Effects of Porewater Flow on Interstitial Algal Composition and Juvenile Unionid Mussel Feeding

By
Victor Fung

A Thesis
Presented to
The University of Guelph

In partial fulfillment of requirements
For the degree of
Master of Science
In
Integrative Biology

Guelph, Ontario, Canada

© Victor Fung, February, 2019
ABSTRACT

EFFECTS OF POREWATER FLOW ON INTERSTITIAL ALGAL COMPOSITION AND JUVENILE UNIONID MUSSEL FEEDING

Victor Fung  
University of Guelph, 2018

An examination of porewater algae and their removal by juvenile unionid mussels (3 – 4 week old *Lampsilis siliquoidea*, Fatmucket mussels) will help facilitate their recovery. Flow cytometry revealed higher fluorescent particle and algal concentrations in interstitial regions (downstream of boulders > upstream of boulders and non-bedform regions) vs. surface waters. Clearance rates (*CR*) based on chlorophyll *a* fluorescence were also higher in mussels exposed to interstitial water in a recirculating flow chamber. *CR* based on identified algal taxa varied with algal concentration. Chesson’s Electivity index revealed that algal removal was consistent with algal concentration in 5 chlorophyte taxa but was higher in 5 diatom taxa; i.e., juvenile mussels selected diatoms across the range of algal flux examined, whereas chlorophytes were taken at random. This study provides evidence of the importance of diatoms and flow in the interstitial habitats of juvenile mussels and the need to conserve this physico-chemical environment.
Acknowledgements

There have been many people who have helped me in my pursuit of my Master’s. First and foremost, I would like to thank my advisor, Dr. Josef Daniel Ackerman, for his guidance, encouragements, advice and mentorship during the course of my studies. I was also very fortunate to have the guidance and support from my advisory committee, Dr. Gerald Mackie, Dr. Todd Morris and Dr. Paul Sibley and thank them for their time and helpful feedback.

My special thanks go to “Grandmaster” Christopher Robert Farrow; for spending hundreds of hours training me in the art of R programming, algae identification, and FlowCAM; Julian Lum, for being my field partner and bearing with my demands (keeping multiple YSI instruments in his waders) and Shaylah Tuttle-Raycraft for dedicating her time to raise juvenile mussels for this study and providing me with invaluable information for life in graduate studies. Without their guidance, support and friendship over the last two years I would not be here today. I would like to acknowledge all the past, and present Ackerman Lab members who have assisted me with my field excursions, care of my mussels and water chemistry.

A profound thank you to my friends in the Integrative Biology department who have been with me through this journey. In particular, Boyan Liu, Rachel Holub, Anne Easton, and Dr. Karl Cottenie for always being available to talk to.

Finally, thanks to friends and family, all whom encouraged and motivated me to finish this thesis.
# Table of Contents

**ABSTRACT** ................................................................. ii

**Acknowledgements** .................................................. iii

**List of Figures** .......................................................... v

**INTRODUCTION** ......................................................... 1

**METHODS:** ................................................................. 11

- **Study Site:** .................................................................... 11
- **Hydraulic Conductivity of Sediments from the Study Site** .................. 13
- **Study Organism** .......................................................... 13
- **Identification of Algal Taxa** .......................................... 17
- **Feeding Electivity** ....................................................... 18
- **Field Study of Algae in Pore and Surface Waters** ...................... 19
- **Statistical analysis** ...................................................... 22

**RESULTS** ........................................................................ 24

- **Field Conditions** .......................................................... 24
- **Algal Taxa in the Thames River** ...................................... 31
- **Feeding study** .............................................................. 41
- **Feeding Electivity** ........................................................ 45

**DISCUSSION** .................................................................. 52

- **Feeding electivity and taxon specific algal flux** ......................... 53
- **Unionid Habitat** ........................................................... 55
- **Spatial dynamics of algae in the riverbed** ............................ 56
- **Implications for the conservation of unionid mussels** ............... 58

**CONCLUSION** ................................................................. 60

**LITERATURE CITED** ...................................................... 61
List of Figures

Figure 1: Schematic diagram of streamlines over a bedform (indicated in black) and associated bed pressure gradients. The lines represent streamlines, the blue represents the flow separation zone where recirculation occurs and the red line represents the advection of water in the interstitial zone. The lower image represents associated pressure gradients. Redrawn from Blois et al. (2014). .............................................. 4

Figure 2: Sediment airlift system and measurement equipment setup: (A) components of the lift system:(a) air pump, (b) airlift apparatus, and (c) recovery bucket. (B) In stream substrate processing components: (a) series of sieves (64, 250, 500, 2000 µm), (b) corer, and (c) measuring equipment scale.12

Figure 3: Racetrack flow chamber system used to measure the clearance rate of juvenile unionid mussels under different flux. (A) photograph of the racetrack flow. (B) schematic of an individual racetrack flow chamber. The black arrow represents the direction of water flow (from Mistry and Ackerman, 2016)..... 16

Figure 4: (A)Map of the Innerkip study site and locations of bedform and non-bedform areas sampled, based on a survey conducted in conjunction with another project. River flow is from left to right in the image.(B) Illustration of different sampling sites. .................................................................................................................. 21

Figure 5: Physical chemical conditions measured in open water, and in porewaters upstream and downstream of boulders and in non-bedform areas in the Innerkip River study reach. A – Temperature (°C), B – dissolved oxygen (mg L⁻¹), C – pH, and D – conductivity (µS cm⁻¹) measured from weekly porewater samples from July to September 2017 across bedform (N = 2) and non-bedform (N = 2) locations (mean ± SE). .................................................................................................................. 26

Figure 6: Nutrient concentrations measured in open water, and in porewaters upstream and downstream of boulders and in non-bedform areas in the Innerkip River study reach. A – Ammonia (mg L⁻¹), B – Phosphate (mg L⁻¹), C – Nitrate (mg L⁻¹), and D – Nitrite (mg L⁻¹) measured from weekly porewater samples from the month of July to September 2017, across all bedform (N = 2) and non-bedform (N = 2) locations (mean ± SE). .................................................................................................................. 28

Figure 7: Substrate size distribution of streambed sediments sampled upstream and downstream of boulders and in non-bedform areas in the Innerkip River study reach: (A) d₁₀ values; (B) d₅₀ values; and (C) d₇₄ values (µm) measured. Bars are the mean ± SE, N = 2 up - and downstream of bedforms, and N = 4 in non-bedform areas. .................................................................................................................. 30

Figure 8: Relative abundance of major algal groups at A – open water, B – non-bedform porewaters, C – porewater upstream of boulder and D – downstream of boulder in 2017......................................................... 32
Figure 9: Variation in the concentration of (A) algal and (B) proportion of algal vs. non-algal particles found at different locations in the Innerkip site of the Thames River. Bars indicate average ± SE measured in open (N = 2) and porewater samples, across all bedform (N = 2) and non-bedform locations (N = 2). Bars sharing the same letter are not significantly different. 

Figure 10: Spatial and temporal patterns in the concentration of (A) Centric diatoms and (B) *Nitzschia* concentration across all bedform (N = 2), non-bedform porewaters (N = 2) and open waters (N = 2) from weekly samples taken between July and September 2017 (Bars are the mean ± SE). 

Figure 11: Spatial patterns in the concentration of (A) *Navicula*, (B) *Scenedesmus*, (C) *Monoraphidium*, (D) *Chlamydomonas*, (E) *Staurodesmus*, (F) *Oscillatoria*, (G) *Pediastrum*, (H) *Chlorella*, (I) *Tetraedron* and (J) Cyanobacteria concentration across all bedform (N = 2) (Bars are the mean ± SE). Bars with the same letter are not significantly different according to a Tukey’s test. 

Figure 12: Clearance rates (CR) of juvenile Fatmucket mussels on river water collected from the Thames River. Average CR in open and porewater treatments based on change in Chlorophyll a fluorescence (Bars are the mean ± SE). 

Figure 13: The average clearance rate (CR) of juvenile Fatmucket mussels on 10 algal taxa identified in the porewaters of the Thames River (Bars are the mean ± SE). 

Figure 14: The square root of the clearance rate (CR) vs. the taxa specific initial algal concentration of 10 algal taxa in open and porewaters by juvenile Fatmucket mussels. 

Figure 15: Chesson’s electivity of A- Centric Diatoms, B- *Nitzschia* C- Unidentified Pennate Diatom, D – *Navicula*, E- Small Centric Diatom, F – *Chlamydomonas*, G – Small Chlorophyte, H – *Coelastrum*, I – *Pediastrum*, and J – *Scenedesmus* (Vertical: ± Confidence intervals, Horizontal: ± SE ) of juvenile Fatmucket mussel at different levels of taxa specific algal flux. Intersection with 0.1 represents random selection. 

Figure 16: The electivity of open water algal taxa small chlorophyte sp., *Nitzschia* and centric diatoms (mean ± SE) calculated by Jacob’s Modified Electivity Index. 

Figure 17: The electivity of juvenile Fatmucket mussels for algal taxa found in open water against algal taxa specific flux.
Figure 18: Jacob’s Modified electivity of A- Centric Diatoms, B- *Nitzschia* and C- Small Chlorophyte (mean ± 95% Confidence intervals) of juvenile Fatmucket mussels at different levels of taxa specific algal flux. Intersection with 0 represents random selection. .................................................................................................................. 51

List of Tables

**Table 1**: Summary of the statistical comparison of algal taxa concentration (cells mL⁻¹) sampled at the Innerkip site at different times and locations: (A) p – value from the two-way ANOVA and (B) p – value from the Kruskal – Wallis Test. .......................................................................................................................................................................................... 41

**Table 2**: Summary of linear regression of clearance rate (CR) versus the initial concentration of the algal taxon using shell length as a covariate. Results are presented for the slope, significance and R² of the regression. Significant relationships are in bold. ........................................................................................................................................................................ 45
INTRODUCTION

Unionid mussels play a vital role in freshwater ecosystems, providing many ecosystem services such as improving water quality through the clearance of particulates, and the creation and modification of river microhabitats (Vaughn et al., 2008). These freshwater mussels are found in wetlands, lakes and streams in multi-species, multi-aged mussel beds (Vaughn, 1997; Haag, 2012). Unionid suspension feeding influences nutrient concentrations in overlying waters and they deposit nutrients from overlying waters into streambeds through their pseudofaeces (rejected particles) and feces (Vaughn et al., 2004; Vaughn and Hakenkamp, 2001). Mussels also create habitats for other benthic organisms through their burrowing activities, which may stabilize streambeds and provide refuge with their discarded shells (Vaughn and Hakenkamp, 2001).

The ecosystem services (e.g., habitat modification and creation, nutrient cycling) performed by mussels depend on the species present, their abundance, and spatial/temporal variation in environmental factors such as temperature, seasonality and water flow (Spooner and Vaughn, 2006; Vaughn et al. 2008; Vaughn 2018). Unionid mussels are unique in that they need a vertebrate (mostly fish) host for their parasitic larvae (glochidia) to complete their development and metamorphose into juvenile mussels (Haag, 2012). Their range is, consequently, limited spatially to where they excyst from their fish host and settle to the bottom (Haag, 2012). Changes in environmental factors related to habitat degradation, pollution, and invasive species as well as commercial exploitation have led to their designation as a highly imperiled group (Strayer et al., 2004; Haag 2012; Haag and Williams, 2014). Efforts have been made to reintroduce and relocate species, but a better understanding of the habitat requirements for juvenile unionid mussels remains an important issue (Haag and Williams, 2014). It is important then to discern the habitat
requirements and basic biological requirements (e.g., feeding) of juvenile mussels to help facilitate their recovery and conservation in general.

**Hydrodynamic Forces and Unionids**

Hydrodynamic forces are important for many aspects of mussel biology (Ackerman 2014). For example, fluid forces such as bed shear stress (force per unit area parallel to the riverbed; Ackerman 2014) alter streambed composition (Harvey et al., 2012), affect the dispersal of glochidia (Schwalb et al., 2010), and the settlement of juvenile mussels post excystment from their hosts onto the riverbed (Morales et al., 2006; French and Ackerman, 2014). Areas with high shear stress have lower mussel densities (Layzer and Madison, 1995) because shear stress affects the ability of juvenile mussels to settle onto the substrate, and may cause resuspension and thus limit settlement (French and Ackerman, 2014). These shear forces also affect stream porewater (water in the interstitial spaces of streambed) exchange (i.e., water exchange between interstitial zone and overlying waters), which is important for the establishment of juvenile unionid mussel habitats, allowing them to occupy interstitial zones (Layzer and Madison, 1995; Blois et al., 2014).

**Hyporheic Zones**

The hyporheic (or interstitial) zone is the interface between overlying river water and groundwater and it varies spatially and temporally (Wetzel, 2001). The exchange of particulates and nutrients between the overlying surface water and the interstitial zone is carried out through molecular diffusion and advection (or water motion) and is determined by hydrodynamic near-bed flows and substrate permeability (Huettel et al., 2003). River flows have been shown to
transport phytoplankton, bacteria and organic detritus particles into the interstitial zone along with dissolved oxygen (DO), which are important for hyporheic habitats (Jones et al., 2014; Cardenas et al., 2016; Roley and Tank, 2016). For example, Clubshell Mussel (*Pleurobema clava*) populations declined in areas with low porewater DO due to sedimentation of interstitial pore spaces decreasing porewater - surface exchange (Roley and Tank 2016). Greater densities of invertebrates were also found in hyporheic zones with greater interstitial porosity and therefore higher oxygen levels and organic content (Strayer et al. 1997).

The size, shape, and composition of riverbed substrates affect the permeability, determine the penetration of organic matter, oxygen availability, and flow patterns in the interstitial space (Boulton et al., 1998). For example, the entry of fine sediments and the growth of biofilm on the surface of riverbed substrates can affect the porosity of the sediment by trapping particles (Vandeveire and Baveye, 1992; Brunke, 1999). In areas with low near-bed flow, fine sediments and organic matter, which deposit through sedimentation in the water column, can accumulate in the interstitial space (Huettel et al., 2003). This lowers hydraulic conductivity (i.e., the ease of a fluid to flow through a porous material) and also creates a solute concentration gradient allowing diffusional processes to dominate (Huettel et al., 2003). Under faster and more turbulent near-bed flows, fine and larger sediments can be re-suspended, creating a more coarse-grained bed, which increases hydraulic conductivity and increases advective flows in the interstitial zone (Schalchi, 1992).

Differences in temperature (i.e., fluid density) and elevation (i.e., hydrostatic [or pressure] head) over a distance creates pressure or hydraulic gradients that cause advection of water and particles within the interstitial zone (Figure 1; Thibodeaux and Boyle, 1987). Bed or bathymetric features such as rocks and coarse woody debris can also affect the transfer between...
surface and porewaters. This is because such features increase local pressure gradients upstream that facilitate the entry of particles and water into the interstitial zone, and as the flow passes over these obstructions there is a decline in the local pressure gradient downstream within the recirculation zone that draws porewater out from the interstitial zone (Huettel et al., 2003). Bedform-induced advective flows allow greater algal penetration into the interstitial zone upstream of bedforms because the bedform pressure gradients force water entry at steep angles (Huettel and Rusch 2000).

**Figure 1:** Schematic diagram of streamlines over a bedform (indicated in black) and associated bed pressure gradients. The lines represent streamlines, the blue represents the flow separation zone where recirculation occurs, and the red line represents the advection of water in the interstitial zone. The lower image represents associated pressure gradients. Redrawn from Blois et al. (2014).

The entry of algae into the interstitial zone is not only limited by porosity of the bed substrate but also by the physical properties of the algal cell such as its size, shape, density, motility and surface characteristics (Huettel and Rusch, 2000). Chemical properties such as ionic strength and pH also affect entry of particles into the interstitial zone by increasing electrostatic particle-particle interactions and thus particle filtration (Ren and Packman, 2002). Strong groundwater upwelling can also prevent advective flow in the interstitial zone, whereas weak upwelling will allow some advective flow but limit the penetration depth in the hyporheic zone (Cardenas et al., 2016).
Particles can be ‘filtered’ in the interstitial zone through physical sieving by pore space, gravitational settling/sedimentation, collision and physico-chemical attachment to streambed substrate (Packman et al., 2000; Bradford et al., 2004, 2006). Under steady hyporheic flows, particle deposition and retention in the interstitial zone is greater than particle resuspension, resulting in a higher concentration of particles in the hyporheic zone (Arnon et al., 2010). Hydrodynamics can conceivably play a large role in the delivery of food resources to interstitial zones where mussels feed (Vanden Byllaardt and Ackerman, 2014). An understanding of how algal composition in the interstitial zone is influenced by hydrodynamics (e.g., near-bed flow and hydraulic conductivity) would provide insight to what food is available to unionid mussels including Species at Risk mussel, which would be valuable for recovery strategies (vanden Byllaardt and Ackerman, 2014).

Benthic algal species in rivers are important primary producers and consist largely of Cyanophyta, Chlorophyta, Bacillariophyta, and Rhodophyta, but other taxa can be found in the assemblage through settlement onto the riverbed or in a dormant form (Stevenson et al., 1996). The algal composition of benthic communities is affected by the immigration and emigration of algal taxa, and is regulated by interspecific differences such as morphology, sinking rates and adhesiveness (Stevenson and Peterson, 1991). The benthic community influences the overlying stream phytoplankton assemblage by serving as a depository, likewise, the stream also serves as a depository for benthic communities (Roeder, 1977).

**Algae as a food source for unionid mussels**

Phytoplankton are an important component of bivalve diets, providing important biochemical resources necessary for development and growth (Wikfors et al., 1992). The nutritional content
of phytoplankton is dependent on environmental conditions such as light, temperature and nutrients (Brown et al., 1997; Gatenby et al., 2003). For example, Gatenby et al. (1997) demonstrated that juvenile mussels fed algal diets had greater growth rates than those reared on fine sediments and associated microbial flora alone. Differences in growth rates were also observed among mussels fed different algal diets because algae may have different nutritional content. For example, diatoms (Bacillariophyta) such as Cyclotella and Nitzschia have high polyunsaturated fatty acids (PUFA) and lipid content, which are important for mussel growth (Gatenby et al., 2003). Conversely, green algae (Chlorophyta) such as Chlorella and Chlamydomonas have lower PUFA content, compared to diatoms, but still contain lipid content mussels need (Gatenby et al., 2003).

**Unionid mussel feeding**

Adult unionid mussels have been found to feed on algae, detritus, bacteria, fine organic particulate matter, and zooplankton through suspension and deposit feeding (Vaughn et al., 2008). Suspension feeding is undertaken through the generation of flow into the inhalant siphon of the mussel by cilia found on the gill surface (Haag, 2012). Deposit feeding occurs through the generation of a current by cilia on the foot into the shell or by pedal feeding, directly sweeping food into the shell with the foot (Yaeger et al., 1994; Haag, 2012). Juvenile unionids feed within the interstitial zone and have been found to be more reliant on pedal feeding for the first few weeks post-metamorphosis, eventually transitioning to suspension feeding (Yaeger et al., 1994; Gatenby et al., 1997).

Insights on unionid feeding can be provided from other bivalve systems, for example marine bivalves have different clearance rates (CR; removal of particles from a volume of fluid
per unit time per individual), ingestion and retention of cell types among species. These differences suggest they are able to select particles that have greater importance in their diet (Shumway et al., 1985). Chemical cues (Ward and Targett, 1989), electrostatic charge (Hernroth et al., 2000), and particle size (Defossez and Hawkins, 1997; Nadaffi et al., 2007) have also been shown to affect marine bivalve selective feeding behaviour (reviewed in Ward and Shumway, 2004).

Particle size (Beck and Neves, 2003) and algal flux (Mistry and Ackerman, 2018) affect the selection of particles by unionids, but to the best of my knowledge, the mode of selection (chemical cues or electrostatic charge) remains unknown. Different CR have been found among species, habitat, and life-stages in unionid mussels (Silverman et al., 1997; Baker and Levinton, 2003; Beck and Neves, 2003). For example, Beck and Neves (2003) fed a mixture of *Scenedesmus quadricula*, *Nannochloropsis oculata*, and *Selenastrum capricornutum* to Rainbow mussel (*Villosa iris*) of 3 age groups (2–3 d, 50–53 d, and 3–6 years). They found that all age groups selected smaller algae, *N. oculata* and *S. capricornutum* (2.8–8.5 µm), and rejected *S. quadricula* (22.3–44.5 µm). Although *N. oculata* and *S. capricornutum* are of similar size, there were significant differences found in their ingestion among all age groups, suggesting there were other particle characteristics influencing selective feeding. Lopes-Lima et al. (2014) examined seasonal phytoplankton composition and stomach contents of *Anodonta cygnea* and found that Chlorophyta and Bacillariophyta were ingested in proportion to their abundance in the environment. In the spring months, however, mussels selected for Cyanobacteria (containing proteins and essential fatty acids) which were in low abundance, whereas they selected Cryptophyta (containing high starch content) in the autumn months (Lopes-Lima et al., 2014). These change in algal preference coincided with gametogenesis and
spawning periods respectively, suggesting that mussels select food based on nutritional needs to meet seasonal physiological demands.

**Algal flux and unionid feeding**

Measurements of mussel suspension feeding are often obtained through controlled laboratory experiments in aerated static (i.e. non-flowing) containers, which are not representative of natural environmental conditions where flow and algal concentrations vary (Ackerman, 1999; Yu and Culver, 1999; Elliott et al., 2008; Ackerman, 2014). Studies have shown that water flow leads to increased water mixing and the replenishment of local resources, which increases bivalve feeding ability as reflected in greater CR at higher velocities (Ackerman, 1999; Elliott et al., 2008). Moreover, CR were on average 20× those obtained in static containers (vanden Byllaardt and Ackerman, 2014). High flows can also equate with high algal flux ($J = UC$, cells cm$^{-2}$ s$^{-1}$ where $U$ [cm s$^{-1}$] is the velocity and $C$ [cells mm$^{-3}$] is the algal concentration) that can saturate the gills due to increased particle handling time, which result in lower filtration efficiency and CR, and a type II functional feeding response (Palmer, 1980; Bontes et al., 2007; Mistry and Ackerman, 2018). Under even faster flow conditions, however, CR may be inhibited due to lift and drag forces causing mussel behavioural and filtration instability (retraction of byssus in the case of dreissenids, siphon and valve closure; Wildish et al., 1987; Ackerman, 1999; Nielsen and Vismann, 2014).

Studies on the combined effects of particle concentrations and flow velocity on bivalves indicates that they are able to respond to food concentration as well as flow velocity (Wildish et al., 1992; Cahalan et al., 1989). For example, vanden Byllaardt and Ackerman (2014) fed *Elliptio complanata, Elliptio dilatata, Fusconaia flava* and *Strophitus undulatus* lab-cultured *Chlorella*
and natural seston at different flux. Clearance rate increased with increasing flux but differed in the same species from different habitats, suggesting adaptation to local hydrodynamic conditions (vanden Byllaardt and Ackerman, 2014). Mistry and Ackerman (2016) demonstrated that increases in algal flux resulted in increased CR of juvenile mussels. Higher algal flux also reduced the ability of adult mussels to discriminate and selectively feed on algal taxa (Mistry and Ackerman, 2018). These findings suggest bivalves have adaptive behaviour, because they can respond (feeding response) to dietary quality, different velocities and concentrations to optimize feeding (Cahalan et al., 1989; Wildish et al., 1992; Ackerman and Nishizaki, 2004). Optimal foraging behaviour may explain these observations, as there may be optimal conditions (i.e., velocities and concentrations) at which handling and capturing of particles is most efficient (Ackerman, 1999).

Optimal foraging theory (OFT) predicts an organism will choose food items that will maximize net energy gain per unit feeding time (Calow and Townsend, 1981). If the abundance of profitable food items is low, then selection may be low or absent, whereas if abundance of profitable food items is high, selection may favor high quality over lower quality food items (MacArthur, 1972). Optimal foraging theory assumes a forager must make decisions to optimize efficiency such as identifying optimal prey or patches and when to move between patches (Bartumeus and Catalan, 2009). Because juvenile unionid mussels have limited mobility, food items encountered are dependent on hydrodynamics and are thus stochastic (Jumars et al., 1981). With this information, it would be interesting to determine if juvenile mussel feeding response to flux found in their natural habitat can be predicted by optimal foraging theory. This determination would require a knowledge of (1) whether juvenile mussels feed differently at different flux as well as (2) the nutritional content of what they ingest. The goal of this thesis is
to determine the first condition, namely are there differences in the feeding response of juvenile mussels at different flux of porewater. Operationally, this will also require that we determine whether there are differences in porewater algal composition among river bed locations.

**Research Question:** Does porewater algal composition differ spatially in streambeds?

**Hypothesis #1:**

Does algal composition (i.e., species and abundance) differ among open water and interstitial waters in different riverbed locations because of differential retention/loss of algae in interstitial water related to pressure gradients and hydraulic conductivity.

**Prediction #1:**

Areas with greater sediment size and adequate hydraulic conductivity (coarser sediments due to resuspension and winnowing) will have greater concentrations of algae.

**Research Question:** Do juvenile mussels feed selectively?

**Hypothesis #2:**

Is the selective feeding of juvenile unionid mussel (i.e., what algal taxa they remove and at what rate) affected by algal flux because of the delivery and replenishment of resources.

**Predictions #2:**

Juvenile unionid selective feeding (i.e., particle selectivity) will increase with algal flux up to a limit when particle handling time limits feeding.
METHODS:

Study Site:

The Thames River near Innerkip, ON (43.25728, -80.73725) was selected using the following criteria: (1) there was evidence of mussel recruitment (presence of juvenile mussels with shell length ≤ 3 cm); (2) there were large bedforms (e.g., >15 cm height above the bottom and >20 cm diameter [large cobble or boulder]) present in a non-armoured area (to allow entry of sampling devices); (3) there were non-bedform areas (e.g., <5 cm height above the bottom, composed mostly of sand and gravel) in a non-armoured area; (4) there was sufficient water flow (e.g., evidence of a strong recirculation zone downstream of large cobble/boulder) and water depth (>5-10 cm above the bedform); and (5) the site was accessible for field work.

The composition of the substrate at areas in which porewaters were sampled on the streambed was examined to provide information regarding the average substrate size and thus permeability. In this case, sediment samples were taken upstream and downstream of the two boulders and non-bedform region of the river bed sampled using a 14.2 cm diameter (i.e., 158 cm$^2$) steel core pushed into the riverbed. Bed material within the corer was excavated manually and using an airlift system (Figure 2) to a depth of 8 cm, which is the maximum depth at which juvenile mussels have been found (Neves and Widlak, 1987). The excavated material was passed through a series of four 20-cm diameter stainless steel sieves (mesh size: 64, 250, 500, 2000 µm) and each was weighed. Material greater than 4 mm was removed manually and weighed separately. A cumulative frequency plot of the mass vs. the grain size was used to determine the particle size distribution ($d_{10}$, $d_{50}$, and $d_{74}$, where $d$ is the effective diameter of the sediment particles and the subscript is the percent of the sample which is smaller) for each sample (Carter, 2008).
Riverbed substrate composition of the study site reach was surveyed through a visual classification of riverbed substrate using the Wentworth Scale (Wentworth, 1922). A fixed grid system was set up, by placing a measure tape in the streamwise direction, and anchoring another measuring tape perpendicular across the stream. Data were collected every 1 m along the cross-stream transect, which was moved 1 m in the streamwise direction upon completing the cross transect (Lum and Ackerman, unpublished). A total of 739 points was collected and used to generate a map of the river bed substrate with ArcMap.
Hydraulic Conductivity of Sediments from the Study Site

To determine porewater velocities in juvenile mussel habitat, a constant-head permeameter was used to measure hydraulic conductivity of streambed substrate collected from the study site (Thames River, Innerkip, ON) using an existing protocol (Mistry and Ackerman, 2016). Briefly, the permeameter (5.08 cm diameter × 45 cm long) was filled with interstitial sediments (grain size ranging from 100 µm – 6.5 cm) collected from the Thames River and tap water (20 ± 1 ºC) was added until the discharge was constant due to full saturation (i.e., no air bubbles). The rate of discharge \( Q \) was determined from the time taken to fill a 1000 mL graduated cylinder below the permeameter, which was undertaken 10 times using the same bed material. Hydraulic conductivity \( k \) was obtained using Darcy’s equation,

\[
k = \frac{QL}{Aht}
\]

where \( L \) is the length of the column (0.45 m), \( A \) is the cross-sectional area of the column (0.07 m\(^2\)), \( h \) is the head difference (0.25 m), \( Q \) is the rate of discharge (m\(^3\) s\(^{-1}\)) and \( t \) is the time of water displacement through the porous medium.

Study Organism

Juvenile Fatmucket mussels (\textit{Lampsilis siliquoidea}) were transformed by infecting known host fish (Rock Bass \textit{Ambloplites rupestris}) with glochidia using an existing protocol (McNichols et al., 2011). Briefly, gravid female Fatmucket were collected from the Thames River at Innerkip, ON (43.25728, -80.73725), placed in a cooler, and transported to the Hagen Aqualab at the University of Guelph where they were maintained at 15-17 ºC and at a 12:12 light cycle before returning to their collection site. Glochidia were flushed from one half of the marsupial gills of the female mussels and checked for viability (ASTM 2006) prior to infesting Rock Bass.
collected in the Grand River, ON. Each Rock Bass was placed into 1.5 L of aerated water and exposed to glochidia for 60 minutes, and then transferred to a separate unit in an Aquatic Habitat (AHAB) where their tanks were monitored for the presence of juvenile mussels.

When newly transformed juveniles were found they were collected and placed into aerated 325 mL Pyrex glass crystallization dishes in age-specific cohorts. Approximately 800 juvenile mussels were transformed for this project. Juvenile survivorship rate was monitored weekly by recording the number of living juveniles using a dissecting microscope. Clearance rate experiments were performed once juveniles were 3-4 weeks old.

**Clearance Rate Experiments**

In order to examine my second hypothesis, I conducted feeding experiments using 3-4 week-old juvenile mussels and porewater from non-bedform areas and surface water at the Innerkip site. This allowed a comparison of particle selectivity (food preferred in a species diet) between porewaters and surface waters at different algal fluxes (Mistry and Ackerman, 2016). Six passive groundwater samplers (Solinst model 615 drive-point piezometers; Solinst Canada) were fit with On/Off valves and push-to-connect adapters and deployed in a non-bedform area 2 cm below the riverbed. For each replicate of the feeding experiments, 86 mL water samples were drawn from 3 piezometers (alternating with each replicate) and placed in a 250 mL amber Nalgene bottle and transported to the University of Guelph where they were kept in the dark at 18° C until use in the experiments, usually within 2 hrs.

Feeding experiments were conducted in a racetrack flow chamber system (Figure 3) comprised of five oval racetracks (12 cm long × 4.5 cm wide) with channels (1.5 cm wide × 1.0 cm deep; volume = 25 mL) containing turning vanes to direct the flow, and paddle wheels that fill the
width and depth of the chamber (Mistry and Ackerman, 2016). The first four racetracks have a drive mechanism geared at 100%, 75%, 50%, 25% of the motor speed, respectively to drive the paddle wheels and the fifth is a no-flow control without a paddlewheel. The racetrack flow chambers were operated at five chamber velocities (U): (a) 0 cm s\(^{-1}\) (i.e., no-flow control); (b) 1 cm s\(^{-1}\); (c) 2 cm s\(^{-1}\); (d) 3 cm s\(^{-1}\); and (e) 4 cm s\(^{-1}\); as determined by measuring the time it took polystyrene beads (200-300 µm; density = 1.06 g cm\(^{-3}\), Polysciences Inc, Pennsylvania USA) to move through a 2 cm length (Mistry and Ackerman, 2016). Racetrack flow chambers were operated between 0 - 4 cm s\(^{-1}\) for the study. These velocities were chosen based on the results of the hydraulic conductivity measurements (see below) and to cover a wider range of flux than those examined in Mistry and Ackerman (2016). The chamber Reynolds number (\(Re = U d_h/\nu\), where \(U\) is the chamber velocity, \(d_h\) is the hydraulic diameter \([4 \times \text{area/wetted perimeter}]\) and \(n\) is the kinematic viscosity) based on \(d_h = 1.7 \times 10^{-2} \text{ m i.e., } [4 \times (1\times1.5) \times 10^{-4} \text{ m}^2/(1 + 1 + 1.5) \times 10^{-2} \text{ m}]\) of the racetrack was laminar because \(Re = 680\) for the fastest velocity \(680 = [4 \times 10^{-2} \text{ m s}^{-1} \times 1.7 \times 10^{-2} \text{ m}] /10^{-6} \text{ m}^2 \text{ s}^{-1}\). Each experimental trial took approximately 1.5 hours (1 hour to run + 15 minute setup + 15 minute clean up using a disinfectant (Superior Solutions, Ontario). No-mussel control experiments were also run to measure any seston loss that was not due to mussel feeding. Experimental and no-mussel control trials were conducted on the same day to minimize any potential changes in algal composition through time.
Figure 3: Racetrack flow chamber system used to measure the clearance rate of juvenile unionid mussels under different flux. (A) photograph of the racetrack flow. (B) schematic of an individual racetrack flow chamber. The black arrow represents the direction of water flow (from Mistry and Ackerman, 2016).

Before each experimental trial, 15 juvenile mussels were isolated for each treatment and their shell lengths were measured. Three juvenile mussels were placed in a small circular 1-mm deep hemispherical depression in the channel floor of each of the five flow chambers, which were filled with 25 mL of treatment water (surface water or interstitial water trials were run separately and chosen randomly). Experimental trials began after a 15-minute acclimation period and were run for 60 min. Water samples (10 mL) taken at $t = 0$ and 60 min were analyzed using a
fluorometer (Turner Designs 10-AU; Sunnyvale, CA, USA) to determine Chlorophyll $a$ concentrations ($C$). The change in Chlorophyll $a$ ($C$) over the course of the experiment was used to determine the clearance rate ($CR$) of the mussels using the Coughlan (1969) equation:

$$ CR = \frac{Vol}{Nt} (\ln \frac{C_i}{C_f} - \ln \frac{C_{ctrli}}{C_{ctrlf}}) $$  \hspace{1cm} (2)

where $Vol$ is the volume of the chamber (25 mL), $N$ is the number of mussels (3), $C_i$ and $C_f$ represent the initial and final Chlorophyll $a$ concentration, and $C_{ctrli}$ and $C_{ctrlf}$ are the respective values for the no-mussel controls.

It was not possible to verify the strength of the relationship between $C$ and $t$ (i.e., $\ln(C)$ vs. $t$ should be linear) because samples were only taken at beginning and end of the experiment. Moreover, in some experimental trials, the loss of $C$ was higher in the control than the experimental trials and these were not included in the analyses. This occurred in 13/30 and 17/30 for open water and interstitial trials, respectively.

**Identification of Algal Taxa**

Water samples taken at $t = 0$ and 60 min were analyzed using an imaging flow cytometer (FlowCam Model VS Series, Fluid Imaging Technologies, Maine, USA) in trigger mode (fluorescence detection after excitation by a 488 nm laser) to determine the algal composition of the treatment waters. Samples (3 mL) were diluted and screened through a 100 µm mesh to prevent clogging of the flow cell and analyzed with 10× objective (~100× magnification) at 0.2 mL min$^{-1}$. In some cases, the samples had to be diluted to prevent over-saturation of the detection system, which can occur when the PPUI (particles per used image) exceed 2 (Spaulding 2012). Imaged algal taxa were identified to the genus level whenever possible using keys from Janse van Vuuren et al. (2006) and Bellinger and Sigee (2010). Higher taxonomic rankings were used.
(e.g., centric diatoms, small chlorophyte) in cases where there were insufficient structural features or the images were too small for proper identification.

The CR of mussels was also determined using the concentration of common algal taxa identified as C in Eqn (2). In this case, algal taxa were used if they were present in high concentration (i.e., > 15 cells mL\(^{-1}\)) at \(t = 0\) in both the treatment and control trials. To determine the relative abundance for each algal taxon at each river bed location, the total particle count for each algal taxon was divided by the total number of algal particles found within their respective sample, multiplied by 100% and averaged for each replicate.

**Feeding Electivity**

A variety of electivity indices have been used to describe a species preferred food item relative to the food availability found within its environment (Lechowicz, 1982). Among these, Chesson’s index (\(\alpha\)) was used to determine electivity in interstitial water treatments because it can address variation in relative abundance of algal taxa in water samples because \(\alpha\) does not change with prey density (Chesson, 1983),

\[
\alpha_i = \frac{r_i/p_i}{\sum_{j=1}^{m} r_j/p_j}
\]

where \(r_i\) and \(r_j\) are the proportion of prey consumed by the organism, \(p_i\) and \(p_j\) are the proportion of prey type found in the environment, and \(m\) is the number of different prey types (Chesson, 1983). If \(\alpha_i = 1/m\) then there is no selective feeding, if \(\alpha_i > 1/m\) there is selective feeding of the food item, whereas \(\alpha_i < 1/m\) indicates that there is avoidance of the food item. Note that \(\alpha_i\) ranges from 0 to 1.

There were far fewer of algal taxa that met the selection criteria for use in clearance rate determination for the open water treatments (see below). Consequently, Jacob’s Modified
Electivity Index \((D)\) was applied because this electivity index calculates the selective feeding for two food types using the following

\[
D_i = r_i - p_i (r_i + p_i) - 2 (r_i \times p_i)
\]

where \(r_i\) is the relative quantity of food item in the digestive track (i.e., removed from the water as in Eqn (3)) and \(p_i\) is the relative quantity of the food item found within the environment (as in Eqn (3)). The value ranges from -1 to +1 with 0 indicating random feeding, positive values (i.e., \(D_i > 0\)) indicating feeding preference, and negative values (i.e., \(D_i < 0\)) indicating avoidance. The relative proportion of food items within the environment \((p_i)\) was determined from the particle counts at \(t_0\) obtained from the flow cytometer, and \(r_i\) was determined from the proportions at \(t_{60}\) assuming that all particles cleared from the water were ingested (i.e., within the digestive track; \(r_i\)). These methodologies cannot distinguish between particles that are ingested and those that are rejected either pre or post ingestion (pseudofaeces and faeces, respectively).

**Field Study of Algae in Pore and Surface Waters**

The porewater upstream and downstream of two large boulders (>15 cm height above the bottom and >20 cm diameter [i.e., large cobble or boulder]) and two non-bedform areas (< 5 cm height above the bottom, composed mostly of sand and gravel) located within the study reach at Innerkip, ON were sampled weekly from July to September 2017 (Figure 4A). A pushpoint piezometer (MHE Products, East Tawas, Michigan, USA) was used to collect 60 mL porewater samples ~ 5 cm upstream and ~ 5 cm downstream of bedforms and in non-bedform areas on each sampling date (Figure 4B). The pushpoint consists of a stainless steel body with a series of interlaced slots that allows the uptake of water, and a guard-rod that provides structural support during the insertion of the body into the sediments. Once the piezometer was inserted into the
sediment (2-8 cm), the guard rod was removed, and a syringe (60 mL; BD Syringe) was attached to the sample port. Water was withdrawn at 60 mL min\(^{-1}\), and the first aliquot was discarded due to turbidity. Representative water samples were taken and oxygen, pH, and conductivity were measured with the YSI multi-parameter probe (YSI QS600; Yellow Springs Instruments, Yellow Springs, OH, USA). Water samples were placed in a 250 mL amber Nalgene bottle and transported to the University of Guelph where they were kept in the dark at 4\(^{\circ}\) C for no longer than 2 days before analysis in the flow cytometer. Ammonia, phosphate, nitrite, and nitrate of open and interstitial water samples were measured using a calorimeter (HACH DR690) in the lab within 2 hrs of collection.
Figure 4: (A) Map of the Innerkip study site and locations of bedform and non-bedform areas sampled, based on a survey conducted in conjunction with another project. River flow is from left to right in the image. (B) Illustration of different sampling sites.

Algal taxa were imaged using the FlowCAM trigger mode as described above. In this case, water samples (1 mL) were screened through a 35 µm mesh and analyzed with 20× objective (~200× magnification) at 0.05 mL min⁻¹ in trigger mode. Samples were diluted with Ultrapure water to maintain a PPUI < 2. To determine the proportion of algal particles to non-algal particles, three samples (12, 21, and 26 September 2017) were also processed using the FlowCAM autoimage mode, which images all particles in the moving fluid at continuous set
intervals. After each sample run, Windex [S.C. Johnson] and deionized water was used to clean and flush the flow cell to prevent contamination of samples.

Statistical analysis

The results of the clearance rate (CR) study were analyzed using analysis of covariance (ANCOVA). Specifically, a one-way ANCOVA was used to examine the effects of independent variables (porewater vs. surface water treatment) and covariates (initial Chlorophyll \(a\) concentration, shell length) on CR (response) determined using the fluorometer data. This model was chosen because other covariates related to algal flux (e.g., initial algal flux and velocity) did not pass the AIC (Akaike information criterion) analysis for use in the analysis. A similar approach was used to analyze the CR data based on the common algal taxa identified using the FlowCam. Specifically, a two-way ANCOVA was used to examine the effects of independent variables (porewater vs. surface water treatment, and algal taxa) and covariates (initial algal concentration and shell length) on CR. Tukey’s post-hoc tests were used to identify significant pairwise differences when significant differences were detected in the ANCOVA. When a significant interaction was found, separate linear regressions were performed, comparing the CR of specific algal taxa against their initial algal concentrations. The assumption of homogeneity of variance was assessed using diagnostic plots and the assumption of normality of residuals was examined using the Shapiro-Wilk Test. In cases where the assumptions were violated, the data were transformed to meet the assumptions of the statistical model.

The feeding electivity results were analyzed separately for pore and surface water (i.e., treatments) because different electivity indices were used. A one-way ANCOVA was used to determine the effects of independent variables (algal taxa) and covariates (flux and shell length)
on the response (electivity (α)) in interstitial waters. The electivity of algal particles was also examined by plotting the estimated marginal means of Jacob’s Modified electivity against algal flux to determine whether and how electivity changed with flux.

To determine electivity of open water taxa, the estimated marginal means of Chesson’s electivity and their respective 95% confidence intervals were plotted against algal flux to see if there was a significant difference from 1/m (where α = 1/m is random selection). A one-way ANCOVA was used to determine the effects of independent variables (algal taxa) and covariates (flux and shell length) on the response (electivity (D)) in open waters.

The relationship among the algal taxa identified in the different streambed locations (e.g., up- and downstream of boulders, non-bedform, and surface water) through time were examined using a permutational multivariate analysis of variance (perMANOVA). A significant interaction between date and location was found, so two-way analysis of variance (ANOVA) were run for each algal taxon using sample location and date as the factors to ascertain which taxa differed in location and in time. Tukey HSD was used to determine significant pairwise differences when significant factors were found. If significant interactions were found between date and location, a plot showing the relationship was created. When the assumption of homogeneity of variance could not be met, non-parametric tests (Kruskal – Wallis test and Wilcoxon test to test for pairwise difference) were used instead. This was the case for 4 algal taxa: *Pediastrum, Chlorella, Tetraedron* and Cyanobacteria.

ANOVA was used to compare the algal concentrations, and proportion of algal to non-algal particles among streambed locations. Tukey’s post-hoc test was used to determine significant pairwise differences when significant differences were detected in the ANOVA. Shapiro-Wilk and assessing diagnostic plots were used respectively to test the assumption of
normality and assumption of homogeneity of variance. When the assumptions of homogeneity of variance, or the assumption of normality could not be met after transformations, non-parametric Kruskal–Wallis Test were used to analyze the data. If a significant difference was detected, pairwise Wilcoxon Rank Sum tests were used to determine significant pairwise differences.

Repeated measure analysis of variance (rmANOVA) were performed for each chemical parameter measured in the temporal study using independent variable location. If significant differences were found a Tukey’s HSD was used for pairwise comparison. When the assumption of homogeneity of variance could not be met, a non-parametric test (Kruskal–Wallis test) and a Wilcoxon test for pairwise difference were used if significant differences were found. This was done for nitrate and water temperature.

**RESULTS**

**Field Conditions**

The physical and chemical conditions of porewaters and open waters, which were measured weekly from July 5th to September 26th, 2017, varied temporally, and spatially. Water temperature was relatively similar in bedform and non-bedform sites, but open water temperatures were cooler (Figure 5A). A Kruskal-Wallis Test revealed significant differences in water temperatures (df = 3, p = 0.007), and pairwise comparisons revealed that open water was cooler than upstream, downstream of bedform and non-bedform sites (p = 0.044, p = 0.018, p = 0.034, respectively). Interestingly, water temperature increased in the month of September due to a warm period, which was also recorded in the Grand River, east of the Thames River, by the Grand River Conservation Authority (data.grandriver.ca). Dissolved oxygen (DO) was relatively similar in bedform and non-bedform sites, however porewater DO was considerably lower than
the levels measured in open water (Figure 5B). It is important to note that interstitial DO concentrations became hypoxic (< 2 mg L⁻¹) in later sampling days. A rmANOVA revealed significant differences in DO among bedform locations (F(3, 99) = 6.33, p < 0.001) with significantly higher DO concentrations in open water vs. upstream, downstream of bedform and non-bedform (p = 0.044, p = 0.0003, p = 0.024, respectively). The pH and conductivity was similar among non-bedform, bedform, and open water sites (Figure 5C and D; pH: F(3,99) = 1.18, p = 0.317; conductivity Kruskal-Wallis Test; df = 3, p = 0.205).
Figure 5: Physical chemical conditions measured in open water, and in porewaters upstream and downstream of boulders and in non-bedform areas in the Innerkip River study reach. A – Temperature (°C), B – dissolved oxygen (mg L⁻¹), C – pH, and D – conductivity (µS cm⁻¹) measured from weekly porewater samples from July to September 2017 across bedform (N = 2) and non-bedform (N = 2) locations (mean ± SE).

Nutrient concentrations were also measured weekly from July 5th to September 26th, 2017. A sharp increase in nitrogen levels was observed among all sampling locations in September and this may have been due to input into the surface waters from agricultural run-off within the watershed (Figure 6A, C, D). Ammonia was lower in open water than in porewaters (F(3, 23) = 2.91, p = 0.055; Figure 6A) and open water was significantly lower vs. downstream of bedform
and non-bedform areas (p = 0.0013, p = 0.0002, respectively) as was upstream of boulders vs. non-bedform (p = 0.049). Phosphate was more variable and differed among locations (F(3, 97) = 6.85, p = 0.0003; Figure 6B) with significantly lower concentrations detected in open water vs. downstream of bedform and non-bedform areas (p = 0.0066, p = 0.0274, respectively) as well as upstream of boulders vs. between non-bedform (p = 0.0274). Nitrate and nitrite declined seasonally at all locations (Figure 6C, D), but did not differ among locations (Nitrate Kruskal-Wallis test: df = 3, p = 0.848; Nitrite: F(3, 83) = 1.083, p = 0.360).
Figure 6: Nutrient concentrations measured in open water, and in porewaters upstream and downstream of boulders and in non-bedform areas in the Innerkip River study reach. A – Ammonia (mg L\(^{-1}\)), B – Phosphate (mg L\(^{-1}\)), C – Nitrate (mg L\(^{-1}\)), and D – Nitrite (mg L\(^{-1}\)) measured from weekly porewater samples from the month of July to September 2017, across all bedform (N = 2) and non-bedform (N = 2) locations (mean ± SE).

Substrate Particle Size Distribution and Hydraulic Conductivity

The substrate at the Innerkip site consisted of a mix of clay, sand, cobble and gravel. The upstream of boulders location tended to be more coarse than the downstream of boulders location, but was similar to the non-bedform location (respectively, \(d_{10} = 494 \pm 104\), \(d_{50} = 2781 \pm\) 104).
55, and $d_{74} = 3365 \pm 25 \mu m$; $d_{10} = 378 \pm 12$, $d_{50} = 2505 \pm 111$, and $d_{74} = 3217 \pm 61 \mu m$, and $d_{10} = 474 \pm 102$, $d_{50} = 2655 \pm 17$ and $d_{74} = 3297 \pm 13 \mu m$; Figure 7). The hydraulic conductivity ($k$) of riverbed substrate determined using Eq (1) ranged from $0.17 \text{ cm s}^{-1}$ and $0.28 \text{ cm s}^{-1}$ with an average of $0.22 \pm 0.01 \text{ cm s}^{-1}$.
Figure 7: Substrate size distribution of streambed sediments sampled upstream and downstream of boulders and in non-bedform areas in the Innerkip River study reach: (A) $d_{10}$ values; (B) $d_{50}$ values; and (C) $d_{74}$ values (µm) measured. Bars are the mean ± SE, $N = 2$ up and downstream of bedforms, and $N = 4$ in non-bedform areas.
Algal Taxa in the Thames River

Temporal study

On average there were 1600 ± 183 particles imaged per mL sample using 20 × magnification in the FlowCam. Twenty-two algal taxa were identified (i.e., Centric Diatom spp. (5 – 35 μm diameter), *Navicula*, *Nitzschia*, *Cymbella*, *Rhoicosphaerica*, *Pinnularia*, *Chlorella*, *Scenedesmus*, *Pediastrum*, *Coelastrum*, *Tetraedron*, *Kirchneriella*, *Chlamydomonas*, *Oocystis*, *Monoraphidium*, *Cosmarium*, *Staurodesmus*, *Cryptomonas*, *Chroomonas*, and *Oscillatoria*, *Merismopedia*, and Cyanobacteria sp.). These taxa were classified into 4 higher taxonomic ranks commonly known as diatoms, chlorophytes, cryptomonads, and Cyanobacteria and their relative abundance was plotted vs. sample date to examine changes in composition through time (Figure 8). Diatoms and chlorophytes were most abundant throughout the study at all locations sampled (July – September 2017).
**Figure 8:** Relative abundance of major algal groups at A – open water, B – non-bedform porewaters, C – porewater upstream of boulder and D – downstream of boulder in 2017.
Algal concentration was generally higher in porewater vs. open water sampling locations (Figure 9A). An ANOVA revealed significant differences among locations ($F_{(3, 44)} = 34.2$, $p < 0.001$) and algal concentrations in downstream, upstream, and non-bedform porewater samples were significantly higher than in open water samples (Tukey’s $p < 0.0001$), whereas downstream concentrations were marginally higher than upstream ($p = 0.051$). Conversely, the proportion of algal to non-algal particles (i.e., FlowCam trigger mode vs. autoimage mode) was generally higher in open water (Figure 9B). These results were significant among location ($F_{(3, 8)} = 62.26$, $p < 0.001$) with higher proportions of algae in open water and downstream vs. upstream and non-bedform locations, and non-bedform vs. upstream.
Figure 9: Variation in the concentration of (A) algal and (B) proportion of algal vs. non-algal particles found at different locations in the Innerkip site of the Thames River. Bars indicate average ± SE measured in open (N=2) and porewater samples, across all bedform (N=2) and non-bedform locations (N=2). Bars sharing the same letter are not significantly different.
Composition of Algal Community in Open and Porewaters at Innerkip

A permutational multivariate analysis of variance (perMANOVA) was used to determine if there were differences in algal abundances among riverbed locations at different times. The perMANOVA revealed phytoplankton abundance differed significantly among locations ($F_{(3,48)} = 37.97, p = 0.001$), sampling dates ($F_{(11,48)} = 5.164, p = 0.001$) and their interaction ($F_{(33,48)} = 2.176, p = 0.001$). Consequently, the analysis was simplified and examined within locations and sampling dates separately.

A subset of the algal taxa could be used to determine whether there were differences in algal concentrations on different dates and locations. Of the 22 algal taxa identified in the temporal study, 12 were present in high concentration in porewater samples (> 15 particles mL$^{-1}$) and were found consistently (found in at least 3 consecutive sampling days). Eight algal taxa (centric diatoms, *Nitzchia, Navicula, Scenedesmus, Monoraphidium, Chlamydomonas, Staurodesmus*, and *Oscillatoria*) were examined using 2-way ANOVA (location and date as factors), whereas four taxa were examined using the Kruskal Wallis Test because they exhibited heterogeneity of variance. The two-way ANOVAs revealed location had a significant effect on all 8 algal taxa examined, as did date (2 were marginally significant) (Table 1 A). A significant interaction between date and location was found for centric diatoms and *Nitzschia* ($p < 0.05$). Interestingly, Tukey’s post hoc test revealed porewater concentrations of all algal taxa were consistently greater than open waters, and for 6 of the 8 (12 in total) algal taxa, concentrations downstream of boulders were significantly greater than all other bedform locations (Figure 11 A-F). For the 4 algal taxa (*Pediastrum, Chlorella, Tetraedron, Cyanobacteria* sp.) in which the assumption of homogeneity of variance could not be met, non-parametric tests revealed a
significant effect of location for all algal taxa examined, but not for date (with the exception of Cyanobacteria) (Table 1B) (Figure 11 G-J).

Centric diatoms varied significantly by date \(F_{(11,48)} = 23.63, p < 0.001\), and location \(F_{(3,48)} = 17.72, p < 0.001\) and there was a significant interaction between date and location \(F_{(33,48)} = 2.82, p < 0.001\) because the patterns of centric diatom concentrations among locations differed through time (Figure 10A). An ANOVA revealed significant differences among locations \(F_{(3,92)} = 9.56, p < 0.001\), and a Tukey’s test determined that there were significantly higher concentrations of centric diatoms in porewaters than in open waters. Similar patterns of variation were observed for the concentration of *Nitzschia* by date \(F_{(11,48)} = 6.85, p < 0.001\), and location \(F_{(3,48)} = 104.19, p < 0.001\), and their interaction \(F_{(33,48)} = 2.88, p < 0.001\) because of greater concentrations in non-bedform sites on July 5th with a shift to greater concentrations in porewaters downstream of boulders. Significant differences were found among locations \(F_{(3,92)} = 43.87, p < 0.001\), and a Tukey’s test determined that there were significantly higher concentrations of centric diatoms in porewaters than in open waters, as well as in non-bedform and downstream of boulders than in upstream of bedform \(p < 0.05\).
Figure 10: Spatial and temporal patterns in the concentration of (A) Centric diatoms and (B) *Nitzschia* concentration across all bedform (N = 2), non-bedform porewaters (N = 2) and open waters (N = 2) from weekly samples taken between July and September 2017 (Bars are the mean ± SE).
Figure 11: Spatial patterns in the concentration of (A) *Navicula*, (B) *Scenedesmus*, (C) *Monoraphidium*, (D) *Chlamydomonas*, (E) *Staurodesmus*, (F) *Oscillatoria*, (G) *Pediastrum*, (H) *Chlorella*, (I) *Tetraedron* and (J) Cyanobacteria concentration across all bedform (N = 2) (Bars are the mean ± SE). Bars with the same letter are not significantly different according to a Tukey’s test.
Table 1: Summary of the statistical comparison of algal taxa concentration (cells mL⁻¹) sampled at the Innerkip site at different times and locations: (A) p – value from the two-way ANOVA and (B) p – value from the Kruskal – Wallis Test.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Date</th>
<th>Location</th>
<th>Date × Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Centric Diatom sp.</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Nitzschia</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Navicula</td>
<td>0.059</td>
<td>&lt; 0.001</td>
<td>0.22</td>
</tr>
<tr>
<td>Scenedesmus</td>
<td>0.054</td>
<td>&lt; 0.001</td>
<td>0.34</td>
</tr>
<tr>
<td>Monoraphidium</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.22</td>
</tr>
<tr>
<td>Chlamydomonas</td>
<td>0.015</td>
<td>0.004</td>
<td>0.36</td>
</tr>
<tr>
<td>Staurodesmus</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.88</td>
</tr>
<tr>
<td>Oscillatoria</td>
<td>&lt; 0.001</td>
<td>0.007</td>
<td>0.62</td>
</tr>
<tr>
<td>(B) Pediastrum</td>
<td>0.06</td>
<td>&lt; 0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>Chlorella</td>
<td>0.81</td>
<td>&lt; 0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>Tetraedron</td>
<td>0.19</td>
<td>&lt; 0.001</td>
<td>N/A</td>
</tr>
<tr>
<td>Cyanobacteria sp.</td>
<td>0.004</td>
<td>&lt; 0.001</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Feeding study

The clearance rate (CR) of juvenile Fatmucket was higher on interstitial vs. surface waters (e.g., 1.42 ± 0.21 and 0.84 ± 0.12 mL mussel⁻¹ h⁻¹, respectively; Figure 12). Significant differences between open and porewaters were found under ANCOVA ($F_{(1,29)} = 13.76, p < 0.001$), shell length was not a significant covariate ($F_{(1,29)} = 0.0482, p = 0.827$), but initial algal concentration was ($F_{(1,29)} = 17.55, p < 0.001$).
Figure 12: Clearance rates (CR) of juvenile Fatmucket mussels on river water collected from the Thames River. Average CR in open and porewater treatments based on change in Chlorophyll a fluorescence (Bars are the mean ± SE).

The algal taxa present in the water samples taken at \( t = 0 \) and \( t = 60 \) min were also used to determine the algal specific CR of \( L. \) siliquoidea. In this case, ten algal taxa (\( Navicula, Nitzschia, Chlaymodomonas, Pediastrum, Coelastrum, Scenedesmus, \) small centric diatom (< 15 µm diameter), large centric diatoms (> 15 µm diameter), pennate diatom (\( Cymbella \) and/or \( Pinnularia) \) sp., and small chlorophytes (\( Chlorella \) and/or \( Chlamydomonas \)) were found in high abundance under 10× magnification (i.e., > 15 cells mL\(^{-1} \)) in \( t = 0 \) samples. Of these, \( Nitzschia, Scenedesmus, \) and small centric diatoms had the highest relative abundance (27, 16 and 15% respectively) and combined to 58% of the total interstitial abundance. Conversely, only two taxa (large centric diatom, \( Nitzschia \) and occasionally a third (small chlorophyte) were found in sufficient abundance in the open waters for use in the clearance rate and electivity analysis.

The CR determined of specific algal taxa ranged from 5.89 ± 0.64 to 13.1 ± 2.53 mL mussel\(^{-1} \) h\(^{-1} \) for centric diatoms and \( Chlamydomonas, \) respectively (Figure 13). Significant
differences in the CR of algal taxa was observed among taxa (F(9,174) = 2.06, p < 0.05), neither shell length nor initial concentration of specific algae were significant covariates (F(1,174) = 3.54, p = 0.06 and F(1,174) = 3.05, p = 0.08, respectively), but there was a significant interaction between algal taxa and the covariate, the initial concentration of each algal taxon (F(9,174) = 2.66, p < 0.05). Linear regressions were, therefore, used to examine the relationship between the clearance rate and initial algal concentration of each algal taxon, using shell length as a covariate (Figure 14). The data were square root transformed to meet the assumptions of homogeneity of variance and normality. The CR of six taxa increased with their initial algal concentration whereas the CR of the other four taxa deceased as initial algal concentration increased – thus explaining the significant interaction. Of these regressions, there were two significant declines in CR versus the initial algal concentration of Scenedesmus and open water centric diatoms concentration (p = 0.0157; p = 0.011 respectively; Table 2), whereas there was one significant positive relationship between CR and initial algal concentration for Navicula (p = 0.0471; Table 2).
Figure 13: The average clearance rate (CR) of juvenile Fatmucket mussels on 10 algal taxa identified in the porewaters of the Thames River (Bars are the mean ± SE).

Figure 14: The square root of the clearance rate (CR) vs. the taxa specific initial algal concentration of 10 algal taxa in open and porewaters by juvenile Fatmucket mussels.
Table 2: Summary of linear regression of clearance rate (CR) versus the initial concentration of the algal taxon using shell length as a covariate. Results are presented for the slope, significance and $R^2$ of the regression. Significant relationships are in bold.

<table>
<thead>
<tr>
<th>TAXON</th>
<th>SLOPE OF REGRESSION</th>
<th>P - VALUE</th>
<th>ADJUSTED $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CENTRIC DIATOM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INTERSTITIAL</td>
<td>-0.58</td>
<td>0.16</td>
<td>0.15</td>
</tr>
<tr>
<td>OPEN</td>
<td>-0.60</td>
<td>0.011</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>NITZSCHIA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INTERSTITIAL</td>
<td>-0.14</td>
<td>0.51</td>
<td>-0.013</td>
</tr>
<tr>
<td>OPEN</td>
<td>-0.37</td>
<td>0.75</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>SMALL CHLOROPHYTE</strong></td>
<td>0.25</td>
<td>0.31</td>
<td>-0.005</td>
</tr>
<tr>
<td><strong>COELASTRUM</strong></td>
<td>-0.24</td>
<td>0.74</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>PEDIASTRUM</strong></td>
<td>0.71</td>
<td>0.21</td>
<td>-0.004</td>
</tr>
<tr>
<td><strong>NAVICULA</strong></td>
<td><strong>1.18</strong></td>
<td><strong>0.047</strong></td>
<td><strong>0.13</strong></td>
</tr>
<tr>
<td><strong>SMALL CENTRIC</strong></td>
<td>0.38</td>
<td>0.31</td>
<td>-0.04</td>
</tr>
<tr>
<td><strong>SCENEDESMUS</strong></td>
<td><strong>-0.83</strong></td>
<td><strong>0.015</strong></td>
<td><strong>0.26</strong></td>
</tr>
<tr>
<td><strong>CHLAMYDOMONAS</strong></td>
<td>0.53</td>
<td>0.35</td>
<td>-0.087</td>
</tr>
<tr>
<td><strong>UNID PENNATE</strong></td>
<td>0.66</td>
<td>0.30</td>
<td>-0.013</td>
</tr>
</tbody>
</table>

Feeding Electivity

The electivity of algal taxa found in interstitial water was determined using Chesson’s Electivity Index and plotted against the flux of the specific algal taxon. Several models of flux (velocity, taxon specific algal flux and total algal flux) were examined to assess these relationships, but taxon specific algal flux was selected since that model had the highest $R^2$ (results not presented). The taxon specific algal flux values were binned into 5 equal increments before being plotted against electivity because algal concentrations varied according to the water sample. The electivity was determined by comparing the electivity value (mean ± 95% confidence interval) to the $1/m = 0.1$ criterion for random selection.
The electivity of interstitial centric diatoms decreased with taxon specific algal flux. The centric diatoms were selected for initially at zero flux (i.e., 0 cm s⁻¹), but turned towards random selection as flux increased (Figure 15a). The electivity of interstitial Nitzschia also changed in response to taxon specific algal flux from avoidance at zero flux, to preference at higher taxon specific algal flux (Figure 15b). The electivity of small centric diatoms fluctuated between preference and random selection with taxon specific algal flux, being selected for at 0-500 and 1200 cells cm⁻² s⁻¹ and randomly selected for at 800 and 2200 cells cm⁻² s⁻¹ (Figure 15e). The chlorophyte Scenedesmus was randomly selected for up to 2200 cells cm⁻² s⁻¹ but was avoided at higher levels of taxon specific algal flux (Figure 15j). The electivity of unidentified pennate diatom, Navicula, Coelastrum, Chlamydomonas, small chlorophytes, and Pediastrum did not change with taxon specific algal flux (Figure 15c,d,f-i). These were all randomly selected across the ranges examined, with the exception of unidentified pennate diatom and Navicula which were selected for across the algal flux range examined (Figure 15c, d).
Electivity

Taxa Specific Algal Flux (mL cm\(^{-2}\) s\(^{-1}\))
Figure 15: Chesson’s electivity of A- Centric Diatoms, B- Nitzschia C- Unidentified Pennate Diatom, D – Navicula, E- Small Centric Diatom, F – Chlamydomonas, G – Small Chlorophyte, H – Coelastrum, I – Pediastrum, and J – Scenedesmus ( Vertical: ± Confidence intervals, Horizontal: ± SE ) of juvenile Fatmucket mussel at different levels of taxa specific algal flux. Intersection with 0.1 represents random selection.

It was only possible to determine Jacob’s Modified Electivity Index for three algal taxa in open water. In this case, L. siliquoidea demonstrated selection for centric diatoms, random use of Nitzschia, and avoidance of small chlorophytes (Figure 16). An ANCOVA revealed there was a significant effect of algal taxon on electivity \( (F_{(2,51)} = 10.88, p < 0.001) \), however, there was an interaction with the covariate (taxon specific algal flux) \( (F_{(2,51)} = 7.46, p = 0.001) \) because the
The electivity of *Nitzschia* increased, centric diatoms slightly decreased, and small chlorophytes decreased with taxon specific flux (Figure 17). Significant differences among taxa were found in an ANOVA used to test the main effect of electivity on taxa ($F_{(2,54)} = 5.51$, $p = 0.007$) and Tukey’s test determined there were significantly higher electivity of centric diatoms than *Nitzschia* and small chlorophytes.

![Jacob's Modified Electivity Index](chart.png)

**Figure 16:** The electivity of open water algal taxa small chlorophytes (*sp.*), *Nitzschia* and centric diatoms (mean ± SE) calculated by Jacob’s Modified Electivity Index.
Figure 17: The electivity of juvenile Fatmucket mussels for algal taxa found in open water against algal taxa specific flux.

Centric diatoms were selected for at no flux, but they became random portions of *L. siliquiodea*’s diet as the taxon specific flux increased (Figure 18A). Conversely, *L. siliquiodea* rejected *Nitzschia* at no flux but selected it at random with increased taxon specific flux. The electivity of open water small chlorophytes was random at all levels of taxon specific flux examined (Figure 18B). Linear regressions were performed for each taxon to determine their relationship with taxon-specific algal flux. There was a moderate positive relationship between the electivity of *Nitzschia* and the flux of *Nitzschia* ($R^2 = 0.36$, $p = 0.001$). Centric diatoms tended to decrease with their flux as did small chlorophytes, but neither was significant.
Figure 18: Jacob’s Modified electivity of A- Centric Diatoms, B- Nitzschia and C- Small Chlorophyte (mean ± 95% Confidence intervals) of juvenile Fatmucket mussels at different levels of taxa specific algal flux. Intersection with 0 represents random selection.
DISCUSSION

Juvenile unionid mussels inhabit the interstitial zone in riverbeds, where the delivery of food is via porewater flow (Strayer, 2008). Unfortunately, little is known about the surface-pore water exchange of algae and/or their use by juvenile or adult unionid mussels (Strayer, 2008), even though this is considered an important functional characteristic of unionid habitat (Strayer 2008). The clearance rates ($CR$) of juvenile mussels has been shown to increase with increasing algal flux using a laboratory culture of *Chlorella vulgaris* (Mistry and Ackerman, 2016). This would indicate the importance of porewater velocity in juvenile feeding. The present study examined the feeding of juvenile unionid mussels using ecologically relevant flow conditions and natural seston found within the interstitial zone. Algal concentrations were higher in the interstitial zone compared to open waters, and the $CR$ of juvenile *L. siliquoidea* was higher in porewaters than open waters. The relevance of porewater velocity was also confirmed in the changes in electivity of specific algal taxa under different algal flux. To the best of my knowledge, this is the first examination of the temporal pattern of algae (food) in the interstitial zone and its use in feeding by recently metamorphosed juvenile unionids.

The concentration of algae is important to juvenile unionid mussels because of the effect on $CR$. Algal concentrations were greater in interstitial waters than in open water, which resulted in greater algal flux and higher $CR$. This is consistent with Mistry and Ackerman (2016) who found that the $CR$ of juvenile *L. siliquoidea* increased with the flux of *Chlorella vulgaris*. Concentration may be more important than velocity in determining the effect of algal flux on $CR$. For example, scallop growth was found to be more dependent on food concentrations than on either velocity or algal flux, but velocity affected growth rates when food concentrations were very high or low (Cahalan et al., 1989). This suggests that the mussels may be more dependent
on the quality and quantity of food available than its delivery, i.e., the nutritional value of the diet, as energetic costs for particle filtration and digestion should be balanced by the energy gain from the food (Willows, 1992).

The higher CR in interstitial waters suggests there is more energy available in the interstitial zone. Moreover, differences in the CR of specific algal taxa may provide clues as to the nutritional needs of juvenile mussels. *Dreissena polymorpha* removed algal taxa at different rates depending on their concentration, because the properties of the algal cells interfered with their consumption (e.g., size, clumping; Morton 1971). Baker et al. (1998) demonstrated that the CR of *D. polymorpha* also depended on algal composition; specifically, *D. polymorpha* randomly selected for *Cyclotella* when offered *Cyclotella* and *Thalassiosira* together but switched to preferential selection for *Cyclotella* when provided a solution containing *Cyclotella*, *Thalassiosira* and *Microcystis*. Rock-pool clams, *Venerupis corrugatus*, switched from clearing smaller particles during low tide to larger particles during high tide because of the greater proportion of larger and organically rich particles at high tide (Stenton-Dozey and Brown, 1992). This suggests that studies using lab cultured diets may underestimate or overestimate CR because they do not include natural seston (Ackerman et al., 2001; Riisgard, 2001).

**Feeding selectivity and taxon specific algal flux**

The objectives of this study were to examine (1) if phytoplankton abundance differed between open water and porewaters (2) to determine if juvenile unionid selective feeding increases with algal flux. The results of this study could not provide sufficient information to determine whether juvenile mussel feeding involves optimal foraging behaviour, in part because the nutritional value of the different dietary components is not fully known. One way to approach the nutritional
value of algae would be to search the literature to determine nutritional values; unfortunately we
do not know the energetic requirements of juvenile unionid mussels and which components they
use. There is, however, evidence to support the second hypothesis that selective feeding
increased with flux of a given algal taxon. Firstly, it is apparent that diatoms were selected by
juvenile *L. siliquoidea* relatively consistently and under higher flux in interstitial waters (Figure
14A-E). *Navicula* and unidentified pennate diatoms were consistently selected for with
increasing fluxes. Although the selection of centric diatoms declined to random selection at
higher levels of flux, and small centric diatom fluctuated between random selection and selection
among flux, *Nitzschia* shifted towards selection at higher flux from random selection. This
indicates that juvenile mussels are able to discriminate among algal taxa and that there are
certain food items, which are more important in their diet (i.e., they use them disproportionately
to their concentration in nature). Alves et al. (2016) found that smaller algae, which were
predominant in natural waters, had similar frequencies in the gastrointestinal tract, whereas other
larger algal species (e.g., diatoms: *Navicula* and *Surirella*) were found in greater frequencies.
The results of that study are consistent with this one, suggesting there were nutritional
advantages to these algal cells. Indeed, diatoms are an important food source for unionid mussels
as it is essential for growth and development since they have a greater content of polyunsaturated
fatty acids (PUFA) which are necessary for mussel growth (Gatenby et al., 1997). In contrast,
*Scenedesmus*, was randomly selected at no flux and was avoided at higher levels of flux. This is
likely due to their large size and shape (spines to discourage ingestion), which led to feeding
avoidance by unionids provided a diet of three algae including *Scenedesmus* (Beck and Neves,
2003). The concentrations that affect selection efficiency are believed to be species specific.
Mechanistically, reduction in sorting efficiency is likely related to increased particle handling
times, which can be affected by cell shape (Foster – Smith, 1975; Ward and Shumway, 2004; Bontes et al., 2007).

**Unionid Habitat**

The characteristics of unionid habitats remain for the most part, elusive, given the wide range of conditions in which individual species can be found. Strayer (2008) proposed eight characteristics of functional mussel habitats: (1) shear stress is not excessive to allow juvenile mussel settlement; (2) there is a supply of food, (3) a supply of essential materials; (4) the substrates must be soft enough for burrowing yet firm for support; (5) there should be substrate stability to prevent scouring; (6) there should be suitable temperatures, (7) protection from predators, and (8) low toxicity. Clearly, oxygen, sediment granulometry, and food are important in determining the distributions of hyporheic animal communities (Strayer et al., 1997). Sediment grain size is correlated with permeability, with coarser substrate having greater permeability, which allows for greater exchange of dissolved and particulate materials (Packman et al., 2004). For example, oxygen concentrations were lower in porewaters across bedform locations compared to surface waters, but these differences were not significant among locations, despite differences in substrate d50. Adult mussels are more resilient to low oxygen levels, but juveniles are more sensitive to hypoxia (Strayer, 2008). Our inability to capture the relationship between sediment grain size (d50) and permeability may be because when smaller grain sizes (< 36 µm) fill the spaces for the larger grains, permeability of the samples will not be as dependent on the mean grain size (Detmer, 1995). Unfortunately, 63 µm was the smallest sediment grain size that we were able to measure in this study. There are also other factors such
as hydrology, disturbance history, biological interactions and seasonal variations which may exert control over the hyporheic community (Strayer et al., 1997).

Some nutrients, such as ammonia, can be toxic to juvenile mussels where the reported 96-hr LC50 for juvenile mussels is 0.04 - 0.28 mg L\(^{-1}\) (Augsperger et al., 2003). It is not uncommon to find higher concentrations of ammonia in the interstitial zone than in surface waters because of decomposition of organic matter and lower oxygen levels (Chambers et al., 1992). Unfortunately, there were periods in our study where the ammonia concentrations were much greater (i.e., \(\times 200 - 500\%\)) than the LC50 in non-bedform and downstream of boulders interstitial waters. Conditions like this do not bode well for the recruitment of juvenile unionids at this site. Other factors such as increased temperature and pH can increase the toxicity of ammonia, however these factors were also found to be similar among interstitial water sampled in our study.

**Spatial dynamics of algae in the riverbed**

The concentration of particles, and algal particles in particular, was higher in porewater than in open water (i.e., in river), suggesting that there is retention and accumulation of particles in the porewaters, this observation supports the first hypothesis. The concentration of algal particles and proportion of algal particles to non-algal particles varied with the location in the stream likely due to spatial differences in hydrodynamics (e.g., resuspension and deposition; Arnon et al., 2010). The penetration of permeable sediments by particles including algae has been reported in the past for the seafloor and coastal marine sediments (e.g., Huettel and Rusch, 2000). Nearbed hydrodynamics has a strong influence on particle transport and entry into the porewaters although much remains to be determined (Cooper et al., 2018). For example, the
threshold value of velocity above or below which algal composition and abundance is significantly altered is not well understood (Bona et al., 2012). The entry of particles into the interstitial zone is affected by the grain size of the riverbed sediments. Smaller grain sizes result in greater concentrations of particles because particles constrict pore space openings, resulting in more particle filtration (Karwan and Saiers, 2012). This is consistent with the porewaters downstream of bedforms, which had the greatest algal concentration, and the smallest d_{50} by mass. There are however other physicochemical conditions which can affect retention rates such as ionic charge (Karwan and Saiers, 2012).

The advective transport of algal particles is dependent on particle morphology and size. For example, *Chlorella*, which is spherical, has been found to move and enter sediment interstitial pores more readily than taxa such as *Scenedesmus*, which are more variable in shape and size (Kloep and Roske, 2004). Particles can also be removed from the bed via resuspension due to high shear stress and scouring (Boncagni et al., 2009, Bona et al., 2012). This is likely the case for the upstream of bedform locations where near bed acceleration results in greater resuspension and lower deposition. Downstream of the bedforms, however, there was flow separation and a recirculating zone where slower velocities would favor the settlement of particles (Hoover and Ackerman, 2004).

Among the porewater locations sampled, algal particle concentrations were highest downstream of boulders. In terms of algal taxa, diatoms were the most abundant algae in the porewaters for a number of reasons. Firstly, they were also most abundant in the river and they are more prone to settling because of the high density of their frustules, and the fact that some species (*Navicula, Nitzschia*) are commonly found benthic in habitat (Potapova et al., 2002; Wehr and Sheath, 2003; Reynolds, 2006). Once in the benthic zone, diatoms and other taxa
could accumulate in greater densities. The presence of diatoms and other algae further promote settlement and stability and cohesion of fine sediments through surface adhesion and bed clogging, thus facilitating further accumulation of particles and limiting resuspension (Jones et al., 2014). It is also possible that particle retention led to lower sediment permeability, which is also known to promote sediment retention (Kloep and Roske, 2004). These findings may suggest there may be areas in the streambed more suitable for mussels to settle. Previous studies have found juvenile mussels in greatest densities downstream of boulders in riffles and runs and this finding was attributed to the deposition of finer particles and organic matter by eddies behind the boulder (Neves and Widlak, 1978). This may also correspond to the higher concentrations of diatoms in the interstitial zones, such as *Navicula* which were found to be preferred by juvenile Fatmuckets.

**Implications for the conservation of unionid mussels**

The results of this study indicate that there are areas in the riverbed that are more suitable for juvenile mussels because of the conditions of the interstitial water including algae, and that changes to stream characteristics could influence algal composition and mussel feeding behaviour. It may be that there are areas in the riverbed that do not have suitable algal composition (i.e., food availability) to sustain juvenile mussels. Modification of stream flow could also alter mussel habitat and food availability. For example, greater diatoms and cryptophyte biomasses were found under low flow velocity and increased water depths compared to fast flowing shallow river sections where chlorophytes were favoured (Bahnwart et al., 1999). Furthermore, increases in overlying streamflow influence porewater velocities and particle filtration by the streambed (Karwan and Saiers, 2012). We found that juvenile unionid mussels
selected for diatoms, and therefore changes in phytoplankton composition that reduced diatom concentration could limit the growth and survival of the juvenile mussels. Specifically, increases in loading of fine sediments may decrease the permeability of river beds thus limiting the upwelling of nutrients and particles into the interstitial zone.

In addition to hydrodynamic conditions, nutrients can also alter phytoplankton composition. For example, Chlorophytes become dominant at high concentrations of nitrogen and phosphorous, whereas Cyanobacteria become dominant at low nitrogen and phosphorous concentration (Zhu et al., 2010). It is likely that conditions that alter the phytoplankton community could also affect the feeding and growth of juvenile unionids. As indicated above, ammonia concentrations can be toxic, and levels that far exceeded those were observed in this study. Whereas there was more energy available in the interstitial zone, the chemical environment was not always favourable for juvenile mussels; it became hypoxic and toxic at times. Juvenile unionids have been found in the Innerkip site of the Thames River, suggesting that the conditions are not consistent, either temporarily or spatially. It is possible that mussels may change the dynamics of the interstitial zone through their burrowing and feeding activities (Strayer, 2008). For example, burrowing activities results in structuring, mixing and flushing of sediments and can increase the depth of oxygen penetration into the interstitial zone (McCall et al., 1995). Feeding facilitates water transport and consequently the transfer of oxygen, CO$_2$, and excretory products, which changes the flux of solutes between the interstitial and water column (Norkko and Shumway, 2011). Future research on mussel habitat should focus on the spatial arrangement of riverbeds for mussel habitat. Riverbeds are spatially and temporally dynamic, and a greater understanding of the underlying processes would allow better categorization of mussel habitat.
CONCLUSION

The CR of juvenile Fatmucket mussels were higher in interstitial waters vs. open waters due to higher concentrations of algae. Results from flow cytometry indicated that juveniles cleared algal taxa differently at rates that depended on the initial algal concentrations (6 increased, 4 decreased). Whereas chlorophytes such as *Chlamydomonas* were cleared at higher rates, results from the feeding electivity revealed that diatoms were removed at higher rates relative to their abundance (i.e, $\alpha > 1/m$). Diatoms appeared to be more important in diets as they were consistently selected (e.g., *Navicula*), but chlorophytes were taken at random. In some cases, flowing conditions increases the selection for *Nitzschia* and but reduced it for centric diatoms. The algal composition and chemical environment at the Innerkip site were spatially and temporally dynamic. Algal concentrations in the interstitial zone were greater than in the overlying surface waters, with greatest concentrations in porewaters downstream of boulders. However, the chemical environment was found to be unsuitable at times for juvenile mussels, due to hypoxia and toxic levels of ammonia. Future studies should focus on the effects of environmental change on the survival of unionid mussels. Habitat alteration from human activities or increased temperature could alter substrate composition, affecting phytoplankton composition and mussel feeding behaviour. This study is important for the conservation of mussels as it demonstrates that changes in aquatic environments may alter substrate composition, in turn changing algal composition in the interstitial zones and affecting the feeding abilities of mussels.
LITERATURE CITED


Harvey, J. W., Drummond, J. D., Martin, R. L., McPhillips, L. E., Packman, A. I., Jerolmack, D. J., … Tobias, C. R. 2012. Hydrogeomorphology of the hyporheic zone: Stream solute and


