pond grow-out of farmed shrimp (Brock and Main, 1994). The maximal concentration of nitrite of 2.32 mg l⁻¹

| Table 2. | Setal characteristics and setations on the segments of the third maxilliped endopod in postlarvae of the three penaeid species. |
|----------|---------------------------------|-----------------|-----------------|
| Segment  | *L. vannamei*                   | *F. chinensis*  | *M. japonicus*  |
| Ischium  | A row of plumose setae, a row of simple setae | Mainly simple setae | A row of simple setae |
| Merus    | Two rows of plumose setae       | A row of simple, serrulate setae | Two rows of simple (stout and long, slender and shorter) setae |
| Carpus   | Two rows of plumose setae       |                 |                 |
| Propodus | Three cuspidate setae distally, two rows of plumose setae | Five cuspidate setae distally, simple and serrulate long setae | Seven cuspidate setae distally, simple long setae |
| Dactylus | 0.7× propodus, two rows of plumose setae | 0.3× propodus, simple and serrulate setae on entire surface | 0.4× propodus, simple and serrulate setae on entire surface |

| Table 3. | Mean values ± SD of body length of animals, endopod length, setal parameters and filtering area on the third maxilliped in the postlarvae of three species of penaeids. Five specimens were examined in each species. A significant difference between species is marked with different letters (p < 0.01). |
|----------|-------------------------------------------------|-----------------|-----------------|
| Parameter | *L. vannamei* | *F. chinensis* | *M. japonicus* |
| Body length of postlarva (mm) | 23.23 ± 1.25 | 22.07 ± 3.31 | 21.18 ± 2.81 |
| Total endopod length (mm) | 1.18 ± 0.13a | 0.84 ± 0.22b | 0.73 ± 0.04b |
| Total number of seta | 226 ± 10a | 154 ± 12b | 187 ± 24c |
| Seta length (μm) | 123.1 ± 23.0a | 79.0 ± 19.7b | 71.1 ± 13.1b |
| Seta distance (μm) | 21.4 ± 1.8a | 29.1 ± 4.7b | 29.3 ± 3.6b |
| Setule length (μm) | 13.7 ± 2.5a | 3.6 ± 0.7b | 6.9 ± 0.9b |
| Setule distance (μm) | 7.6 ± 0.7a | 2.3 ± 0.2b | 9.6 ± 4.2a |
| Filter area (cm²) | 32.1 ± 2.2a | 16.8 ± 2.2b | 11.4 ± 1.8b |
observed in the biofloc treatment of *L. vannamei* was also assumed to not affect the survival of this species because *L. vannamei* could have a high tolerance to extremely high nitrite concentrations in the short term in no exchange systems. For example, Cohen et al. (2005) observed a 97.5% survival rate in a 50-day nursery trial under limited discharge with *L. vannamei*, whose juveniles were exposed to 26.4 mg l$^{-1}$ of NO$_2$-N for one week. Similarly, Samocha et al. (2007) reported an 85.8% survival rate of *L. vannamei* juveniles exposed to 31.7 mg l$^{-1}$ NO$_2$-N for one week in a zero exchange system.

Although the biofloc culture media in the present study was provided with biofloc water sources in different seasons or years, there were no significant differences in TSS concentrations between biofloc treatments. Such high TSS concentrations did not appear to affect the survival and growth performance in *F. chinensis* and *M. japonicus* because the survival rate and SGR between BF-f and SW-f were similar.

Table 4. Specific growth ± SD and survival rates ± SD of postlarvae in four experimental groups of *L. vannamei*, *F. chinensis*, and *M. japonicus*. A significant difference between species in each experimental group is marked with different letters. A significant difference between the same factors of each species is marked with an asterisk (*p < 0.05, **p < 0.01*). BF-f, biofloc water with feeding; SW-f, seawater with feeding; BF-nf, biofloc water without feeding; SW-nf, seawater without feeding; SGR, specific growth rate. Data of BF-f and SW-f in *L. vannamei* were cited from Kim et al. (2014).

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Factor</th>
<th><em>L. vannamei</em></th>
<th><em>F. chinensis</em></th>
<th><em>M. japonicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>BF-f</td>
<td>SGR (% bw day$^{-1}$)</td>
<td>15.9 ± 0.9$^a$</td>
<td>4.0 ± 0.6$^b$</td>
<td>5.1 ± 0.4$^b$</td>
</tr>
<tr>
<td></td>
<td>Survival rate (%)</td>
<td>91.5 ± 2.9$^a$</td>
<td>32.0 ± 1.4$^b$</td>
<td>75.0 ± 1.6$^c$</td>
</tr>
<tr>
<td>SW-f</td>
<td>SGR (% bw day$^{-1}$)</td>
<td>11.9 ± 1.0$^{**}$</td>
<td>4.9 ± 0.6$^b$</td>
<td>5.3 ± 0.5$^b$</td>
</tr>
<tr>
<td></td>
<td>Survival rate (%)</td>
<td>82.5 ± 8.4$^a$</td>
<td>52.0 ± 0.1$^b$</td>
<td>83.0 ± 2.5$^a$</td>
</tr>
<tr>
<td>BF-nf</td>
<td>SGR (% bw day$^{-1}$)</td>
<td>4.3 ± 2.1$^{**}$</td>
<td>10.6 ± 4.4$^{bs}$</td>
<td>4.2 ± 3.8$^a$</td>
</tr>
<tr>
<td></td>
<td>Survival rate (%)</td>
<td>33.0 ± 4.9$^{**}$</td>
<td>1.0 ± 0.6$^{bs}$</td>
<td>9.0 ± 1.5$^{bs}$</td>
</tr>
<tr>
<td>SW-nf</td>
<td>SGR (% bw day$^{-1}$)</td>
<td>4.6 ± 1.9$^{**}$</td>
<td>10.9 ± 1.3$^{bs}$</td>
<td>2.9 ± 0.7$^a$</td>
</tr>
<tr>
<td></td>
<td>Survival rate (%)</td>
<td>2.0 ± 1.0$^{**}$</td>
<td>1.0 ± 0.0$^{a}$</td>
<td>7.0 ± 2.0$^{bs}$</td>
</tr>
</tbody>
</table>
but may not provide any beneficial or detrimental effects on growth and survival of *F. chinensis* and *M. japonicus* PLs. TSS in the BFT systems is closely related to the optimal levels of DO and inorganic N compounds (Ray et al., 2010). BFT systems with high TSS require more oxygen due to the abundantly present respiring bacteria. Under these conditions, gill fouling by dense particles and relatively lower oxygen supply to shrimp can limit animal health (Liltved and Cripps, 1999; Brune et al., 2003; Hargreaves, 2006). Therefore, concentrations of these particles must be controlled to optimize the performance of the system (Ray et al., 2010).

Ray et al. (2010) and Schweitzer et al. (2013) proposed 400-600 mg l\(^{-1}\) as suitable TSS levels for *L. vannamei* in limited exchange systems. However, Mishra et al. (2008) reported a 96.2% survival rate with a TSS concentration as low as 126 mg l\(^{-1}\) in an intensive nursery culture with limited exchange. In the present study, *L. vannamei* showed a 91.5% survival under 502.7 mg l\(^{-1}\) TSS, similar to previous authors. *L. vannamei* can tolerate a wide range of optimal TSS concentrations from 120 to 600 mg l\(^{-1}\). However, with the exception of *L. vannamei*, the TSS ranges for other penaeid shrimps remain unclear.

The only other study of BFT application with *M. japonicas* was reported by Zhao et al. (2012), who observed the higher survival and SGR of this species in biofloc than in control treatments. They reported that floc volume (FV) in biofloc treatment gradually increased to the peak (11.6 mg l\(^{-1}\) during 106 days, without mentioning TSS concentration. Although the conversion of FV to TSS may vary by conditions, Liu et al. (2014) reported that 11 ml of FV in an integrated *L. vannamei* culture system was consistent with 200 mg l\(^{-1}\) TSS. In the present study, however, the survival and SGR of *M. japonicus PLs were not significantly different between biofloc (mean 577.6 mg l\(^{-1}\) TSS) and seawater (mean 16.9 mg l\(^{-1}\) TSS) treatments. This inconsistency may be due to differences in the culture period and ages, particularly larval and growing stages.

The survival rate in BF-f of *F. chinensis* was lower than that of *L. vannamei* and *M. japonicus* (Table 4). However, such a low survival rate in *F. chinensis* might not be caused by bioflocs because it was not significantly different with SW-f treatment. We stocked *F. chinensis* at a density of 20,000 PL m\(^{-3}\) and obtained 32 and 53% survival rates in BF-f and SW-f treatments, respectively. However, there are no studies on the survival rate of *F. chinensis* with such high density conditions. Low survival rates of this species during the larval production were frequently observed from commercial hatcheries. Li et al. (2006) observed 66 and 39% survival rates in the nursery culture of *F. chinensis* at densities of 200 and 600 PL m\(^{-3}\), respectively. The density tolerance may be species-specific and related to many factors such as available foods, feeding habit, behavioral interactions and water quality parameters (Foster and Bread, 1974; Abdussamad and Thampy, 1994; Otoshi et al., 2006; Araneda et al., 2008). The low survival rate of *F. chinensis* postlarvae observed in this study may be largely affected by cannibalistic habit, which will be discussed later.

Features of Maxilliped Three and Biofloc Feeding Efficiency of Postlarvae

In the current study, we analyzed the morphological structure of the third maxilliped because this appendage affects the harvesting and feeding behaviors of crustaceans (Hamner, 1998; Kent et al., 2011). The third maxilliped consists of a large five-segmented endopod, an exopod, and an epipod. This structure functions in manipulating or passing food particles with numerous setae on endopod segments and in grooming other parts of the head (Garm, 2004a, b). In particular, the length and distance of the setae and setules (secondary setae) on the third maxilliped endopod could determine the feeding type and filter-feeding mesh size, i.e., the feeding efficiency of the crustaceans (Boyd et al., 1984; Suh and Nemoto, 1987).

The observation of the morphological structures and the characterization of various setae on the third maxilliped suggests useful information to determine harvesting efficiency of bioflocs as food sources in the penaeid postlarvae used in the current study. This study provides a detailed description of the setation on the third maxilliped of the three species postlarvae (Fig. 1 and Table 2). We measured the length and distance of setae and setules and counted the number of setae on the third maxilliped endopods of the three species, of which the postlarvae have a similar mean body length of 21.18-23.23 mm (Fig. 2 and Table 3). The total seta number, seta length and setule length in *L. vannamei* were significantly longer and more abundant than those in *F. chinensis* and in *M. japonicas*. The same significant differences between *L. vannamei* and the other two species were also found in the majority of the five endopod segments on the third maxilliped. As calculated using these setal measurements and with the modified equation (see Materials and Methods; Suh and Nemoto, 1987), the total filtering area in the maxillipodal endopod of *L. vannamei* was 32.05 cm\(^2\), which is approximately two and three times wider than that of *F. chinensis* (18.82 cm\(^2\)) and *M. japonicas* (11.36 cm\(^2\)), respectively. The mesh size formed by the setae is determined by the distance of the setae (Boyd et al., 1984; Suh and Nemoto, 1987). Typically, most bacteria are free-living and extremely small, with a diameter of approximately 1 μm. However, in dense microbial biomass, bacteria tend to congregate and create flocs with a diameter ranging from 100 μm to several mm (Avnimelech, 2012). The seta distance (21.4 μm) of *L. vannamei* postlarvae measured in this study may be narrow enough to allow this animal to capture bioflocs. Based on an examination with a scanning electron microscope, however, Kent et al. (2011) reported a mesh size of 10 μm in the corresponding region of the same species. The difference in mesh size or seta distance may be the result of different measurement methods because the setae of the third maxilliped endopods are too small and pliable to handle. Furthermore, the seta distance of *L. vannamei* was significantly smaller than that of the other species. The present result of the larger filtering area and the smaller seta distance in the third maxilliped of *L. vannamei* implies that this species may more effectively filter and capture the suspended bioflocs than *F. chinensis* and *M. japonicus*. 

\( p > 0.05, \text{Table 2} \). However, *L. vannamei* showed a significantly higher SGR under in biofloc treatment than in seawater. This result implies that bioflocs with such TSS concentrations may contribute to the growth of *F. chinensis* PLs but may not provide any beneficial or detrimental effects on growth and survival of *F. chinensis* and *M. japonicus* PLs.
This postulation is also supported by different survival rates in non-feeding experiments (Table 4). The survival rate of *L. vannamei* in BF-nf treatment reached 33% higher than *F. chinensis* (1%) and *M. japonicus* (9%). The survival rate of *L. vannamei* in BF-nf treatment was the highest (33%) in all of the non-feeding treatments, while that in SW-nf was as low as 2%. This clearly suggests that *L. vannamei* postlarvae use bioflocs to survive. The survival rate of *F. chinensis* and *M. japonicus* was not significantly different between BF-nf and SW-nf treatments, suggesting an inefficiency of bioflocs for survival in these species. Due to the small filtering areas of the third maxilliped in these species postlarvae, they were unsuitable to catch particulate bioflocs and therefore starved. It is noteworthy that in both BF-nf and SW-nf, only 1% of survivors of *F. chinensis* obtained an SGR that was twice as high as that of the other two species, explained by a stronger cannibalistic habit of *F. chinensis* compared to *L. vannamei* and *M. japonicus*.

The cannibalistic activity may be linked to the setal morphology of their appendages. *F. chinensis* has many serrululate setae on the inner margin in the carpus, propodus, and dactylus, compared with mainly plumose setae and simple setae on the corresponding regions in *L. vannamei* and in *M. japonicus*, respectively (Fig. 1 and Table 2). Consequently, this setal structure of *F. chinensis* provides a better opportunity to capture or grasp prey, particularly weaker or smaller animals (Garm, 2004a). The frequent cannibalism of *F. chinensis* has also been reported in an observational study; Zhang et al. (2008) found that the postlarvae of *F. chinensis* showed more active foraging activity than the postlarvae of *L. vannamei* in high stocking density, although animals of both species were provided with sufficient *Artemia* nauplii and with artificial feed. Many studies have demonstrated the carnivorous feeding habit of adult *M. japonicus*. During juvenile and adult stages, the protein requirement of *M. japonicus* is as high as 45-57% (Deshimaru and Kuroki, 1975; Teshima et al., 2001), compared with 45% in *F. chinensis* (Wu and Dong, 2002) and 30-40% in *L. vannamei* (Pedrazzoli et al., 1998). Some pond culture studies have shown that *M. japonicus* is an opportunist that prefers to prey on insect larvae, rather than formulated feeds in earthen ponds (Gunderman and Pope, 1977; Lumare et al., 1985; Reymond and Lagardere, 1990), whereas *L. vannamei* consumes plant materials such as organic detritus and benthos (Martinez-Cordova and Pena-Messina, 2005). The postlarvae of *M. japonicus* are much more similar to *F. chinensis* than *L. vannamei* in setal morphology and measurements. In contrast to *F. chinensis*, however, the cannibalism did not occur in the non-feeding treatments of *M. japonicus*, as evidenced by the lack of significant differences in the survival rate and SGR between BF-nf and SW-nf treatments. Griffith et al. (1992) and Rodríguez et al. (1994) reported that feeding behavior of *M. japonicus* is similar to omnivorous *L. vannamei* during the postlarval stage. The setal feature of the postlarvae of *M. japonicus*, with simple setae on the third maxilliped in contrast to the plumose or serrulate setae of the other two species, suggests that this species may depend on a certain opportunistic feeding strategy: neither filter-feeding nor predatory habits. Interestingly, the survival rate of *M. japonicus* was significantly higher than that of *L. vannamei* under starvation (SW-nf, Table 4). Under the condition of starvation or low preferred foods, penaeid shrimp can use glyco- gen or protein as a primary energy source for their survival (Cuzon et al., 1980; Muhlía-Almazán and García-Carreño, 2002). The energy metabolism of shrimp responding to periods of starvation is species-specific (Sánchez-Paz et al., 2006) and requires further studies to better understand the differences between species.

Most culture trial studies based on BFT (biofloc technology) have focused on growth performance with *L. vannamei* because this technology has been applied to aquaculture. The present study provides useful information regarding the biofloc effects on growth and survival in the postlarval stages of three penaeid species that are commercially farmed in East Asian countries. This study also suggested a feeding mechanism of bioflocs or food particles based on morphological structures in the postlarvae of the three species. The results showed that the bioflocs contributed to the growth of *L. vannamei* postlarvae but did not affect the survival and growth of *F. chinensis* and *M. japonicus*.

The different contribution of bioflocs is closely related to the morphological features of the setae on the third maxilliped of these species. Setal features such as number, length and distance of setae and setules may determine the area and mesh size of the ‘setal net,’ which can filter or capture suspended particles such as bioflocs. In this respect, the postlarva of *L. vannamei* had two and three times larger filtering areas of the net than that of *F. chinensis* and *M. japonicus*, respectively. This study furthers understanding the different feeding efficiencies of bioflocs in the postlarvae of different species, as related to the morphological features of the third maxillipeds.

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