



Filtration Capacity and Behaviour of Juvenile Green-Lipped Mussels in Small-Volume Bioassays

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ABSTRACT

This research specifically observes the duration of valve opening and closure in three different size classes of juvenile mussels (*Perna canaliculus*) over a 60-minute period after exposure to the microalgae *Isochrysis galbana*. Current study would like to understand this behaviour as a precursor to using flow cytometry (FCM) to accurately measure the juvenile mussels' filtering capabilities under the FCM set up. There is still limited information on the use of experimental set up of flow cytometry on measuring the filtration of the juvenile mussels. Two factors used in the experimental set up; i.e. the size class of juvenile mussels, and the observation time (T_1 - T_{60}). The results indicated that both mussel size and observation time significantly influence the proportion of time valves remain fully open, with juvenile mussels generally exhibiting different behaviour patterns (such as more crawling). Current study also measures the clearance of the microalgae, which shown the larger size class of mussels exhibit higher cells clearance compared to the smaller size of mussels. Ultimately, the study concludes that while valve observation indicates filtering, further refined methods are needed to ensure FCM accurately measures the actual particle removal capability.

Keywords: Juvenile mussel, Valve opening, Valve closure, Filtering behaviour, Flow cytometry

ABSTRAK

Penelitian ini mengamati durasi pembukaan dan penutupan cangkang kerang pada tiga kategori ukuran berbeda dari juvenil kerang (*Perna canaliculus*) selama periode 60 menit setelah terpapar mikroalga *Isochrysis galbana*. Studi ini bertujuan untuk memahami perilaku tersebut sebagai langkah awal dalam menggunakan *flow cytometry* (FCM) untuk mengukur kemampuan filtrasi mikroalga pada benih kerang secara akurat dalam pengukuran jumlah mikroalga terfilter oleh kerang dengan menggunakan FCM. Informasi tentang penggunaan *flow cytometry* dalam penelitian untuk mengukur filtrasi juvenil kerang masih terbatas. Dua faktor yang digunakan dalam penelitian ini yaitu; kategori ukuran benih kerang, dan waktu pengamatan (T_1 - T_{60}), dengan masing-masing diulang sebanyak lima kali. Hasil menunjukkan bahwa baik ukuran kerang maupun waktu pengamatan secara signifikan mempengaruhi proporsi waktu cangkang tetap terbuka sepenuhnya, dengan benih kerang umumnya menunjukkan pola perilaku yang berbeda (seperti lebih banyak bergerak dan berpindah tempat). Studi saat ini juga mengukur jumlah filtrasi mikroalga, yang menunjukkan bahwa kelas ukuran kerang yang lebih besar memiliki nilai filtrasi sel yang lebih tinggi dibandingkan dengan kerang berukuran lebih kecil. Pada akhirnya, studi ini menyimpulkan bahwa meskipun pengamatan cangkang menunjukkan proses filtrasi, metode yang lebih terperinci diperlukan untuk memastikan FCM dapat mengukur kemampuan kerang pada filtrasi partikel secara akurat.

Kata kunci: Juvenil kerang, Bukaan cangkang, Penutupan cangkang, Tingkah laku filtrasi, Flow cytometry

1. Introduction

Mussels feed by filtering particulate material from seawater. Seawater contains a diverse range of microscopic particles, some of which are removed by the filtering activity of mussels, including microalgae, bacteria and protozoans. There are various factors which are able to influence filtering rate in mussels, including particle concentration, size, water velocity and turbidity (Argente, 2024). In optimal conditions, such as an adequate concentration of food particles, mussels usually filter at a high rate of filtration (Riisgård et al., 2011). Filtration can be stopped in mussels by completely closing their valves (Riisgård et al., 2014). Mussels can selectively retain and ingest organic-rich particles, often rejecting inorganic or less nutritious particles as pseudofaeces, thereby enriching their diet (Pospelova et al., 2025). The efficiency of this selection process is affected by the size and composition of available particles (Rosa et al., 2017). While the filtration behavior of adult mussels and their responses to environmental factors have been studied extensively (Argente, 2024; Hatton et al., 2005), most research has focused on adult or mixed-age populations, with less attention to how juvenile mussels of different sizes respond to controlled experimental setups and artificial diets (Argente, 2024; Baker et al., 1998; Defosse, 1997; Dionisio et al., 2004). There is limited information on the filtering capabilities and behavior of juvenile mussels, particularly under the specific conditions required for flow cytometer analysis (e.g., after starvation and in well plates with axenic microalgae) (Gui et al., 2016a). Although flow cytometer has been validated for distinguishing particle ingestion in adult bivalves (Cucci et al., 1985), its application to juvenile mussels, especially in the context of hatchery or laboratory conditions, remains underexplored (Gui et al., 2016).

The overall aim of the research is to determine the filtering behavior of juvenile mussels of *Perna canaliculus* under the flow cytometry set up condition by using live particles. These live particles are quickly ingested and are essential for meeting the nutritional needs of mussels, especially in captivity or restoration projects (Eryalçın et al., 2024). The effective use of the flow cytometry set up on juvenile mussels to be actively feeding will accommodate the accurate measuring of filtering capabilities of juvenile mussels when placed in the experimental conditions required for flow cytometry measurements. This information will bring a beneficial impact on the effectiveness of measuring filtration rate using flow cytometry on juvenile mussels in the hatchery or aquaculture practice. The specific aim of this study was to determine the filtering behaviour of juvenile *P. canaliculus* of a range of sizes when placed in the experimental

conditions required for FCM measurements, i.e., starved for 24 hours then placed in a well plate with a mixture of sterile seawater which was added with axenically cultured microalgae *Isochrysis galbana*.

2. Materials and methods

2.1 Juvenile mussel collection and preparation

Wild juvenile green-lipped mussels were collected from Muriwai Beach in the north-western New Zealand and held in the seawater facilities in School of Biological Science (SBS). The size of juvenile mussels that will be used in the research ranged in shell length from 1 to 8 mm. All the juvenile mussels were held in a static 2 L conical aerated tanks, which had been filled with UV treated seawater. The mussels were held in seawater at 18 °C until the start of the experiment. The mussels were starved for 24 hours before observation in the experiment to promote their feeding once returned to a suspension of microalgae. The mussels were sorted under the microscope into three size classes relating to changes in the morphological development of their filtering apparatus; 1 - 3 mm (post-settlement juvenile), 4 - 5 mm (early juvenile), 6 - 8 mm (juvenile) of shell length (Sim-Smith et al., 2005; Gui et al., 2016).

2.2 Laboratory experiment

A BD Falcon 24 well plate with 4 ml volume of wells was used as the experimental container for observing the feeding behavior of juvenile mussels under the microscope as it is proposed that these would ultimately be used for the FCM measurements of particle capture in mussels. The starved mussels were firstly measured under a binocular microscope and then placed in a randomly selected well on the well plate. The number of mussels which were added in each well were; 1 - 3 mm (1 individual), 4 - 5 mm (1 individual), 6 - 8 mm (1 individual). There were 15 individuals in total with five replicates in each size of mussel. Each well on the well plates was then filled with 2 ml of cultured microalgae solution (*Isochrysis galbana*) with a proximity concentration at $\sim 4 \times 10^5$ cells. ml⁻¹ up to 7×10^5 cells. ml⁻¹, and 1 ml of 1 µm filtered and UV sterilized seawater at 18 °C. The behavior of the individual mussels inside each well was then observed continuously under a binocular microscope (Wild Heerbrug type 376788, Switzerland) for the first minute and the percentage of time the mussel valves were open was recorded with a stopwatch. Thereafter, each mussel was observed again for one minute at 10 (T₁), 20 (T₂), 30 (T₃), 40

(T₄), 50 (T₅) and 60 (T₆) minutes after the initial observation was made.

2.3 Data Analysis

There are three variables measured in this study; percentage of fully open valve within 60 second in each interval of observation time, percentage of non-crawling behavior within the interval of observation time, and number of cells filtered by the juvenile mussels. All of the data are normality and homogeneity tested by using Kolmogorov-Smirnov and Levene's-test prior the statistical analysis by using Two-way ANOVA. Percentage data were arcsine transformed prior to the statistical analysis.

3. Results and Discussion

3.1. Behavior of Juvenile Mussels *Perna canaliculus* under limited Bioassay volume

3.1.1 Percentage of opening valve

The results on the percentage of valve opening in mussel seeds (*P. canaliculus*) (%) show that there are differences in the response of mussel shell opening and closing at different observation times (i.e., ~1-60 min). The range of shell opening percentages in all mussel seeds was between 50 - 83% ($P < 0.05$) (**Fig. 1**). Significant fluctuations showed in the percentage of valve opening as the observation time increases. Valve opening reached its highest value at 60 minutes of observation, with a percentage at 83%. Similarly, at the 30 minutes of observation time, giving the same response at 82% of valve opening. The lowest

valve opening was observed at 40 minutes, with a percentage of around 55%.

Although there was an increase in the percentage of valve opening at the initial of observation time (i.e., ~1 – 30 min), the valve then showed a closing at 40 minutes, which applied to all sizes of juvenile mussels. The pattern of shell opening continued to increase at 50 and 60 minutes (*Tukey-HSD*, $P < 0.05$) (**Fig. 1**).

The mean percentage of valve opening based on three size categories of juvenile mussels (i.e., 1-3 mm, 4-5 mm, and 6-8 mm) revealed that the size class of juvenile mussels performed different opening valve activities ($P < 0.05$) (**Fig.2**).

The mussel size category of 4-5 mm showed the highest average of valve opening, approximately at 83%. Whereas, both the 1-3 mm and 6-8 mm size class of juvenile mussels observed to have the similar response on the percentage of valve opening at 65% and 62 % respectively (*Tukey-HSD*, $P < 0.05$) (**Fig. 2**).

3.1.2. Crawling Behaviour

Different pattern performed between largest and the smaller sizes of juvenile mussels on crawling behavior ($P < 0.05$) (**Fig.3**). The smallest size of the mussels (i.e. 1 – 3 mm) of shell length tend to have less movement at $15.83 \pm 2.11\%$ than the larger size of the mussels (i.e., 4 – 5 mm and 6 – 8 mm) of shell length at $26.43 \pm 3.57\%$ and $35.97 \pm 3.87\%$ respectively (*Tukey-HSD*, $P < 0.05$) (**Fig. 3**).

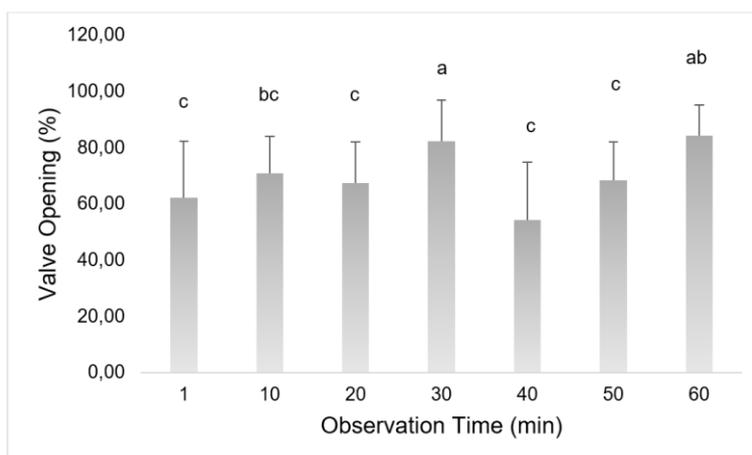


Figure 1. The mean ($\bar{x} \pm SD$) of valve opening percentage within 1 min for each observation time. Different letter above each bar graph, indicated significant result (*Tukey-HSD*, $P < 0.05$).

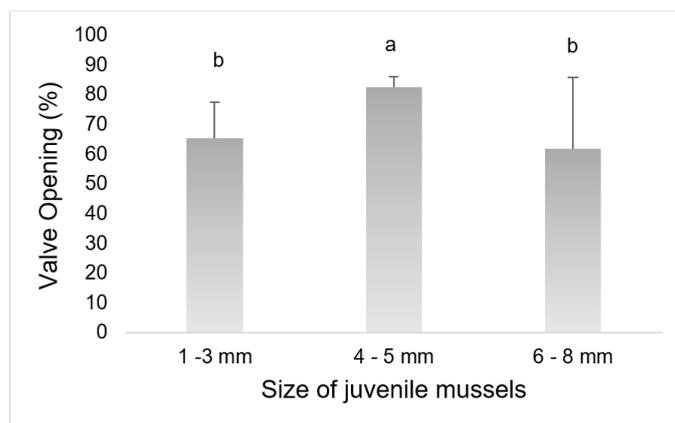


Figure 2. Mean ($\bar{x} \pm SD$) of proportion data of completely opening-valve for 60 second within each observation time for each size class of juvenile mussels. Different letter above each bar graph, indicated significant result (*Tukey-HSD*, $P < 0.05$).

Fig. 4. depicts a linear decline in cell concentration over a 60-minute interval ($R^2=1$), representing a filtration or depletion of the *I. galbana* cells by the mussels which showed 5.7×10^5 cells. ml^{-1} and 3.8×10^5 cells. ml^{-1} respectively for T_0 and T_{60} , resulting the delta of the cells concentration at 1.8×10^5 cells. ml^{-1} (*Tukey-HSD*, $P < 0.05$).

The filtration rate of juvenile mussels varied significantly across the three size classes examined ($P < 0.05$) (**Fig. 5**). Mussels in the 4–5 mm size range exhibited the highest filtration capacity, with the mean rate of approximately 6.7×10^3 cells. min^{-1} . This rate was significantly greater than those recorded for both the smaller and larger size classes (*Tukey-HSD*, $P < 0.05$). In contrast, the smallest (1–3 mm) and largest (6–8 mm) juveniles showed markedly lower filtration rates, ranging at 1.1×10^3 to 1.6×10^3 cells. min^{-1} , respectively (*Tukey-HSD*, $P < 0.05$).

Statistical analysis indicated no significant difference between the 1–3 mm and 6–8 mm groups suggesting that filtration efficiency peaks at the early juvenile stage (4-5 mm) before declining as the mussels exceed 5 mm in size (**Fig. 5**).

4. Discussion

Current result indicated that interval time of observation and mussel size category may affect the valve opening and closure behaviour of juvenile *Perna canaliculus*. Overall, the juvenile mussels experienced fluctuate percentage in opening their valve, and the highest value was found in the first minute of 30 and 60 minute of the experiment across all the juvenile mussels size classes at 82-83% of percentage on valve opening (**Fig. 1**). In the

same way, different size class of the mussels revealed to perform various percentage of valve opening. The early juvenile of the mussels size class 4-5 mm perform the highest proportion of percentage in fully opening their valve within all the observation time compare to the post settlement juvenile (i.e. 1-3 mm) and (6-8 mm) the largest mussel classes (**Fig 2**). Meanwhile, the smallest and largest mussels size class seemed performing selective filtration by performing limited opening valve. The variation of valve gaping on mussels could be triggered by various factors i.e., internal (Gui, et al., 2016) and external (Cheng et al., 2024; Sanjayasari et al., 2021; Sanjayasari & Jeffs, 2019). Similar result performed by blue mussels that their valve only slightly open before exposed to microalgae and required certain time until they experience fully open valve (Riisgård et al., 2014). *Mytilus galloprovincialis* observed to have opening amplitudes most frequently 60–90% of maximum and closures unsynchronised across individuals during 10 days of the experimentation which monitored every minute (Comeau et al., 2018). Adult *Perna canaliculus* showed gaping magnitude increased up to ~84% of maximum under some conditions such as acute environmental change (Cheng et al., 2024).

The phenomena of unsynchronised valve gaping on mussels were influenced by various factors (Cheng et al., 2024; Sanjayasari & Jeffs, 2019), in the current study, the juvenile mussels were placed in limited well plate volume (~4 ml). The observation of valve gaping of juvenile mussels was conducted immediately on the first minute once the mussels place in the well plate. Hence, the low percentage of opening valve of

the mussels in the beginning of the observation period (i.e. 1, 10, and 20 min) (**Fig 1.**) could be due to the juvenile mussels are required some times to adjust with the environment. *Margaritifera margaritifera* performed efficient ctenidial filter feeding once juveniles reach ~2.2 mm, marking a shift from pedal to true filter feeding. Very small juveniles thus require developmental “time” before they can filter effectively (Schartum et al., 2017). Early juvenile size in the study (i.e., 1 – 3 mm) observed to have lower percentage in opening their valve at 65%. This might be due to smaller size of mussel possess inefficient of filtering apparatus; therefore, they limited opening their valve rather than the larger size of mussels (Gui et al., 2016).

Pronounce crawling behaviour of all size categories of juvenile mussels within the well plate observed throughout the period of time (**Fig. 3**). Although, the smallest size class of the mussels showed the highest percentage on remain settled inside the well plate, all of juvenile mussels tend to crawl, attach and detach themselves for several times.

This result similar to (Wu et al., 2025), early juveniles of several mussel species detach, crawl, and re-attach many times in “secondary migration,” especially the one between ~0.5–5 mm shell length. Substrate characteristics may also influence the attachment of juvenile mussels in *Perna viridis* (Sanjayasari et al., 2021) and other species of mussels (Wu et al., 2025). The well plate which used as the observation chamber tend to have smooth surface, this might lead the juvenile mussels to attach and detach themselves within the well

plate and crawl before deploying themselves by using byssus string.

A study on juvenile *Perna viridis* showed that the mussel prefers to attach on the coconut coir rope compare to other substrate which enrich with macroalgae extract rich in terpenoid (Sanjayasari et al., 2021). Hence, it indicated that the mussels prefer to attach on the texture substrate compare to the smooth substrate, this may cause the mussels to move around inside the well plate. The mean of the number of cells removed from the well plate by the juvenile mussels between initial and final of observation time indicated that all of the mussel size classes are actively filtered the microalgae (*Isochrysis galbana*), with the R^2 value 1 of linear relationship between T_0 and T_{60} (**Fig. 4**).

Current study revealed that the amount of cells concentration filtered by juvenile mussels could be influenced not only by the size class of the mussels but also other triggering factors (**Fig. 5**). Juvenile mussels generally do not start filtering at full capacity immediately after environmental change or placement. They can begin feeding within minutes to hours, but full, stable filtration often requires hours to days, and under strong stressors (e.g., low salinity) weeks of acclimation (Filgueira et al., 2007).

Juvenile of *P. canaliculus* under 6 mm of shell length might have difficulty in filtering the particle due to undeveloped ctenidial structure (Gui, Zamora, et al., 2016b). Poor capture efficiency may lead to increased metabolic rate for example increasing their filtration rate by frequently opening their valve to obtain sufficient algal cells (Gui, 2012).

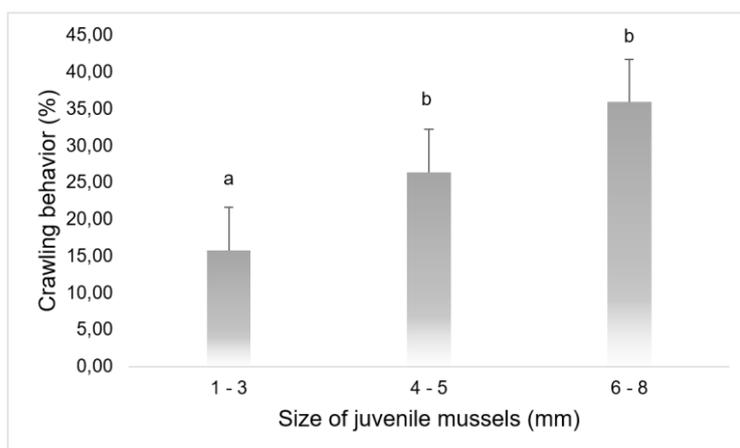


Figure 3. Mean ($\bar{x} \pm SD$) of proportion data of crawling behavior for 60 second within each observation time for each size class of juvenile mussels. Different letter above each bar graph, indicated significant result (*Tukey-HSD*, $P < 0.05$).

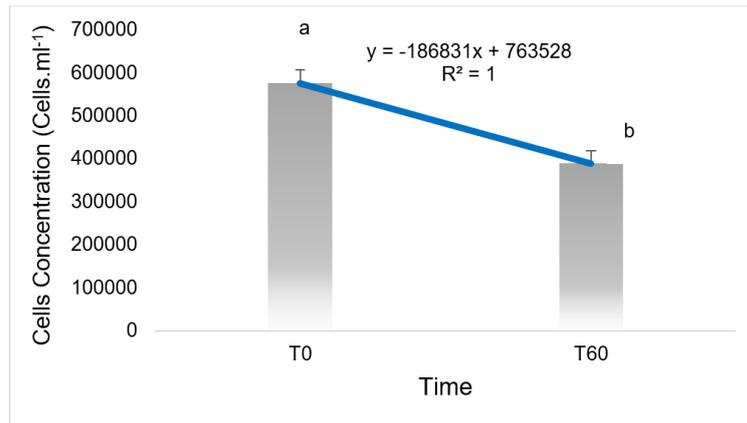


Figure 4. Mean ($\bar{x}\pm$ SD) of concentration of microalgae cells *I. galbana* within initial and final of observation time. Different letter above each bar graph, indicated significant result (*Tukey-HSD*, $P<0.05$).

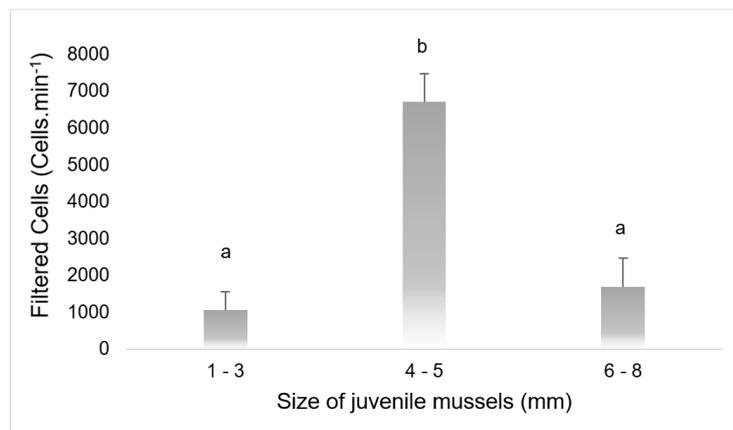


Figure 5. Mean ($\bar{x}\pm$ SD) of filtering capacity across all juvenile mussels group size. Different letter above each bar graph, indicated significant result (*Tukey-HSD*, $P<0.05$).

In several bivalves (*Cardium edule*, *Mytilus edulis*, *Mya arenaria*), initially unfed animals open valves and start filtering soon after food is added, but maximum filtration is reached only after a short response time; when food returns after starvation, maximum filtration is restored within 5–20 min (Riisgård et al., 2003). This condition was also observed at current experiment, which was showed by the largest size class of the mussels. Juvenile mussel experienced adjustment time to open their valve in the beginning of observation time, compare to the size of 4-5 mm in shell length. The well plate in the experiment set up was filled with *I. galbana* microalgae with 5.7×10^5 cells.ml⁻¹. This concentration was considered to be able to fulfil the adequate number of cells for the mussels. It also could be seen from visual observation that the mussel's tent to open their valves once place in the well plate. The highest clearance was observed at the early stage of juvenile (4-5 mm) shell length compare to two other size

groups. This result was linear with the percentage of the mussels opening their size, which also maximize within this group ~83%. This might be due to this size class of mussels try to obtained as many as food particles compare to the smallest and largest size of the mussels. The smallest size of the mussels might have some difficulties to filter the microalgae cells, as it possesses less complex of gills structure (Gui et al., 2016). Whereas, the largest size of mussels might require longer time to adjust with the environment compare to the other size class of mussels. Similar result observed in *Mytilus edulis*, mussels were given ~1 h of acclimation in aerated, food-rich water before measurements of valve opening and filtration behaviour, indicating to expect a settling period to reach routine activity (Peterson et al., 2025).

In terms of this experimental set up to be applied on the flow cytometry measurement

arrangement, this study suggested that these size classes of mussels tend to move and crawl inside the well plates. In addition, remembering that flow cytometry equipped with suction needle to collect and measure the cells number, this may become a hinder and bring detrimental impact not only to the safety of the mussels but also to prevent the flow cytometry from the blockage of the mussels in the suction needle. However, combining between flow cytometry and continues observation of the mussels on filtering the particle under the microscope might become a promising method to measure the filtration rate of the mussels in more rapid and accurate measurement which consider the length duration of the mussels continuously opening their valve.

5. Conclusion

Although the opening-valve duration observation in a well plate setting indicates filtering activity in juvenile mussel, this study shows that not all of the mussel are fully open their valve during the observation. In addition, to observe the constant move and crawl of juvenile mussels might require a method which could observed them throughout the experiment. This present work led to other research method such as using microphotography combine with FCM analysis or overall adjustment between indirect measurement and direct measurement of particle capture in order to get the closer result to juvenile mussel filtering biology. Hopefully, this initial work will stimulate such future study.

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