The Effect of Flow on Filtering and Gaping Behavior in *Mytilus galloprovinicalis*

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Abstract

As ecosystem engineers, mussels create habitat for many species by ameliorating harsh environments, like extreme wave forces and temperatures. In low flow conditions, however, mussel aggregations may alter their local chemical environment via respiration and calcification, creating hypoxic conditions within the interstitial gaps between mussels. Thus, the local flow regime may play a large part in whether mussel aggregations are beneficial or detrimental to other species. However, the extent to which mussel filtering and gaping behavior may prevent the development of harsh chemical conditions by increasing mixing in the intertidal zones and the surrounding water is still unknown. We quantified the effects of flow on mussel filtering behavior to better understand how they interact to influence local water chemistry. We used Hall effect sensors to quantify mussel gaping and found a positive linear relationship with filtering speed. Flow (up to 20 cm/s) had no significant effect on gaping, although mussels positioned further downstream within an aggregation tended to gape wider. Our results indicate that individual variation in behavior due to spatial position maybe be more important than flow in determining gaping activity, thus having consequences for how mussel behavior influences change in chemistry within the interstitial zone.

Introduction

Ecosystem engineers serve to modify their immediate environment and generate environmental heterogeneity in habitats at the landscape level (Jones et al. 1997). By doing so, mussels increase biodiversity by creating new niches for other organisms. Coastal marine conditions can be harsh and stressful for organisms, especially in intertidal habitats where organisms are alternately exposed to extreme wave forces and aerial temperatures with the rise and fall of the tide (Arribas et al. 2014; Raffaelli and Hawkin, 1999). Mussels are a great model species for coastal ecosystem engineers as they play an important role on rocky, coastal shores. They are a popular model organism for ecological, physiological, and biomechanical studies (Carrington et al. 2014), in part because they are known to live in a wide variety of environmental conditions and have displayed an impressive capacity to withstand extreme environmental changes (Bayne, 1976). Mussels are also recognized as ecological facilitators for local taxa and these ecosystem engineers can help other intertidal organisms succeed in their environment (Bruno et al. 2003). In the intertidal zone, mussels are known to stabilize the sedimentary environment by aggregating into beds that protect the intertidal sediment (Meadows et al. 1998). Combined with their propensity to mitigate severe abiotic stresses like wave forces and temperature extremes (Bruno et al. 2003; Bulleri 2009; Ninokawa 2019), mussels produce a hospitable environment for many species.

As habitat-modifying creatures that support numerous ecosystems, foundation species control carbon fluxes, nutrient cycling, soil accretion, and other ecological processes (Angelini et al. 2011; Dayton, 1972; Ellison et al. 2005). Through their own engineering activities and by further enhancing biodiversity by expanding niche, mussels have the capacity to change an ecosystem's multifunctionality (Angelini, 2014). They possess the ability to alter the chemistry of their local seawater environment via respiration and calcification (Ninokawa et al. 2019; Gutiérrez et al. 2003). These processes allow for the increase in CO_2 and decrease in O_2 and DO, both of which can increase or decrease the pH and carbonate ions (CO_3^{2-}) in their environment (Ninokawa et al. 2019).

What is less well understood is how local-scale chemical modifications are affected by mussel behavior such as filtering. Roughly fifteen gallons of water is filtered a day by a single mussel, concentrating phytoplankton (food) and other impurities from the environment. This filtering behavior can potentially affect a mussel's ability to alter the chemical environment in its immediate habitat (Murie 2022; Crimaldi et al. 2007; Frechette 2012). When a mussel is submerged in water, its valves open, or gape, allowing for water exchange for feeding and the maintenance of aerobic respiration; when the valves are intermittently closed the mussel does not feed and may switch to anaerobic metabolism (Nicastro et al. 2010; Widdows et al. 1979; Famme and Kofoed 1980). When emersed in the air at low tide, these closed valves reduce evaporative water loss, a common abiotic stressor within intertidal habitats, at the cost of inefficient exploitation of organic energy (Nicastro et al. 2010).

Despite a mussel's ability to filter large volumes of water, water circulation might be limited to areas above a mussel aggregation, while corrosive conditions may develop in interstitial spaces within a mussel aggregation. This is because ambient flow slows down within mussels beds, creating stagnant water within interstitial spaces, ranging from 0.1% to 10% of free-stream velocity (Carrington et al. 2008). As such, there may be a noticeable difference between the interstitial seawater and the seawater adjacent to the habitat due to metabolic processes (Ninokawa et al. 2019; Hurd 2015). This can be exacerbated by low flow conditions, such as slack tide or calm weather, which further limits the exchange of water, and as a result, decreases in exchange may restrict not only the replenishment of food and nutrients but also the elimination of metabolic wastes (Ninokawa et al. 2019). Water stagnation is a problem for many habitat-forming organisms such as oysters, seagrass, and coral but is potentially more extreme in mussel beds because they form denser aggregations. As such, mussel beds are an ideal system to study how chemical gradients develop in biogenic habitats.

A key consideration is the feedback local water conditions may have on mussel behavior. For example, high flow may decrease the impact of mussel activity on the interstitial microclimate by flushing interstitial spaces with bulk seawater while low flow may cause water to stagnate and result in steep concentrations of pH and DO (low flow) (Murie 2021; Ninokawa et al 2019). Moreover, due to elevated CO_2 concentrations and global warming, seawater in coastal environments have acidified and deoxygenated, both of which negatively affect the behavior and morphology of mussels (Gaylord et al 2011; Newcomb et al. 2019), alterations to a mussel's environmental condition can cause mortality, slower growth rates, and reduced byssal thread production. (Newcomb et al. 2019). Understanding the extent to which mussels can positively affect their local chemical environment via filtering is important in understanding how to combat these effects.

In this study, we examined the relationship between flow and gaping behavior in *Mytilus galloprovincialis* under different flow conditions. We first used Particle Image Velocimetry (PIV) to establish gaping behavior as a proxy for filtering activity, then used magnetic gape sensors to quantify the gaping activity of mussel aggregations in a laboratory flume at flow speeds ranging from 0 – 20 cm/s. We also evaluated the relationship between gaping behavior and a mussel's proximate position downstream of the flow.

Methods

M. galloprovincialis were sourced from Penn Cove Shellfish in Coupeville, Washington. Mussels were maintained in a saltwater live box with constantly flowing water in order to maintain a suitable temperature (13-15 C) for mussels. They were fed daily and roughly 1-2 hours before the trials began.

To understand the relationship between flow and gaping behavior, mussel aggregations (roughly 51 cm long, using 51-60 mm shell length mussels) were created using two acrylic plates relatively the same size. Magnetometer gape sensors, which enable high-resolution tracking of individual mussel valve movement, (Luke and Dowd, 2017) were attached to six individual mussels using Z-spar and left overnight to allow the epoxy to completely cure and to allow mussels to lay down their byssal threads to adhere to the plates. Afterward, the plates were placed into the flume (roughly 90 cm long working section). Mussels were numbered according to the gape sensor it was attached to. There were six sensors total, however, sensor two was unavailable, so, the last sensor was labeled seven. The six mussels were placed in different positions of the aggregation: two at the upstream side, two in the middle, and two on the downstream side. Six different flow speeds were used during the 3-hour trials. Zero cm/s was the starting speed and was gradually increased at different intervals (0 cm/s, 2 cm/s, 8 cm/s, 12/cm/s) until it reached 20cm/s. Data were sampled at 1 Hz for 3 hours, capturing mV readings that correlated to gape distance between the magnet and the sensor.

Particle image velocimetry (PIV) methods from a previous REU student (French, 2021) were used to establish the relationship between filtering and gaping. A camera was set to capture footage at ~24 frames per second for easier analysis in MatLab.

After all trials were completed, the gape sensor for each mussel was calibrated by using calipers to measure valve separation (mm) and recording the corresponding mV reading. The data were recorded and plotted using Excel and an exponential curve fit was used to generate an equation to convert mV readings from each sensor to gape distance from the three-hour trials.

Time series graphs were created for all trials and used to create frequency distributions to characterize each mussel's preferred gape distance open and closed in mm. We then tested for the effect of flow on percent time gaping using Welch's ANOVA test using R. We used linear regression to test for the effect of downstream position on preferred gape distance.

<u>Results</u>

Individual variation in gaping behavior in steady flow.

The gape sensor calibrations were successful using an exponential curve, with an R^2 ranging from 0.98-0.99 (Figure 1). Individual mussels tended to have their own preference for gape distance under certain flow conditions. Mussels 3-6 preferred to be open at approximately 5mm, mussel 1 preferred to be open at approximately 7 mm, mussel 6 at appx. 2 mm, and mussel

7 at appx. 4 mm (Figure 2a, b). Mussel 1 was positioned furthest downstream and preferred to be open at approximately 7 mm when experiencing 8 cm/s flow conditions. Though mussel 6 was not the furthest upstream, it was still relatively close to the source of the flow and preferred to be open at approximately 3 cm/s. This was also observed during the two-day trial.

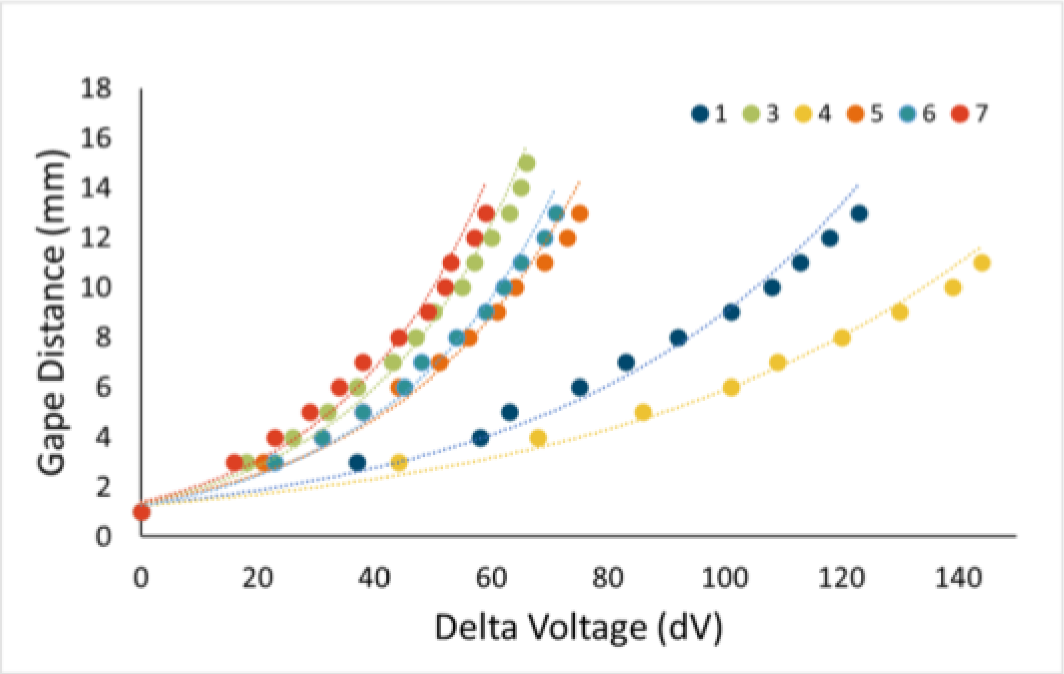

Figure 1. Calibration curve. The x-axis indicates voltage (dV) and y-axis indicates gape distance

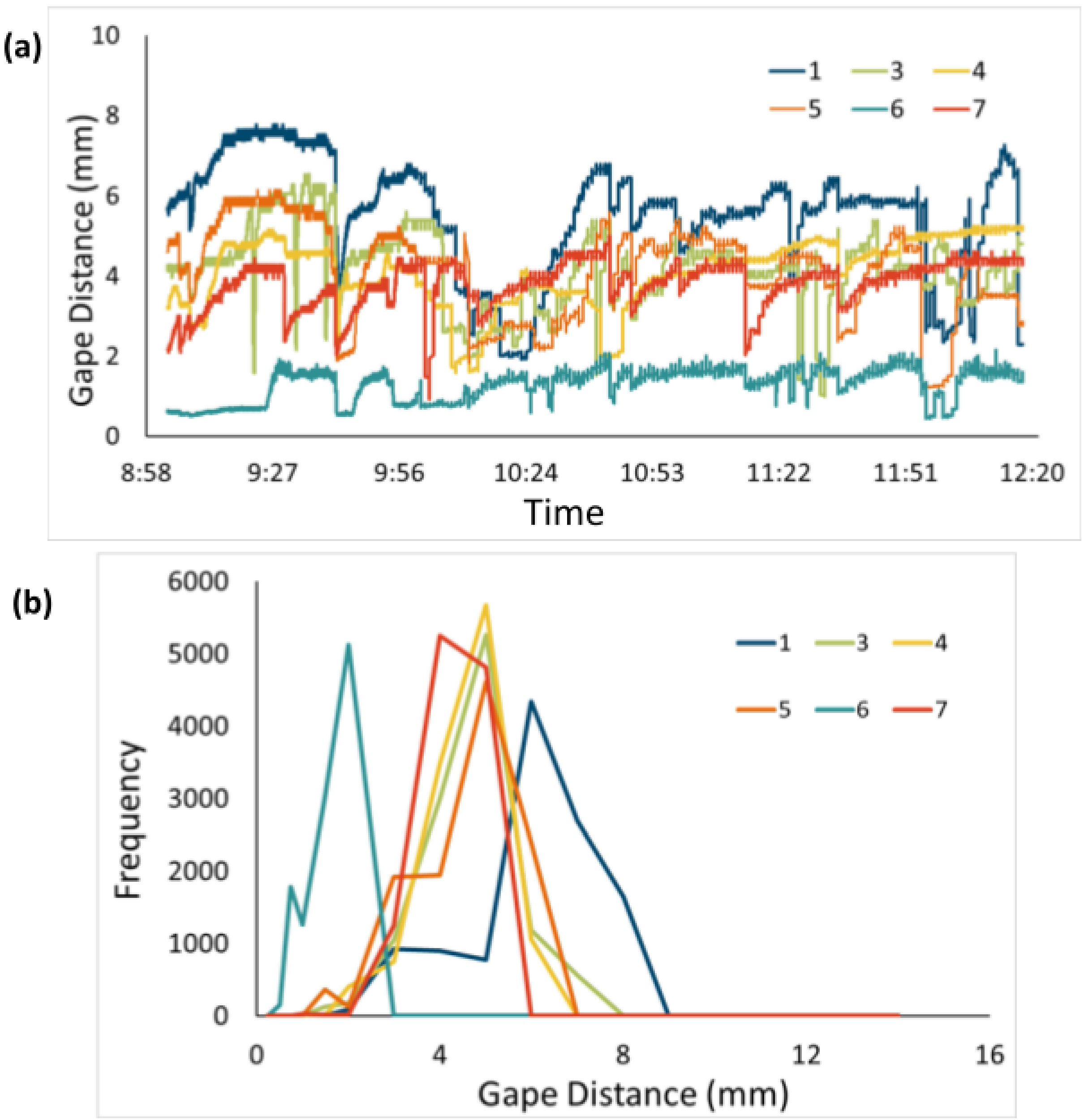

Figure 2. (a) Representative time series of gape distance (mm) over a 3-hour trial at 8 cm/s. Each trace represents a different mussel sampled at 1Hz. (b) The frequency distribution of gape distances for each mussel, generated from time series data for the 8 cm/s trials.

Relationship between filtering and gaping

There was a positive linear relationship between mussel gape (measured as an uncalibrated voltage (mV) and their pumping speed (cm/s). There was a slight variation in the data, however, the regression line indicated that 43% of the variance in gape voltage was explained by their maximal pumping speed.

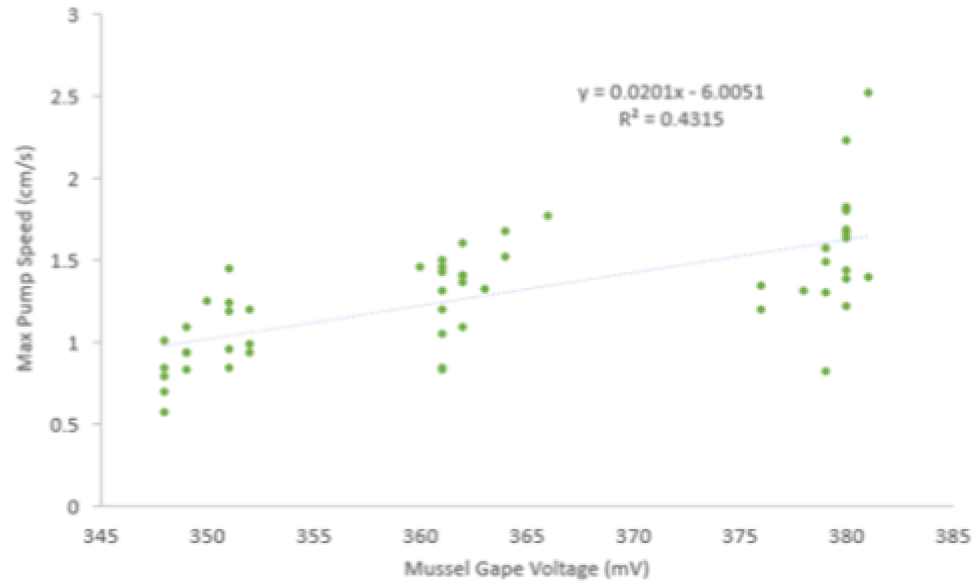

Figure 4. Maximal pumping speed, quantified using PIV, as a function of mV output from a single mussel. Line is a linear regression, with $r^2 = 0.43$.

Gaping behavior as a function of flow speed

Mussels tended to stay open most of the time rather than closed, and there was no clear trend with increasing flow speed (Figure 5). There was, however, a significant difference in percent time open at a flow speed of 5 cm/s compared to 2 cm/s and 8 cm/s, which were consistently high (Welch's ANOVA; p=0.002). Gaping behavior at flow speeds of 0, 12, and 20 cm/s was highly variable and can therefore were intermediate between these two extremes, as indicated with ‘ab’ (Post-hoc Games-Howell; p ≥ 0.05).

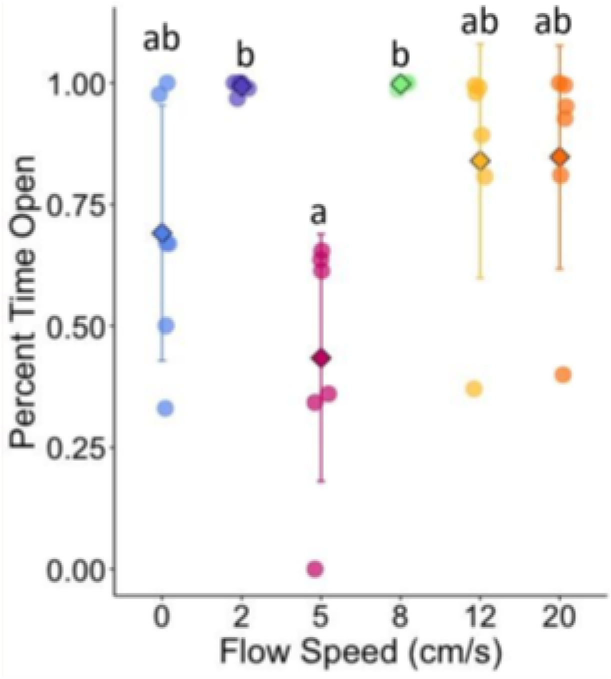

Figure 5. Percent time open in relation to flow speed. Welch’s ANOVA test generate using R. P-value=0.002 indicating significant difference between 2 cm/s, 5 cm/s and 5 cm/s, 8 cm/s. Diamond indicates mean flow speed and whiskers represent the interquartile range (IQR). Circle indicates all percent time data for each mussel. Letters that are different from one another (ab, a, b) denote significance.

Spatial Patterns in Gaping

Each trace in the graph follows almost the same pattern indicating behavioral synchrony in the longer trial (Figure 6a). Mode gape distance (mm) increased as the position of mussels moved further downstream (Figure 6b); approximately 55% of the variance in gape distance was explained by position downstream (Figure 6c). The patterns go up and down together, however, the range is bigger for those positioned downstream.

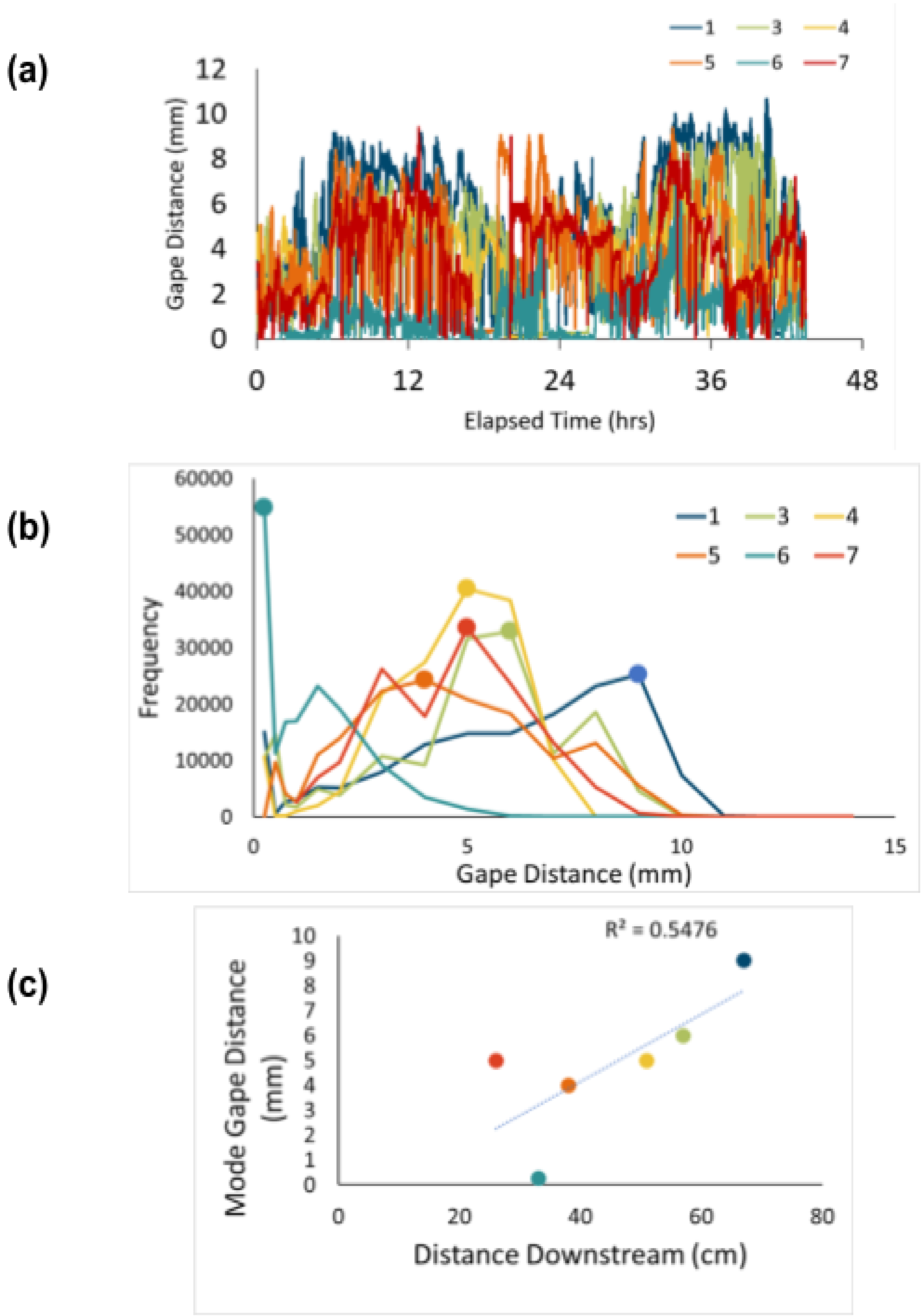

Figure 6. (a) Gape activity of six mussels during an extended trial (roughly 48 hours) at 2 cm/s. Each trace represents a different mussel. (b) Gape distance frequency distribution for each of the six mussels in the ~48-hour trial at 2 cm/s. Filled circle indicates the mode gape distance for each mussel. (c) Mode gape distance (mm) as a function of distance downstream (cm) for the six mussels in the ~48-hour trial at 2 cm/s. Dashed line is a linear regression, with r^2=0.5476.

Discussion

Under a given flow condition, mussels tended to have their own preference for gape position, ranging from 0-8 mm in this study. There was no positive or negative correlation between gape distance and position downstream, however, it was observed that mussels positioned further downstream tended to be open wider than those positioned upstream. Mode gape distance increased as mussels moved further downstream. This could suggest that mussels farther away from the source of the flow prefer to stay more open since they are not receiving the brunt force of the flow, while those further upstream receive more surface shear stress and would therefore close up or prefer a smaller gape distance. This finding is consistent with that of Wildish and Miyares (1990), where flow velocity greatly reduced the filtration rate of mussels facing into the unidirectional flow. The flume used in our study was also unidirectional, so this could explain why some mussels were gaping less than others. Mussel filtration rates are also known to decrease when the flow reaches 10-30 cm^{-1}, and at low velocities such as 0-5 cm^{-1} as it greatly reduces ambient particle concentrations (Newell et al. 2001). It was also observed that when mussels were fed, especially in ambient (0 cm/s) flow conditions, they tended to pump at a faster rate.

When mussels gaped more, their max pumping speed increased (Figure 4). This could mean that to create greater pump force, mussels must gape further. However, there was variation below and under the line of best fit. This could imply that rather than altering the distance between their shells, mussels were altering the physical shape of their exhalant siphon. Further trials are needed to establish whether gape distance is a reasonable proxy for filtering behavior. This information will be valuable for field investigations of mussel gaping, where direct measures of feeding currents (using PIV) are not possible.

Though there were significant differences in gaping behavior (measured as percent time open) between flow speeds of 2 cm/s and 5 cm/s as well as 5cm/s and 8 cm/s, there was no linear relationship over the full range flow speeds investigated. This could be due to the small sample size (n = 6 mussels) and high mussel behavior variance. Additional trials are needed to adequately evaluate the effect of flow on mussel gaping behavior.

Synchrony was observed in the 48-hour trial. Synchrony is the precise occurrence of two or more behaviors at the same time (Ravignani 2017). The temporal organization of individual acts, typically in a non-interactive context, is the focus of spontaneous individual rhythms, also known as synchrony. The term "spontaneous" in this context refers to a general methodological trend of watching or documenting an animal's spontaneous behavior as opposed to its responses to experimental manipulations (Ravignani 2019). This is interesting because it not only exhibits a sort of social behavior, but it can also predict how mussels interact with one another. This could suggest the influence of their neighbor, however, the idea of mussels responding to their neighbor by mimicking its gaping behavior is not well understood, though it would be interesting to explore it in the future as it could help us better understand mussel behavior and how it's influenced by its environment.

Overall, this study showed that individual mussels have a preferred gape distance that increases with position downstream. Mussels preferred to stay open for the majority of the duration of the trials, however, there was no clear trend with increasing flow. It is important to focus on foundation species such as mussels because they have a controlling influence on population and community dynamics as well as ecosystem processes, often by bioengineering macroscale environments to provide microhabitats for a diversity of species. Understanding how the effects of flow influence its filtering behavior could help address global societal concerns such as hypoxia, ocean acidification, and ocean warming as well as help inform a computational fluid dynamics model that aims to predict when concentration gradients develop within mussel beds. Active filtration of excess nutrients and pollutants can inform when and where they would provide effective and sustainable bioremediation and as global warming brings down pH levels in the ocean, a mussel's ability to filter is an important factor to consider and observe to maintain the health of mussels and the habitat they create.

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