

# Effects of pH on filtration of freshwater pearl mussel Lamellidens marginalis, Lamarck (Bivalvia: Unionidae) under laboratory conditions

# SHAFIQUL ISLAM, MD. TAUHID HASAN, MOHAMMAD FERDOUS SIDDIQUE, MOHAMMAD MONIRUZZAMAN<sup>1</sup>, MD. SAZZAD HOSSAIN<sup>2</sup> AND M. JASIM UDDIN<sup>\*</sup>

Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh <sup>1</sup>Bangladesh Fisheries Research Institute, Mymensingh 2201, Bangladesh

<sup>2</sup>Department of Aquaculture, Bangladesh Agricultural University, Mymensingh2202, Bangladesh \*Email: jasimfm@bau.edu.bd

**Abstract**. The study was conducted to evaluate the effects of pH on the filtration rate of freshwater pearl mussel *Lamellidens marginalis* under laboratory conditions. Three treatments were used in triplicate; treatment 1 (T<sub>1</sub>) at pH 7.5, treatment 2 (T<sub>2</sub>) at pH 8.0 and treatment 3 (T<sub>3</sub>) at pH 8.5. Ten mussels were stocked in each aquarium with continuous aeration. After 48 hours, 40 ml eutrophicated water having 34.75 x  $10^5$  to 36.0 x  $10^5$  cells/ml of phytoplankton were provided in each aquarium. Initial concentration of phytoplankton was  $14400\pm360.55$ ,  $13900\pm360.55$  and  $14133\pm404.14$  cells/ml, whereas the final concentrations were  $10300\pm100.00$ ,  $8300\pm200.00$  and  $10333\pm57.73$  cells/ml in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively. The reduction rate was consistently lower in T<sub>2</sub> when compared to other treatments. Mussels of T<sub>2</sub> (pH 8.0) exhibited significantly higher filtration rate (p<0.05) over T<sub>1</sub> (pH 7.5) and T<sub>3</sub> (pH 8.5) at all sampling times. The minimum filtration rate  $58.03\pm32.79$  ml/mussel/h was found in T<sub>3</sub> after 1 h exposure, whereas the highest filtration rate  $110.94\pm21.68$  ml/mussel/h was obtained in T<sub>2</sub> after 2 h exposure. The results concluded that the pH has a clear effect on feeding habit of freshwater pearl mussel and pH 8 provides environment friendly to maximize the filtration of *L. marginalis*.

Keywords: Lamellidens marginalis, pH, Phytoplankton, Unionidae

# Introduction

Bivalves remove particulate organic matter (POM) and suspended algae from the water column. Mussels process large volumes of water and exposed to dissolved toxic substances such as heavy metals (Jana and Das 1997, Kadar *et al.* 2002). On other hand, mussels are efficient filter feeders and can siphon nutrients from water column by means of depleting phytoplankton (Soto and Mena 1999, Areekijseree *et al.* 2004). They act as natural water cleaner and bioindicators of water body which would help to maintain the river and stream ecosystem (Areekijseree *et al.* 2004, Dan *et al.* 2001). Freshwater mussels are the most important components of food webs which linked to multiple trophic levels (Nobles and Zhang 2015). Furthermore, freshwater mussels are declining from the ecosystem due to water pollution, reduction of fish hosts as well as indiscriminate harvesting of mussels from natural habitat for commercial purposes. Nowadays, freshwater mussels have great interest in laboratory-based propagation and rearing techniques to better understand their ecological and eco-toxicological effects (Patterson *et al.* 2018). However, most important challenges in freshwater mussel culture are maintaining phytoplankton levels and stable water qualities in culture environment (Kunz *et al.* 2020).

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Filtration rate is a fundamental parameter in bio-energetic studies of suspension feeding of bivalves community. A vast number of methods and measurements of bivalve filtration rates in relation to factors such as ambient algal concentration and temperature were carried out since the beginning of the 21st century (Soucek *et al.* 2001). Bivalve molluscs can utilize their calcium carbonate (CaCO<sub>3</sub>) reserves to buffer the impairments in haemolymph pH (Heming *et al.* 1988). It was reported that the higher temperature with alkaline pH of water can increase the filtration rate in freshwater mussels than the lower temperature with lower or acidic pH (Loayza-Muro and Elias-Letts 2007). Temperature and body size of mussels were also affected on the filtration rate (Pestana *et al.* 2009). Bivalves filter water continuously and feed on plankton (Mandal *et al.* 2007). Bivalves, particularly mussels, are efficient filter feeders that are capable of depleting the water column of phytoplankton (Dame *et al.* 1991).

There are two species of pearl producing bivalves are available in Bangladesh such as Lamellidens marginalis and L. corrianus, of which L. marginalis is the most preferred freshwater bivalve species that are used as food by some ethnic groups in Bangladesh, India and Nepal (Dan et al. 2001). In Bangladesh, L. marginalis are collected only from natural sources like rivers, ponds, etc. and are predominantly used for the production of lime (CaCO<sub>3</sub>), and for poultry and aquaculture feeds (Siddique et al. 2020). The L. marginalis is an important pink pearl producing freshwater mussel having an increasing demand in pearl producing countries (Miah et al. 2000). Environmental factors such as, temperature, salinity, DO, pH, alkalinity etc. are playing vital roles in growth, survival, respiration, feeding and reproduction of bivalves (Uddin et al. 2013, Niogee et al. 2019, Siddique et al. 2020). Temperature and pH are most important factors that may effect on the physiology of freshwater mussels (Areekijseree et al. 2004, Sangsawang et al. 2019). The pH may impact on the physiology of L. marginalis by changing extracellular acid-base balance, metabolic activities and feeding behavior (Heming et al. 1988). In this study, the effects of pH on filtration rate of freshwater pearl mussel was found suitable because filtration rate is considered as an adequate sensitive sublethal endpoint for evaluating the biological effects of stress parameters like pH (Loayza-Muro and Elias-Letts 2007). Based on the above point of view, the present study was conducted to establish the effects of different levels of pH on filtration rate of freshwater pearl mussels L. marginalis under laboratory conditions.

# **Materials and Methods**

Collection and maintenance of L. marginalis. The study was conducted in the Aquatic Ecology Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Adult freshwater pearl mussels L. marginalis were collected from a perennial pond by hand-picking. Mussels were transported to the laboratory using buckets with respective pond water that were continuously agitated. The experimental mussels were acclimated in fiberglass tanks (100-L capacity) for one week in dechlorinated and filtered tap water. Continuous aeration was provided in the tanks up to near saturation. Mussels having similar shell length ranged from 65 to 70 mm were selected for the experiment to avoid size-specific effects. The shell length, shell height and shell width were measured by using digital calipers in mm and recorded.

Experimental design and data collection: For evaluating the effects of pH on filtration rate of L. marginalis, three treatments were designed in triplicate; treatment 1 (T<sub>1</sub>)- pH 7.5, treatment 2- pH 8.0 and treatment 3- pH 8.5 in nine aquaria (30 cm x 30 cm x 30 cm). Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) or sodium hydroxide (NaOH) were added for maintaining constant pH of the aquaria throughout the experimental period. The pH range of this experiment was selected based on Loayza-Muro and Elias-Letts (2007) method. Each aquarium contained 10 L of dechlorinated and filtered tap water with no planktonic particles. Ten mussels were stocked in each aquarium with continuous gentle aeration. No planktonic food was provided for first 48 hours in all the treatments to deplete the gastric planktonic contents. After 48 hours, 40 ml eutrophicated water having 34.75x10<sup>5</sup> to 36.0x10<sup>5</sup> cells/ml of phytoplankton were provided in each aquarium. Water samples were collected at 0, 1, 2, 3, 4 and 5 h after addition of planktonic algae and cell concentrations were counted by using S-R cell under a microscope (Binocular microscope, Olympus, Model B-2, Japan) and expressed as cells/ml.

The filtration rate (FR) was obtained via the indirect method of measuring the decrease in algal concentrations in the aquarium using the following formula of Coughlan (1969):

$$FR = (V/nt) \ln (C_0/C_t)$$

Where, V indicates volume of water per aquarium (ml) = 10000 ml, n is the number of individuals per aquarium = 10, t denotes time (hour), t is log base t, t and t are algal concentrations (cells/ml) at time 0 and at time t (h), respectively. Water quality parameters such as temperature and dissolved oxygen of each aquarium were monitored at 1 h interval during the experimental period.

Statistical analysis: All the values were tabulated in Microsoft excel spread sheet and expressed as mean  $(\pm SD)$ . Filtration rates in different treatments were analyzed by one-way ANOVA followed by Turkey's post hoc test to evaluate statistically significant differences among different treatments. Statistical analyses were performed using SPSS Version 14.0 (SPSS Inc., Chicago, IL).

# **Results**

The mean shell length, shell width and shell height of experimental mussels were  $69.33\pm1.05$ ,  $30.82\pm0.42$  and  $19.27\pm0.53$  mm in  $T_1$ ;  $66.96\pm3.34$ ,  $30.28\pm1.64$  and  $19.61\pm0.80$  mm in  $T_2$ ; and  $67.91\pm1.25$ ,  $30.23\pm0.51$  and  $19.34\pm0.19$  mm in  $T_3$  (Table I). The concentration of phytoplankton gradually decreased with the progression of time in each treatment. Among the treatments, density of phytoplankton was remarkably lower in  $T_2$  (pH 8.0) in comparison to other treatments (Fig. 1). The filtration rate was decreasing gradually although the magnitude was not similar in all the treatments. The minimum filtration rate ( $58.03\pm32.79$  ml/mussel/h) was found in  $T_3$  after 1 hour of exposure, whereas the highest filtration rate was obtained in  $T_2$  after 2 hours of exposure (Table II).

Mussels stocked in  $T_2$  () exhibited significantly higher filtration rate (p < 0.05) when compared with  $T_1$  () and  $T_3$  () at all sampling times. However, there was no significant difference in filtration rate (p > 0.05) between  $T_1$  and  $T_3$  (Fig. 2). Temperature was monitored at 1 hour interval during 5 hours exposure time. Water temperature was more or less similar in all

the treatments examined (Table III). During the experimental period, dissolved oxygen (DO) contents were monitored, that were more or less consistent in all the treatments (Table III).

Table I. Biometric measurements (mean  $\pm$  SD) of Lamellidens marginalis in different treatments

Treatments	Shell length (mm)	Shell width (mm)	Shell height (mm)
T <sub>1</sub> (pH 7.5)	69.33 ± 1.05	30.82 ± 0.42	19.27 ± 0.53
T <sub>2</sub> (pH 8.0)	$66.96 \pm 3.34$	$30.28 \pm 1.64$	$19.61 \pm 0.80$
T <sub>3</sub> (pH 8.5)	67.91 ± 1.25	$30.23 \pm 0.51$	$19.34 \pm 0.19$

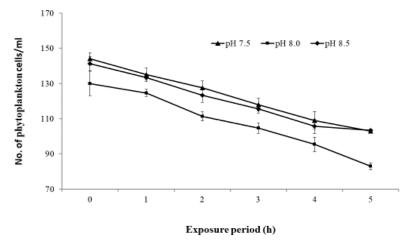


Fig. 1. Changes in density of phytoplankton over time in different treatments.

Table II. Filtration rates of *L. marginalis* at different pH levels at 1 h interval for 5 h exposure time

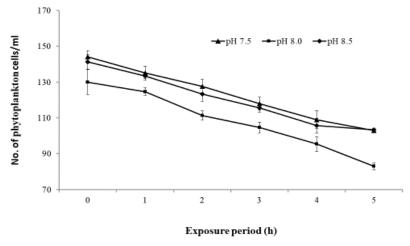
Treatments	Filtration rate (ml/mussel/h)						
	1 h	2 h	3 h	4 h	5 h		
T <sub>1</sub> (pH 7.5)	$64.61 \pm 10.25^{a}$	$60.23 \pm 34.10^{a}$	66.40 ± 16.17 <sup>a</sup>	69.73 ± 14.57 <sup>a</sup>	66.97 ± 3.18 <sup>a</sup>		
T <sub>2</sub> (pH 8.0)	$108.69 \pm 30.59^{b}$	$110.94\pm21.68^{b}$	94.58± 12.27 <sup>b</sup>	94.37 ± 12.51 <sup>b</sup>	103.12± 5.95 <sup>b</sup>		
T <sub>3</sub> (pH 8.5)	$58.03 \pm 32.79^{ab}$	$68.15 \pm 19.32^{ab}$	$66.76 \pm 14.74^{a}$	$72.76 \pm 2.99^a$	$62.57 \pm 5.68^{a}$		

Different superscripts (a, b, ab) in the same column indicate significant difference (p<0.05)

### Discussion

In the study, variation in filtration rate of freshwater pearl mussels *Lamellidens marginalis* at different pH levels was compared. The pH is important parameters for mussels growth as they prefer alkaline water due to better growth of their calcareous shell (Areekijseree *et al.* 2004). The results of the study clearly indicated that mussels exhibited highest filtration at pH 8.0. Likewise, Loayza-Muro and Elias-Letts (2007) reported that filtration rate was highest at pH 8.0 compared to low water pH (4, 5, 6 and 7) in freshwater mussel *Anodontites trapesialis* which is in agreement of the present study. From the study, it can be corroborated that among the different environmental factors, pH was significantly affected the respiratory mechanism of *L. marginalis*. No mortality was observed during the experimental period at different level of pH exposure, reflects that freshwater mussels can survive within the pH ranged from 7.5 to 8.5, however, they may become stressed between pH 7.5 and 8.5 in relation to water filtration. In contrast to the study, Dimock and Wright (1993) as well as Pynnonen and Huebner (1995) reported the adverse effect of acidic pH in freshwater mussels which endorsed the selection of pH ranges in the present study.

The decreased filtration rate at low pH observed in the study could be explained by temporal and partial closure of shells. Influx of H+ ions which produce tissue irritation and stimulate adductor muscle contraction, resulting in a temporal valve closure. Similarly, this was observed for *Anodonta cygnea* (Pynnonen and Huebner 1995) and other freshwater unionids under acid stress (pH 4.0 to 5.0) for extended periods of time. In a related study, Loayza-Muro and Elias-Letts (2007) reported filtration responses of the mussel *Anodontites trapesialis* to different environmental stressors. The filtration rate was calculated from the clearance of algae, fed to mussels at different temperature, pH and metal concentrations. The researchers reported that highest filtration rate at pH 8 at 20°C and decreased at low temperature and pH level. In the study, the highest average filtration rate of *L. marginalis* was reported at pH 8.0 and the lowest filtration rate was observed at pH 8.5 which are in agreement with the above study.



**Fig. 2.** Filtration rates of *Lamellidens marginalis* (Mean±SD) at different pH conditions in aquaria during 5 hours exposure time.

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Table III. Changes in temperature and dissolved oxygen (DO) level (mean  $\pm$  SD) in the study period

Paramete	Treatments	Hours of exposure					Mean±SD	
r		0	1	2	3	4	5	
Temp.	T <sub>1</sub> (pH 7.5)	26.73 ± 0.35	26.3 ± 0.10	26.36 ± 0.05	26.46 ± 0.15	26.43 ± 0.15	26.76 ± 0.25	26.50 ± 0.10
( C)		26.36 +	26.56 +	26.63 +	26.43 +	26.7 +	26.7 +	26.56 +
	$T_2$ (pH 8.0)	0.72	0.20	0.15	0.15	0.17	0.15	0.22
T <sub>3</sub> (pH 8.5)	$26.07\ \pm$	$26.66~\pm$	$26.53~\pm$	$26.63~\pm$	$26.36~\pm$	$26.63~\pm$	$26.48 \pm$	
	13 (p11 0.5)	0.26	0.15	0.15	0.05	0.05	0.30	0.10
DO (mg/L)	T <sub>1</sub> (pH 7.5)	$5.38 \pm 0.22$	$5.42 \pm 0.34$	$5.70 \pm 0.31$	$5.72 \pm 0.1$	$5.58 \pm 0.04$	$5.43 \pm 0.13$	$5.53 \pm 0.12$
	T <sub>2</sub> (pH 8.0)	5.41 ±	5.46 ±	5.80 ±	5.46 ±	5.63 ±	5.52 ±	5.54 ±
	12 (pri 6.0)	0.10	0.27	0.15	0.15	0.06	0.14	0.07
Ta	T <sub>3</sub> (pH 8.5)	$5.64 \pm$	$5.64 \pm$	$5.36 \pm$	$5.65 \pm$	$5.63 \pm$	$5.44 \pm$	$5.56 \pm$
	13 (p11 0.3)	0.10	0.10	0.32	0.10	0.12	0.23	0.09

In the study, 40 ml eutrophicated water having 34.75x10<sup>5</sup> to 36.0x10<sup>5</sup> cells/ml of phytoplankton was inoculated in each aquarium to estimate the filtration rate at different pH levels. Likewise, Loayza-Muro and Elias-Letts (2007) was evaluated the clearance rate of Crypthecodinium sp. suspensions by A. trapesialis at a concentration of 2x10<sup>5</sup> cells/ml. The food source concentration used in the study is similar to that of assess filtration in Mimachlamys asperrima (O'Connor et al. 2000). The filtration rate of L. marginalis ranged from 58 to 110 ml/mussel/h, where it was 347 to 567 ml/mussel/h in Corbicula sp. (Savina and Pouvreau 2004), 40 to 375 ml/mussel/h in *Dreissena polymorpha* (Horgan and Mills 2011), and 133 to 350 ml/mussel/h in Limnoperna fortune (Pestana et al. 2009). Present finding was within the ranges of the above reports when compared. The differences can be possible as the filtration rate may vary considerably among different species of bivalves. Temperature and DO have also considerable effects on the filtration rate of bivalves. Pestana et al. (2009) suggested that the relationship between filtration rates and temperature might depend on the size of the filtered particles in Limnoperna fortunei (Bivalvia, Mytilidae). de Villiers et al. (1989) reported highest filtration rate of infaunal estuarine bivalve Solen cylindraceus with a thermal optimal range of 15-35°C, declining at higher and lower temperatures. Tang and Riisgard (2017) obtained a high and constant filtration rate of blue mussel (Mytilus edulis) when exposed to oxygen concentrations decreasing from 9 to 2 mg/l however, at oxygen concentrations below 2 mg/l, M. edulis responded by gradually closing its valves, resulting in a rapid decrease of filtration rate, concurrent with a rapid reduction of respiration rate. In our experiment, we observed more or less constant temperature and DO levels throughout the 5-h period might suggest that these parameters might not affected considerably the filtration rate of L. marginalis.

**Conclusions:** Filtration rate of freshwater pearl mussel *L. marginalis* exposed to different pH up to 5 h was determined to understand the filtration capacity in relation to alkaline pH level. The filtration rate was decreasing consistently with the progression of exposure time. Mussels stocked at pH 8.0 exhibited significantly higher filtration rate and showed minimum rate when stocked in pH 8.5 after 1 h of exposure whereas the highest filtration rate was obtained in pH 8.0 after 2 h of exposure. The results indicated that pH has a clear effect on freshwater pearl

mussel especially at pH 8.0 where the maximum filtration of *L. marginalis* is observed. The study is of importance to provide better environmental conditions for optimum growth of this species. However, the physiological mechanisms involved in the filtration of freshwater mussels at different pH should be ascertained.

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