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The Effect of Mayfly (Hexagenia (Ephemeroptera:Ephemeridae)) Nymphal Burrowing Activity on Egg Hatching and Subsequent Development of Early Instar Individuals: Implications for Intercohort Interactions

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THE EFFECT OF MAYFLY (*HEXAGENIA* (EPHEMEROPTERA: EPHEMERIDAE)) NYMPHAL BURROWING ACTIVITY ON EGG HATCHING AND SUBSEQUENT DEVELOPMENT OF EARLY INSTAR INDIVIDUALS: IMPLICATIONS FOR INTERCOHORT INTERACTIONS

By

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AUTHOR'S DECLARATION OF ORIGINALITY

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ABSTRACT

Bioturbation can cause the burial of eggs in sediments, isolating them from oxygen and consequently preventing development. I investigated the effects of burrowing on egg hatching and nymph development in burrowing mayflies (*Hexagenia* spp.). First, I examined the effects of burial on egg hatching. More eggs hatched on the sediment's surface than at any other depth. Furthermore, egg burial contributed to greater size variation among hatchlings, possibly from delays in hatching of buried eggs. Subsequently, I investigated the effect of burrowing on the vertical distribution and hatching of eggs within sediments. Reduced hatching and greatest hatchling size variation occurred in trials containing higher densities of nymphs, suggesting that benthic activity facilitates burial of eggs and reduces synchrony of hatching. Under natural conditions, such activities may contribute to the formation of multiple cohorts that characterize *Hexagenia* populations. These studies show how indirect consequences of ecological engineering can influence life history patterns.

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CHAPTER 1: GENERAL INTRODUCTION

Intercohort Interactions:

Intraspecific competition occurs when members of the same species compete for the same resources and most often occurs in populations with high densities (e.g., Hart 1987; Lamberti et al. 1987; Peckarsky and Cowan 1991; Hanes and Ciborowski 1992). In many species, intraspecific competition occurs between life stages or cohorts, especially when individuals differ in size between stages or if individuals in a certain stage are defenseless, as is often the case in embryonic and juvenile stages (Polis 1981). The strength of intraspecific interactions can vary widely among species, and can either be direct or indirect. For example, in the talitrid amphipod, *Orchestoidea tuberculata*, adults prey on juveniles when food is limited (Duarte et al. 2010). Conversely, in fairy basslets (small marine fish), adults prevent juveniles from feeding at prime positions at the center of aggregations and consequently increase the risk of predation on juveniles by forcing them into areas occupied by predators (Webster 2004). Similarly, in tidepool sculpins, adults occupy lower tide pools where food and cover are most plentiful, while relegating juveniles to upper tide pools (Azabo 2002). Intercohort competition can also affect development as is seen in juvenile sea urchins, which exhibit slower growth rates in the presence of adults (Nishizaki and Ackerman 2004). Thus, interactions between life stages can greatly influence populations by affecting the survival and size frequency structure of a cohort as well as the development and distribution of individuals.

In burrowing mayflies (Ephemeroptera: *Hexagenia* spp.) eggs and nymphs of various cohorts occupy the same habitats (Hunt 1953), increasing the potential for interactions and possibly competition between individuals at different developmental

stages. *Hexagenia* are bioturbating organisms (Matisoff and Wang 2000; Bachteram et al. 2005) and can therefore alter the surrounding environment. This change in environment can, in turn, affect egg hatching and development (Gerlofsma 1999). The purpose of this thesis was to determine the potential for intercohort interactions in *Hexagenia* by examining the effect of bioturbation caused by partially-grown nymphs on egg hatching and subsequent hatchling development.

Bioturbation:

Bioturbation is the mixing and displacement of sediment particles, resulting from the activities of organisms such as sediment reworking, bioirrigation, burrow construction and foraging activities involving particle ingestion and egestion (McCall and Tevesz 1982; Krantzberg 1985; Meysman et al. 2006). These activities alter the physical and chemical properties of substrates in what is known as "ecosystem engineering", which can have strong impacts on other organisms (Mermillod-Blondin and Rosenberg 2006; Meysman et al. 2006). Bioturbation, for example, can affect the flow of resources, such as dissolved oxygen, in sediments (Mermillod-Blondin and Rosenberg 2006) and contribute to the suspension of sediments in the water column (Matisoff and Wang 2000; Bachteram et al. 2005). Suspended sediments can make sediment-bound contaminants (Rosa 1985; Reible et al. 1996) and nutrients (Graneli 1979; Holdren and Armstrong 1980; Petterson 1998; Chaffin and Kane 2011) available to other organisms and can also reduce light penetration into the water, altering primary production and oxygen levels (Luzier and Summerfelt 1997). Fine sediment particles can also congest or damage gill structures (Cordone and Kelley 1961) and affect filtration rates in organisms (MacIsaac

and Rocha 1995; Madon et al. 1998). Furthermore, as sediment settles to the bottom, it can bury eggs and newly-hatched nymphs, thereby impeding development and possibly increasing mortality rates (Marcus 1984; Marcus et al.1994; Hairston et al. 1995; Albertsson and Leonardsson 2001; Kefford et al. 2010; Ventling-Schwank and Livingstone 1994; Greig et al. 2005; Wyatt et al. 2010).

The way in which these systems are altered depends on the nature of the bioturbator and the mode of ecosystem engineering used. For example, oligochaetes (aquatic worms) can mix sediment layers to a depth of up to 10 cm, but do not directly contribute to the turbidity of the water column (Fisher et al 1980; Matisoff and Wang 1999). Burrowing mayflies, on the other hand, mix sediments and increase levels of suspended sediment particles (Matisoff and Wang 2000; Bachteram et al. 2005), which can consequently settle and bury eggs, slowing or arresting their development (Gerlofsma 1999).

Dormancy and Egg Banks:

Dormancy can contribute to the persistence of populations when conditions are unsuitable (Bilton et al. 2001), regulate development so that growth occurs at peak seasonal times (Danks 1987), and help maintain genetic diversity in populations (Bilton et al. 2001). In some species, developmental arrest is directly triggered by the onset of adverse conditions in the surrounding environment (quiescence). Development resumes once external conditions are no longer limiting (Danks 1987). In others, it is an endogenously programmed response (diapause) that does not necessarily correspond with

adverse environmental factors, although external factors can indirectly modify developmental pathways (Danks 1987).

The "natural store" of dormant stages in ecosystems is known as a "bank". The existence of "seed banks" in both terrestrial and aquatic plants is well documented (Bakker et al. 1996). Similar systems have also been observed in aquatic invertebrates, most notably the egg banks found in zooplankton. Copepod eggs, for example, can remain dormant in aquatic sediments for decades before hatching, thereby increasing genetic diversity and helping populations to persist in adverse conditions (Marcus 1984; DeStasio 1989; Marcus et al. 1994; Hairston et al. 1995; Bilton et al. 2001).

Effect of Bioturbation on Egg Bank Dynamics:

In aquatic systems, egg burial has been considered a factor contributing to egg dormancy, most likely because occurrence of anoxia, reduced temperatures and other changes in sediment chemistry at greater depths can affect development, hatching and recruitment into populations (Gerlofsma 1999).

The burial of eggs in sediment can be the result of sediment deposition on the lake bed, disturbance from wave action and current flow, or the result of the feeding and burrowing activities of benthic organisms (Marcus 1984; Kearns et al. 1996; Albertsson and Leonardsson 2000; Albertsson and Leonardsson 2001; Viitasalo 2007). Direct mixing and disturbance of the sediments by benthic organisms can relocate eggs or they could be buried by the settling of sediments that were suspended by bioturbation. Buried eggs can subsequently be returned to the surface, either through storm-caused turbulence stirring up sediments or by zoobenthic activity, whereafter development can resume

(Marcus and Schmidt-Gengenbach 1986; Kearns et al. 1996; Gerlofsma 1999). The varied times at which groups of eggs may become buried and subsequently resurface could result in differential hatching times as well as form multiple cohorts within a population. Although *Hexagenia* is typically said to be univoltine or semivoltine (i.e., populations that require one or two years to complete a generation - Hunt 1953; Manny 1991), nymphs within populations exhibit broad size frequency distributions and extended emergence periods suggestive of multiple cohorts (Schloesser and Hiltunen 1984; Heise et al. 1987; Giberson and Rosenberg 1994; Corkum 2010). Gerlofsma (1999) postulated that this variation could reflect the differential timing of egg hatching caused by burial and subsequent return to the surface.

Bioturbation, as a mechanism of egg transport, has been demonstrated in several benthic species, and each species contributes to egg bank dynamics in difference ways. For example, the activities of chironomids (Kearns et al. 1996), amphipods (Albertsson and Leonardsson 2000) and bivalves (Viitasalo 2007) tend to cause egg burial, whereas tubificids (Kearns et al. 1996) transport eggs upwards in the sediment. These examples all refer to interspecific interactions, specifically the burial of copepod eggs by other benthic invertebrates. No studies to date have looked at these types of interactions within species, or more specifically, the effects of one life stage on another, which could have profound effects on the dynamics of populations.

Study Organism:

Hexagenia are burrowing mayflies commonly found in the depositional zones of shallow waters throughout North America (McCafferty 1975; Edmunds et al. 1976).

Among other factors, their distribution is highly influenced by bottom substrate characteristics. They require areas with soft sediments consisting primarily of silt and clay, suitable for burrowing (Lyman 1943; Hunt 1953; Eriksen 1968). Burrowing mayflies were very common in the western basin of Lake Erie until the 1950s, when populations became extirpated due to episodic anoxic conditions resulting from eutrophication and extended periods of thermal stratification (Britt 1955a; Krieger et al. 1996). In the early 1990s, *Hexagenia* began to recolonize the basin, and had returned to historic densities in many locations late in that decade (Krieger et al. 1996; Corkum 2010). Two species are commonly found in Lake Erie: *Hexagenia limbata* (Serville) and *H. rigida* McDunnough (Corkum 2010). In this study, they will be treated as a single functional taxon since they are morphologically and ecologically similar; they occupy the same habitats, utilize the same resources and exhibit similar life history patterns (McCafferty 1975; Edmunds et al. 1976).

Life Cycle:

The *Hexagenia* life cycle consists of both aquatic and terrestrial stages. Sexually mature female adults (imagos) deposit their fertilized eggs on the water and eggs sink to the sediment surface where they remain until hatching (Hunt 1953; Edmunds et al. 1976). Hatching depends on water temperature and dissolved oxygen concentrations (Fremling 1967; Gerlofsma 1999) and under optimal conditions, hatching can occur in 2-3 weeks (Hunt 1953). If oxygen or temperature levels are low, however, eggs can remain dormant for up to a year (Fremling 1967; Giberson and Rosenberg 1992b; Gerlofsma 1999; Bustos and Corkum 2007). Once eggs hatch, nymphs (initially about 0.83-0.88 mm in

length; Hunt 1953) immediately burrow into the sediment (Hunt 1953; Edmunds et al. 1976). The nymph stage typically lasts from 1-2 years (Hunt 1953; Manny 1991; Giberson and Rosenberg 1994; Corkum et al. 1997), during which time the nymphs undergo multiple moults. The growth of *Hexagenia* nymphs is gradual and continuous; larvae pass through an estimated 27-30 instars (Needham et al. 1935; Hunt 1953). The duration of the nymphal stage as well as survivorship and development depend on a number of factors including water temperature (Hunt 1953; Corkum and Hanes 1992; Giberson and Rosenberg 1992a), oxygen concentration (Winter et al. 1996), quantity and quality of food (Hunt 1953; Giberson and Rosenberg 1992a; Hanes and Ciborowski 1992), and time of year in which eggs are deposited (Flannagan 1979). When nymphs are fully developed (typically 17-35 mm in length) and are ready to emerge, they swim to the surface, where they moult into a winged, sexually immature adult (subimago) characterized by dark, cloudy wings (Hunt 1953). The subimago flies to shore where it rests for approximately 1-2 days (temperature dependent) before moulting a final time into an imago (Lyman 1944). In the evenings, males form swarms through which females will fly to mate with the males. After mating, females fly to the water to deposit their eggs (Hunt 1953).

Burrowing Behaviour:

Hexagenia nymphs construct U-shaped burrows in the sediment that are approximately 4.5 cm deep on average (Charbonneau et al. 1997; Charbonneau and Hare 1998), although burrows as deep as 12 cm have been observed (Charbonneau and Hare 1998). Nymphs use their forelegs to burrow into the sediment (McCafferty 1975; Keltner

and McCafferty 1986) and once the burrows are constructed, they use their abdominal gills and frontal process to push sediment particles out of the burrows and irrigate burrows with oxygenated water (Needham et al. 1935; Lyman 1943; Eriksen 1963; Edmunds et al. 1976; Wang et al. 2001). Nymphs remain in their burrows for several hours before constructing new burrows below previous ones (Charbonneau et al. 1997; Charbonneau and Hare 1998). This burrowing activity contributes to the mixing of the upper layers of sediment as well as sediment suspension in the water column (Matisoff and Wang 2000; Bachteram et al. 2005). Both the size and density of nymphs influence turbidity (Bachteram et al. 2005). Larger nymphs displace more sediment when constructing burrows, and higher densities of nymphs increase the net amount of excavation occurring in a particular area over time.

Contribution to Egg Burial:

Eggs oviposited at the water's surface settle onto the surface of the sediment, but they have been observed at deeper depths within sediments (Hunt 1951). Gerlofsma (1999) found that in egg-supplemented cores, significantly fewer eggs could be retrieved from the upper layers of field-collected cores that had contained large *Hexagenia* nymphs. Since she examined only the upper 2-5 mm of sediment, Gerlofsma postulated that the nymphs' burrowing activity contributed to the burial of eggs deep in the sediment. *Hexagenia* nymphal activity could contribute to egg burial either through the direct mixing of sediments, sedimentation or by bringing eggs down into the burrows as they are formed. The pore water around buried eggs lacks the oxygen necessary to allow embryonic development (Gerlofsma 1999). However, development of *Hexagenia* eggs is

only suspended under anoxia. Eggs can withstand long periods of burial and resume hatching when conditions become suitable (normoxic) (Fremling 1967; Gerlofsma 1999; Bustos and Corkum 2007). If burrowing activity contributes to egg burial and subsequently causes a delay in their hatching, then the time of nymphal development might also be staggered (Gerlofsma, 1999). This would be reflected in the size frequency distributions of the nymphal populations. In semivoltine populations, activity of burrowing nymphs could be responsible for the burial and subsequent relocation of eggs back to the surface where development resumes. This variability in hatching time could account for the multiple cohorts that typify these species (Heise et al. 1987).

Egg Survival and Development:

In *Hexagenia*, both the nymphal and embryonic stages are highly oxygen sensitive. At oxygen concentrations of 1.0 mg/L or less, nymphs will die within 30-48 h (Hunt 1953). Winter et al. (1996) also demonstrated that even at slightly subsaturated oxygen concentrations, nymphs exhibit a decrease in growth, most likely because energy must be allocated to satisfying respiratory requirements instead of development. Egg development is suspended under anoxia, but resumes when eggs are returned to normoxic conditions. Eggs can withstand anoxia for up to 380 d (Fremling 1967). However, duration of exposure as well as exposure during later stages of embryonic development reduces overall viability (Fremling 1967; Gerlofsma 1999). The ability of *Hexagenia* embryos to survive without oxygen may permit a population to persist or recover even when all nymphs are eradicated from otherwise suitable habitats by transient periods of anoxia (Britt 1955b).

In aquatic systems, conditions as little as 2 mm below the sediment surface can be anoxic, although this depends on factors such as sediment heterogeneity, bioturbation and trophic state (Jorgenson and Revsbech 1985; Martin et al. 1998; Wang et al. 2001). Even though burial of eggs may isolate them from oxygenated layers of the sediment (Gerlofsma 1999), nymphs irrigate their burrows with oxygenated water, increasing the depths to which oxygen can penetrate (Wang et al. 2001). This may allow buried eggs to hatch, albeit more slowly than eggs situated on the sediment surface. Hunt (1951) found that eggs buried 5-6.5 cm below stream silt, were delayed in hatching by 2-3 weeks compared to unburied eggs.

Significance:

Studies that have examined bioturbation as a mechanism for antagonistic interactions have traditionally focused on relationships between species. No studies to date have looked at these relationships within a species, specifically the effect of one life stage on another. In burrowing mayflies, nymphal activity could be responsible for burial and subsequent relocation of eggs to the surface where development resumes. The consequent differences in hatching time could account for the appearance of multiple cohorts that typify these species. Whereas eggs have evolved traits that allow them to persist through unfavourable conditions, an ancillary consequence of the burial of eggs by nymphs may be splitting of cohorts resulting in reduced competition for resources in subsequent life stages. The role of direct competition for food or space and its effects on size frequency distributions of insects is well documented (e.g. Hart 1987; Peckarsky and Cowan 1991; Hanes and Ciborowski 1992). However, this study represents a first

documentation of how cohort-splitting might be accomplished through the indirect consequences of ecological engineering. This study will contribute to the understanding of how the actions of conspecifics can affect life history patterns and size structures in populations.

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CHAPTER 2: THE EFFECT OF EGG BURIAL ON HATCHING AND SUBSEQUENT NYMPH DEVELOPMENT IN *HEXAGENIA* SPP.

Introduction:

Developmental arrest in some species is often triggered by unfavourable conditions in the surrounding environment (Danks 1987). The occurrence of resting stages allows populations to persist until conditions are once again suitable for survival and development (Bilton et al. 2001). In aquatic systems, benthic eggs can enter a dormant state when exposed to hypoxia or anoxia either through thermal stratification or egg burial. Water becomes stratified in calm conditions, causing a decrease in oxygen concentrations in the epibenthic zone due to respiration of sediment-associated microbes (Adams et al. 1982). Egg burial also restricts levels of oxygen available to developing embryos because there are limits in the depths at which oxygenated water can penetrate sediments (Jorgensen and Revsbech 1985). Thus, buried eggs may become isolated from the upper oxic layers of sediment and cease development (Gerlofsma 1999). Duration of burial can have various effects on hatching, survival and development.

The depth to which oxygen penetrates sediments depends on several factors. One such factor is the presence of bioturbators. Mixing of sediments as well as bioirrigation can increase the depths to which oxygen can penetrate (Wang et al. 2001). Another factor is trophic state, which contributes to differences in sediment oxygen demand. For example, Martin et al. (1998) showed that in Lake Baikal (Siberia, Russia), an oligotrophic lake, oxygen penetration into sediments exceeded 50 mm. Lake Malawi

(East Africa), on the other hand, was mesotrophic and oxygen penetration depths of no more than 3 mm were observed.

Several factors could contribute to the redistribution of eggs in sediment including disturbance from wave action, current flow, or the feeding and burrowing activities of benthic organisms (Marcus 1984; Marcus and Schmidt-Gengenbach 1986; Marcus et al. 1994; Kearns et al. 1996; Albertsson and Leonardsson 2000; Albertsson and Leonardsson 2001; Viitasalo 2007). Hypoxic or anoxic conditions within the sediment can arrest egg development and reduce survival. Buried copepod eggs enter a dormant stage that can last for several years, thereby increasing genetic diversity and helping populations to persist when conditions are unsuitable for survival (Marcus 1984; DeStasio 1989; Marcus et al. 1994; Hairston et al. 1995; Bilton et al. 2001). Other invertebrate species such as chironomids (*Chironomus cloacalis*) and gastropods (*Physa acuta* and *Gyraulus tasmanica*) exhibit reduced incidence of both hatching rate and survival in proportion to egg burial depth (Kefford et al. 2010). Similarly, in fish species such as rainbow smelt (*Osmerus mordax*) (Wyatt et al. 2010), Atlantic salmon (*Salmo salar*) (Greig et al. 2005) and whitefish (*Coregonus sp.*) (Ventling-Schwank and Livingstone 1994), burial in sediment reduces embryo survival by decreasing available oxygen.

Hexagenia are burrowing mayflies whose larvae are commonly found in soft sediments of the Great Lakes (Edmunds et al. 1976). They were abundant in the western basin of Lake Erie until the 1950s, when the population became extirpated, largely due to increasing frequency of anoxia, resulting from eutrophication and extended periods of thermal stratification (Britt 1955a; Reynoldson et al. 1989; Krieger et al. 1996). They

began to recolonize the basin in the early 1990s and reached their former densities in many locations by the late 1990s (Krieger et al. 1996; Corkum 2010).

In *Hexagenia*, both the nymphal and embryonic stages are oxygen sensitive. When dissolved oxygen concentrations decline to 1.0 mg/L or below, nymphs will die within 30-48 h (Hunt 1953). Furthermore, Winter et al. (1996) demonstrated that growth rates of nymphs are proportional to dissolved oxygen concentrations, most likely because increasing energy must be allocated to ventilatory activity instead of development. Eggs become dormant when subjected to anoxia (Gerlofsma 1999) but resume development when returned to normoxic conditions. The length of time for which eggs can tolerate anoxic conditions varies, but increased periods of exposure, as well as exposure during advanced stages of embryonic development, leads to decreases in overall survival (Fremling 1967; Gerlofsma 1999). The ability of *Hexagenia* embryos to survive extended periods of anoxia may contribute to the persistence of populations through periods of adverse conditions. It can also allow populations to recover when nymph populations crash.

Eggs deposited at the water's surface by female imagos (sexually mature adults) sink to the surface of sediments where the embryos develop and hatch (Hunt 1953; Edmunds et al 1976). Under optimal conditions (normoxia; temperatures above 20^{o} C), hatching occurs after 2-3 weeks (Hunt 1953; Giberson and Rosenberg 1992). During this time, however, some eggs can become subject to hypoxia or anoxia. Episodes of stratification in western Lake Erie are usually brief, typically lasting between 1 and 4 days (Bartish 1984). This period of exposure to anoxia can be detrimental to nymph populations, but remaining eggs can hatch and repopulate the area (Britt 1955b,

Gerlofsma 1999). Burial of eggs, on the other hand, may result in much longer periods of exposure to anoxia. Anoxia can occur in as little as a few mm below the sediment surface, although this depends on factors such as sediment heterogeneity, bioturbation and trophic state (Jorgenson and Revsbech 1985; Martin et al. 1998; Wang et al. 2001).

Buried eggs can subsequently be returned to the surface where development can resume, either through turbulence caused by storms or through benthic activity (Kearns et al. 1996, Gerlofsma 1999). The varied times at which groups of eggs are buried and resurface could result in differential hatching times, possibly leading to the development of multiple cohorts, which are commonly found in *Hexagenia* populations (Heise et al. 1987).

The purpose of this study was to assess the effect of egg burial on egg hatching and subsequent nymph development in *Hexagenia*. I expected that when eggs were buried, fewer eggs would hatch, and consequently produce fewer hatchlings. Protraction of the hatching period when eggs are buried should cause wider size frequency distributions in the population of developing nymphs. Furthermore, *Hexagenia* are bioturbators; when eggs hatch, hatchlings immediately burrow into the sediment. Nymphal burrowing activity contributes to mixing of upper layers of sediment and suspends sediments in the water column (Bachteram et al. 2005) which, in turn, could alter the distribution of eggs within the sediment.

Methods:

Experimental Design:

I conducted a lab experiment to determine the effects of egg burial on hatching and subsequent nymph development in *Hexagenia* spp. The experiment consisted of two treatments in which eggs were placed at various depths within the sediments of 8 L aquaria (30 x 15 x 18.5 cm; 5 replicate aquaria per treatment) (Fig 2.1). In one treatment, eggs were placed at depths of 0, 2, and 4 cm below the sediment surface (aka 'some eggs buried'). This treatment reflects what might be observed in natural field conditions following sediment disturbance by storm or benthic activity (Marcus 1984; Marcus et al. 1994; Kearns et al. 1996; Albertsson and Leonardsson 2000). In a second treatment, all eggs were placed on the sediment surface (aka 'no eggs buried'), where they typically would come to rest after being deposited at the water surface by female imagos (Hunt 1953; Edmunds et al. 1976). Each tank was sampled five times over a 9-week period (weeks 1, 3, 5, 7 and 9). The state of eggs (unhatched, hatched, damaged) and the size of nymphs were enumerated at each sampling event.

Egg Collection:

Eggs were collected from ovipositing female imagos collected during peak emergence periods at the head of the Detroit River in Windsor (N 42.33^{o} W 82.93^{o}) and Tecumseh (N 42.30^{o} W 82.86^{o}), Ontario in June 2010. Female imagos attracted to lights at night were collected from surfaces on which they were resting and placed into 2 L polyethylene bags (50 females/ bag) filled with aerated, dechlorinated water into which they deposited their eggs. Females were removed from the bags the following morning.

Eggs were gradually cooled (Friesen 1981) and then stored in the collection bags at 8^{o}C until needed.

***Sediment Collection and Storage*:**

Sediment was collected in July 2010 from the shore of the Chenal Ecart at the mouth of the St. Clair River (MacDonald Park, Ontario; N 42.61^{o} W 82.47^{o}), which supports a large population of *Hexagenia*. A D-frame dip net was used to scoop sediment (upper layers; ~5 cm) at a water depth of 0.5-1 m. Sediment was placed into plastic buckets with tight-fitting lids. In the lab, sediment was frozen at -20^{o}C for 48 h, thawed and then sieved through a 250 µm mesh brass sieve to kill and remove any macroinvertebrates that could affect distribution of eggs in the sediment (Plant et al. 2003; Bachteram et al. 2005). The sediment was then stored at ~4^{o}C until needed. Extended periods of cold storage do not significantly affect sediment quality (Othoudt et al. 1991).

Preparation of Aquaria:

Before the start of experiments, each aquarium (30 x 15 x 18.5 cm) was filled with a depth of 6 cm of sediment and covered with 12 cm of dechlorinated water (~22 ^{o}C). Airstones were placed in the centre of tanks approximately 2 cm below the water surface. Ammonia, pH and dissolved oxygen concentrations were monitored daily in each aquarium to insure that water quality was appropriate for survival of eggs once they were transferred into tanks. Dissolved oxygen and pH were measured using a YSI 556 multi-probe meter, and ammonia concentration was measured using a salicylate-based

aquarium testing kit. If ammonia or pH levels were too high (>0 mg/L and 7.0, respectively) or if dissolved oxygen was too low (<6.0mg/L), one-half of the water in the tank was removed every other day and replaced with fresh, oxygenated, dechlorinated water. This was continued (approximately 5 times) until all variables in all tanks were at target levels.

Transfer of Eggs into Experimental Aquaria:

Eggs were removed from cold storage and placed into Petri dishes containing oxygenated water. They were then counted under a dissecting microscope (at very low light) to ensure that an equal number of eggs (n = 1,500; ~33,300 eggs/m^2) were placed in each tank. In the western basin of Lake Erie, natural densities of eggs are approximately 14,890 (± 2,510)/m^2 (Plant et al. 2003). A total of 1500 eggs/tank was chosen to ensure a high enough density of eggs in each core sample to distinguish differences in hatching.

In the first treatment (some eggs buried), the water was siphoned from the tanks into a clean storage bucket. The top 2 cm of sediment as well as the next (middle) 2 cm were then scooped out using a metal spoon and stored in separate buckets. Eggs were divided (500 eggs/layer) and distributed over the surface of the remaining sediment using a pipette. Using the spoon, the middle 2 cm of sediment were then carefully placed on top of the bottom layer and spread evenly on top of the eggs. Five hundred additional eggs were placed on this layer, after which the original top 2 cm of sediment was returned to the aquarium. The remaining 500 eggs were pipetted onto the sediment surface, and then the water was returned to the tank. The airstone was replaced in the tank 30 min later to allow time for any suspended eggs to settle onto the sediment.

In the second treatment (no eggs buried), eggs were transferred into experimental tanks by pipette placed just below the water surface to allow for even distribution across the sediment. Airstones were removed for 30 min to allow eggs to settle.

Monitoring of Hatching

The precise dates of egg hatching cannot be monitored easily in sediments because nymphs are initially tiny, transparent, and burrow into the sediments. As a proxy for monitoring hatching rates in tanks, eggs were placed in Petri dishes containing aerated, dechlorinated water in parallel with the main hatching study. The eggs in these dishes were examined daily beneath a dissecting microscope. Fresh water was added to the dishes daily to keep the eggs well oxygenated. The experimental aquaria were first sampled on the day that at least 50% of the eggs in the Petri dishes had hatched.

Hatchling Feeding:

Food for hatchlings was added into aquaria once a week for the duration of the experiment (Appendix I). As soon as the eggs in the Petri dishes began to hatch, 2 mL of food solution (40 mL dechlorinated water blended with 1.5 g cereal grass media, and 1.0 g of fish flakes), were pipetted into each experimental tank just below the water surface. The amount was increased by 1 mL every week up to a maximum of 6 mL/week per tank.

Sediment Suspension:

Spectrophotometry was used to estimate suspended sediment concentrations, which served as a measure of burrowing activity (bioturbation) in tanks. These

measurements were used to determine whether or not burrowing contributed to changes in egg distribution, further affecting egg hatching in sediments. Two 5 mL samples were taken from the water column in each tank three times a week. Samples were poured into a cuvette and read at 750 nm in an SP1 Spectrometer 20+, using distilled water as the reference liquid (Bachteram et al. 2005). Absorbance (optical density) readings were recorded.

Sampling Procedure:

Once at least 50% of the eggs in Petri dishes had hatched, two core samples (3 cm in diameter) were taken from the sediments of each aquarium. Additional pairs of cores were collected from each aquarium every two weeks, until each tank had been sampled five times. Materials removed from the tanks were replaced with sediment from a holding tank. Sampled locations were mapped to avoid resampling. For the purpose of this study, only one core sample in each pair was processed and analyzed. The second core sample in each pair was archived and used as reserve in case the first sample was compromised. A total of ~424 cm^3 of sediment was removed from tanks (~16% of total sediments in tanks).

Immediately after each core sample was collected, the sediment in each tube was carefully extruded, 1 cm at a time (Albertsson and Leonardsson 2000). Each 1 cm aliquot of sediment was preserved in a formal ethanol solution (5:2 95% ethanol: phosphate-buffered 100% formalin) diluted 1:1 with water. Several drops of lignin pink dye solution were added to each sample to help distinguish eggs and nymphs from the sediment (Gerlofsma 1999).

Each section was sieved using a 90 µm mesh brass sieve to remove as much of the sediment as possible without risk of egg loss (Gerlofsma 1999) as *Hexagenia* eggs measure approximately 160-190 µm wide and 280-320 µm long (Hunt 1953). The number of eggs and hatchlings found in each sediment aliquot was recorded. Eggs were classified as being whole (unhatched), hatched or damaged (Fig 2.2). Eggs that have hatched are transparent and are characterized by a vertical slit extending about ¾ of the way down the egg, from which the nymph emerges (Hunt 1953; Gerlofsma 1999). Damaged eggs are eggs that are soft or break when they are handled. Whole eggs are firm and undamaged and are assumed to be potentially viable (Gerlofsma 1999). The head width (across the outside of the eyes) and body lengths (from the tip of the head to the end of the abdomen) (Bachteram et al. 2005) of hatchlings were measured to quantify development. Measurements were made with an ocular micrometer in the eyepiece of a dissecting microscope (to the nearest micrometer) and then converted to millimeters.

Early instar size estimates were based on measurements of head width and body length; the instar number was not identified. *Hexagenia* are hemimetabolous (gradual growth with no pupal stage) and the number of instars is variable, although it probably ranges between 27 and 30, based on observations of other species (Needham et al. 1935; Hunt 1953). The number of instars also appears to vary across populations (Brittain 1982).

After enumeration of a sample, the sediment that had not passed through the sieve was captured by filtering the sample through a Whatman Grade 1 filter paper and dried in an oven (Precision Scientific Thelco Lab oven: Model 15) at ~150^{o}C for 24 h. The dry mass of sediment was measured and the mean mass of all samples was calculated. To

account for variation among samples in relation to volume of sediment (i.e. size of the sample) collected, the number of eggs and hatchlings extracted from each sample was expressed as standard numbers adjusted to the overall mean sediment dry mass (eggs or nymphs /1.732 g).

Before statistically analyzing samples, the sediments from depths of 1-2 and 2-3 cm below the sediment surface were pooled. The same thing was done for depths 3-4 and 4-5 cm since buried eggs had been manually placed directly in the middle of these sample depths. The new depth aliquots in each sample for both treatments became 0-1, 1-3 and 3-5 cm.

To determine densities of eggs already present in sediments collected from the field, additional core samples were removed from tanks that contained only sediment. However, no eggs were recovered from these cores.

Oxygen Penetration:

After experiments had concluded, the depth of the oxygenated layer in each tank (characterized by a lighter brown colour (Aller and Aller 1986) visible through the aquarium wall) was measured to the nearest mm using a ruler. The minimum and maximum depths in each tank were recorded.

Statistical Analysis:

The number of eggs (sum of whole, hatched and damaged) enumerated at each depth within a core was added together to provide an estimate of the total number for each core sample. The same tabulation was performed for hatched eggs alone. For further

statistical analysis, the number of hatched eggs in each sample was expressed as a proportion of the total number of eggs to account for variations in the total number of eggs among cores retrieved from each tank. A repeated measures ANOVA was then performed to determine if there were differences in the proportions of hatched eggs between treatments. Hatched eggs (as a proportion of total number of eggs) was the dependent variable, treatment was the independent variable (between-subjects variable) and sampling event was the repeated measure (within-subjects variable). Differences in proportion of hatched eggs between treatments on specific sampling dates were analyzed using a series of univariate ANOVAs.

The number of hatchlings collected at each depth was also summed to determine the total number of hatchlings found in each core sample. A repeated measures ANOVA was performed to determine differences in number of hatchlings between treatments with # of hatchlings and treatment as the dependent and independent variables, respectively, and sampling event as the repeated measure. Prior to performing the ANOVA, a preliminary analysis of sphericity (Mauchly's sphericity test), revealed that the variances were not equal ($W = 0.046$, $p < 0.02$). All values were therefore square root transformed ($W = 0.262$, $p < 0.5$) for the ANOVA. Differences in the number of hatchlings between treatments at each sampling event were then analyzed using a Mann-Whitney U test.

To determine spatial trends in hatching at each depth, the mean proportion of hatched eggs (hatched/total eggs in core sample) at each depth was determined for each treatment at each sampling time. The percentage of eggs hatching at each depth was then calculated.

A regression analysis was used to determine the relationship between head width and body length in hatchlings. Differences in size of hatchlings between treatments were then calculated by determining the mean size (for both head width and body length) of nymphs in each tank and then performing a one-way multivariate ANOVA on the means for each sampling period. Mean head width and body length were the dependent variables and treatment was the fixed variable.

Size frequency distributions were plotted by pooling hatchling body lengths within treatments on each sampling date. Standard deviation and kurtosis values were used to compare differences in the variation of hatchling size between treatments. To determine if density-dependent growth occurs in populations, differences in nymphal growth between each sampling event (calculated by ln (mean $length_x$/mean $length_{x-1}$) where x is the sampling date) were plotted against hatchling density.

The differences in sediment suspension between tanks were analyzed using a non-parametric Mann-Whitney U Test with absorbance as the dependent variable and treatment as the fixed variable. Although, bioturbation measurements were taken three times a week, only the sampling time immediately prior to each core sampling event was analyzed for actual differences between treatments. This included days 5, 23, 37, 51 and 62 of sampling.

The differences in oxygen penetration between treatments were assessed by calculating and comparing the mean minimum and maximum depths for each treatment.

Results:

The mean (±SE) dry mass of all sediment aliquots collected was 1.732 (±0.033, n = 239) g. The numbers of eggs and hatchlings found were adjusted so that the dry mass of all individual samples was equal to the mean.

Egg Hatching:

There was no significant difference in the proportion of hatched eggs found between treatments ($F_{(1,8)} = 0.809$, $p < 0.4$). An analysis of each sampling event, however, revealed significantly more hatched eggs in the 'no eggs buried' treatment during the fourth (7^{th} week) sampling event ($F_{(1,8)} = 6.934$, $p < 0.05$) (Fig 2.3). Proportions of eggs hatching during the remaining time periods were similar between treatments.

The number of hatchlings found was significantly different between treatments ($F_{(1,8)} = 17.448$, $p < 0.01$). An analysis of each sampling event revealed that there were consistently more nymphs in the 'no eggs buried' treatment (Fig 2.4). However, differences between treatments were only significant at five and nine weeks ($U = 1.000$, $p < 0.02$ for both).

Greater percentages of eggs hatched on the surface of sediments (0-1 cm sediment aliquot) than at greater depths for both treatments during all sampling events (Fig 2.5). The percentage of hatched eggs found at greater depths, however, increased over time, specifically in the "no eggs buried" treatment, indicating a change in egg distribution in sediments.

Hatchling Development:

The head width and body length of nymphs were highly correlated ($R^2 = 0.964$, $p < 0.001$, $n = 214$) (Fig 2.6). Therefore, only the results of body length analyses will be discussed in the remainder of this study.

The mean body length of hatchlings recovered from tanks was similar during the first five weeks of the experiment (Fig 2.7). By the seventh week, however, hatchlings found in the treatment in which some eggs were buried, were significantly larger than those in the treatment where no eggs were buried ($F_{(1,8)} = 16.609$, $p < 0.005$). This was true at nine weeks as well ($F_{(1,8)} = 6.493$, $p < 0.03$). Growth rates were lower at higher hatchling densities (Fig 2.8).

The standard deviations and kurtosis values calculated for hatchling length showed that variation in size was greater when eggs were hatched at various depths in the sediment, than if they were placed only at the surface (Fig 2.9). The first two sampling events were excluded from this comparison because only one nymph was recovered from all tanks in the 'some eggs buried' treatment in both cases. Hatchling size in the 'some eggs buried' treatment had higher standard deviations in all cases included in the analysis. Furthermore, kurtosis values of hatchling size distributions in this treatment indicated that values were more platykurtic (more broadly distributed around the mean) than the other treatment in which eggs were hatched on the sediment surface.

Suspended Sediments:

There was no significant difference in water turbidity between treatments at any of the selected time periods (day 6, 23, 37, 51 and 62) (Fig 2.10), although suspended

sediment concentrations increased earlier when no eggs were buried and remained slightly higher than the other treatment until day 62. Levels of suspended sediments in both treatments, rapidly increased after approximately seven weeks (~day 46).

Oxygen Penetration:

There were no large differences in oxygen penetration observed between treatments. The minimum depths for the 'some eggs buried' treatment and the 'no eggs buried' treatment were equal at 5.6 mm (± 0.4 and ± 0.3 S.E. respectively). Maximum depths were only slightly deeper in the 'no eggs buried' treatment at 10.2 mm (± 0.8 S.E.) as opposed to 10.0 mm (± 1.0 S.E.) when some eggs were buried.

Discussion:

Previous studies have shown that anoxia affects the development and survival of *Hexagenia* both at the larval and embryonic stages. Hypoxia reduces survival and growth of nymphs (Hunt 1953, Winter et al. 1996) and suspends embryonic development, reducing overall viability (Fremling 1967; Gerlofsma 1999). Since anoxia can occur as little as a few mm below the sediment surface (Jorgenson and Revsbech 1985), egg burial is likely to affect hatching and viability in *Hexagenia* populations.

Overall, the number of hatched eggs did not differ between treatments. When each sampling event was analyzed separately, however, a significantly higher proportion of hatched eggs were found in the 'no eggs buried' treatments than in the buried treatment at seven weeks (Fig 2.3). Consistently more hatchlings were found when eggs

were situated exclusively on the surface of sediments than within the sediments (Fig 2.4). These differences were significant after 5 weeks.

The lack of more pronounced differences in hatched egg proportions found between the two treatments may be due to hatched eggs becoming damaged in sediments, resulting in misclassifications when identified under a microscope. Over 40% of eggs retrieved from tanks were classified as damaged. It is unlikely that eggs are ingested by *Hexagenia* nymphs during feeding because *Hexagenia* are deposit feeders (Zimmerman and Wissing 1978) and ingest only fine organic matter (Needham et al. 1935). Gut analysis of nymphs has revealed plant fragments, algae, grains of sand and sediment (Hunt 1953). Zimmerman and Wissing (1980) reported that for nymphs approximately 8-14 mm in length, the average size of particles ingested was 1.94×10^4 um^2, although particles as large as 12×10^4 μm^2 were also observed. Eggs are typically 4.48×10^4- 6.8×10^4 μm^2 (Hunt 1953), which is larger than the average particles found in the guts of smaller nymphs, but still within the size range of particles that could possibly be ingested. The average size of nymphs by the end of this experiment, however, was less than 8 mm (the smallest nymph size used in Zimmerman and Wissing's 1980 study). Therefore, hatchlings are unlikely to have ingested eggs during the course of this experiment. General burrowing activity, however, may damage eggs, and this could account for the large variation in proportions of hatched eggs that were observed over time (Fig 2.3). One would expect hatching to continuously increase or to reach a maximum value. However, Fig 2.3 showed both increases and decreases in the proportions of hatched eggs found. The number of hatchlings, however, continuously increased in both treatments (Fig 2.4).

Conversely, if we assume that there are no differences in hatching between the two treatments, then greater hatchling mortality must have occurred when eggs were hatched at various depths. Proportions of hatched eggs were initially very similar between treatments. However, there were eventually more hatched eggs in the 'no eggs buried treatment' than in the 'some eggs buried' treatment (Fig 2.3). Hanes and Ciborowski (1992) found that at very low densities, mortality in *Hexagenia* nymphs was higher than at intermediate densities, and ascribed these observations to an Allee effect. When *Hexagenia* eggs hatch on the sediment surface, the hatchlings immediately burrow into the sediment. They use their legs and frontal process to clear burrows of sediment and undulate their bodies to ventilate the burrows with oxygenated water (Lyman 1943; Hunt 1953; Keltner and McCafferty 1986). Mayfly nymphs have been observed to excavate tightly-clustered burrows, suggesting that living in close association with one another may provide some benefit to survival. This may include a collective increase in the circulation of oxygen and food in the burrows, thus increasing initial survival (Hanes and Ciborowski 1992). Therefore, since there were eventually more eggs hatching in the 'no eggs buried' treatment, there would have been a higher density of nymphs present. This could have fostered greater hatchling survival as a result of increased oxygen circulation in tanks. However, considering the fact that proportions of hatched eggs only differed towards the end of the experiment and because the estimated numbers of eggs hatching varied greatly over time (Fig 2.3), it is more likely that eggs were damaged or misclassified. Therefore the number of hatchlings seems to be a better indicator of overall hatching in experiments, rather than proportion of hatched eggs.

Higher percentages of hatched eggs were found on the sediment surface than at greater depths in both treatments, suggesting that egg burial does affect hatching (Fig 2.5). This is consistent with egg hatching patterns observed in several other aquatic invertebrate (Kefford et al. 2010) and fish species (Ventling-Schwank and Livingstone 1994; Greig et al. 2005; Wyatt et al. 2010). Furthermore, although all eggs in the 'no eggs buried' treatment were placed on the surface of sediments, a higher percentage of hatched eggs were found at deeper depths in this treatment, than when eggs were manually buried in the sediment. The low percentage of eggs hatching at greater depths when eggs were buried suggest that hatched eggs found at deeper depths in the other treatment most likely hatched near the surface and were subsequently buried.

The relocation of eggs could in part be attributed to core sampling. The edges of the coring tube used to remove sediment plugs may have pushed some eggs deep into the sediment, for both treatments. However, hatchling burrowing activity may also have caused egg burial. *Hexagenia* burrowing activity contributes to mixing of the top layers of sediment as well as sediment suspension in the water column (Bachteram et al. 2005). Suspended sediments may cause burial of eggs or the eggs may be transported down into burrows as the burrows are excavated (Gerlofsma 1999).

Bioturbation as a mechanism of egg transport, has been demonstrated in many groups of zooplankton, especially copepods. Kearns et al (1996) showed that the activity of oligochaetes and chironomids contributed to the vertical distribution of copepod eggs in the top 2 cm of sediment. Albertsson and Leonardsson (2000) and Viitasalo (2007) found that activities of the amphipod *Monoporeia affinis* and the bivalve *Macoma balthica* buried eggs in sediments. In the treatment where eggs were hatched on the

surface of sediments, there was a higher hatchling density. This could have contributed to increased sediment mixing and sediment suspension, resulting in increased egg transport.

Initially there were no significant differences in sizes of hatchlings between treatments (Fig 2.7). By the fifth week, however, hatchlings were significantly larger in the treatment where eggs were hatched at various depths. Since there were fewer nymphs when eggs were hatched within sediments, it is possible that they were not subjected to the same level of competition that hatchlings in the other treatment would have encountered. Consequently, hatchlings in this treatment would have been able to allocate more energy to growth. This is consistent with the findings of Hanes and Ciborowski (1992), who observed density-dependent growth in mayflies. Similar trends have also been observed in odonates and caddisflies (Pierce et al. 1985; Hart 1987; Van Buskirk 1987; Anholt 1990; Feminella and Resh 1990). When changes in hatchling size were plotted against hatchling density, growth declined at higher densities of hatchlings (Fig 2.8).

If there were a cooperative effect occurring between hatchling density and survival in this experiment, it does not appear to extend to hatchling size. The relatively small number of hatchlings found when some eggs were buried could have been attributed to low circulation of oxygen from low hatchling density (Fig 2.4). However, the hatchlings found in this treatment were significantly larger than those retrieved from the other treatment (Fig 2.7). Hanes and Ciborowski (1992) found that although lower survival occurred at lower nymph densities, nymphs reared at low densities were larger by the end of the experiment than those reared at high densities.

Variation in hatchling size was greater when eggs were situated at various depths in the sediment (Fig 2.9). The standard deviations were larger in this treatment and the distributions were more evenly dispersed around the mean. When no eggs were buried, distributions displayed higher peaks (Table 2.1). Hunt (1951) observed that *Hexagenia* eggs buried 5-6.5 cm beneath stream silt were delayed in hatching by 2-3 weeks when compared to unburied eggs. Delays in hatching could lead to the formation of multiple cohorts within a species and subsequently increase the variation in the size of nymphs present. *Hexagenia* emergence can occur over several weeks, often reflecting the occurrence of multiple cohorts present within populations (Heise et al. 1987; Schloesser and Hiltunen 1984; Giberson and Rosenberg 1994).

There was no significant difference in the levels of suspended sediments between treatments (Fig 2.10). However, the sediment concentration in the water column rapidly increased after about seven weeks, which is consistent with higher numbers and increased size of hatchlings in tanks. Furthermore, tanks with eggs hatching exclusively on the surface initially had higher levels of suspended sediments. At approximately nine weeks, levels of suspended sediments increased in tanks where eggs were buried. Bachteram et al. (2005) demonstrated that sediment suspension increases as a function of nymph density and size. Although more hatchlings were found in the surface-only treatment, nymphs in the other treatment were significantly larger by the end of the study. Larger nymphs will excavate larger burrows, thus moving larger volumes of sediment (Hunt 1953; Bachteram et al. 2005). These burrows also require more water to be pumped through the burrows to oxygenate them. This activity could have contributed to the similar concentrations of suspended sediments measured between treatments.

Since there appeared to be more egg relocation occurring in the "no eggs buried' treatment (Fig 2.5) and there was no significant difference in the levels of turbidity between treatments (Fig 2.10), the mixing of sediments, rather than sedimentation, is more likely contributing to egg burial.

There was no difference in the depths of oxygen penetration between treatments. The maximum depth of the oxic layer was approximately 10 mm in both treatments and the minimum was 5.6 mm. Therefore, eggs found deeper than 10 mm would have experienced delays in hatching in both treatments. The fact that hatched eggs were found below this layer even at the beginning of experiments suggests that burrowing activity may cause burial of eggs in sediments.

Conclusion:

Egg burial does appear to affect the timing of hatching in *Hexagenia*. More hatchlings were found in tanks where eggs were placed exclusively on the sediment surface. Furthermore, more hatched eggs were found near the surface of sediments for both treatments. The relocation of eggs in the sediment, especially when eggs were placed only on the surface of sediments suggests a relationship between burrowing activity and egg burial. Greater densities of nymphs increased the depths to which hatched eggs were buried indicating the presence of a density-dependent mechanism between life stages. Trials in which some eggs were buried exhibited delayed hatching and subsequently greater variation in hatchling size frequency distribution. In nature, such phenomena, could lead to the formation of multiple cohorts, which are commonly found in these species.

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Table 2.1: Descriptive statistics of lengths of hatchlings retrieved from experimental tanks in the 'no eggs buried' treatment (NEB) and the 'some eggs buried' treatment (SEB). Week refers to the number of weeks after the experiment started (i.e. when eggs were transferred into tanks).

Week	***Treatment***	***# of hatchlings***	***Mean Body Length (mm)***	***Std. Deviation***	***Kurtosis***
1	SEB	1	1.8	N/A	N/A
	NEB	7	1.54	0.292	0.533
3	SEB	1	0.72	N/A	N/A
	NEB	12	0.89	0.079	- 0.267
5	SEB	10	2.92	0.700	-1.256
	NEB	36	2.86	0.658	0.587
7	SEB	7	5.65	2.113	- 0.231
	NEB	38	4.12	1.638	1.151
9	SEB	28	7.33	2.625	0.370
	NEB	59	5.12	1.864	2.460

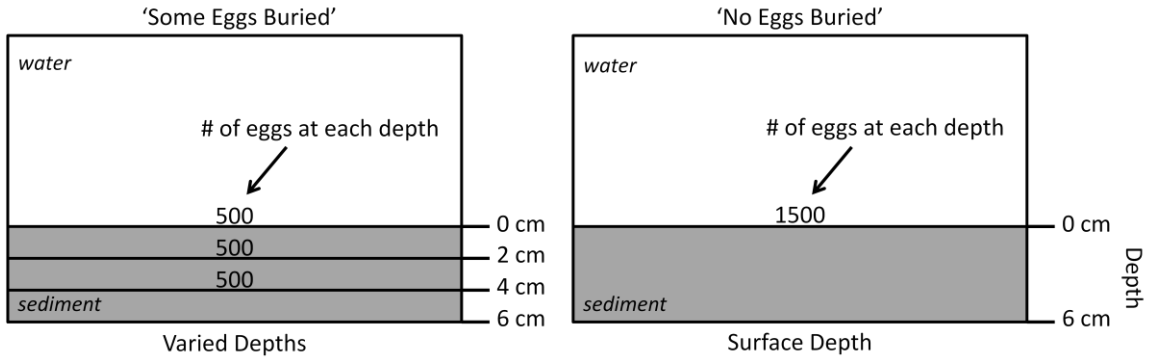

Figure 2.1: Experimental design of aquaria. In treatment 1 ('some eggs buried'), eggs were placed at three different depths in the sediment (specifically 0, 2 and 4 cm below the sediment surface) with 500 eggs/layer. In the second treatment ('no eggs buried'), all eggs (1500) were placed on the sediment surface (0 cm).

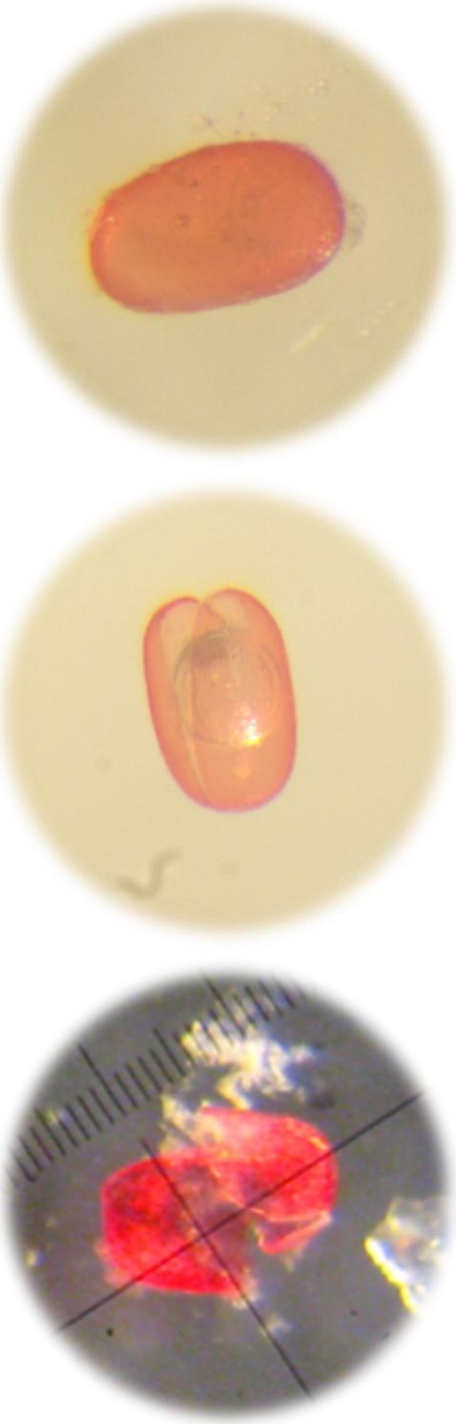

Figure 2.2: Classification of eggs retrieved from core samples.

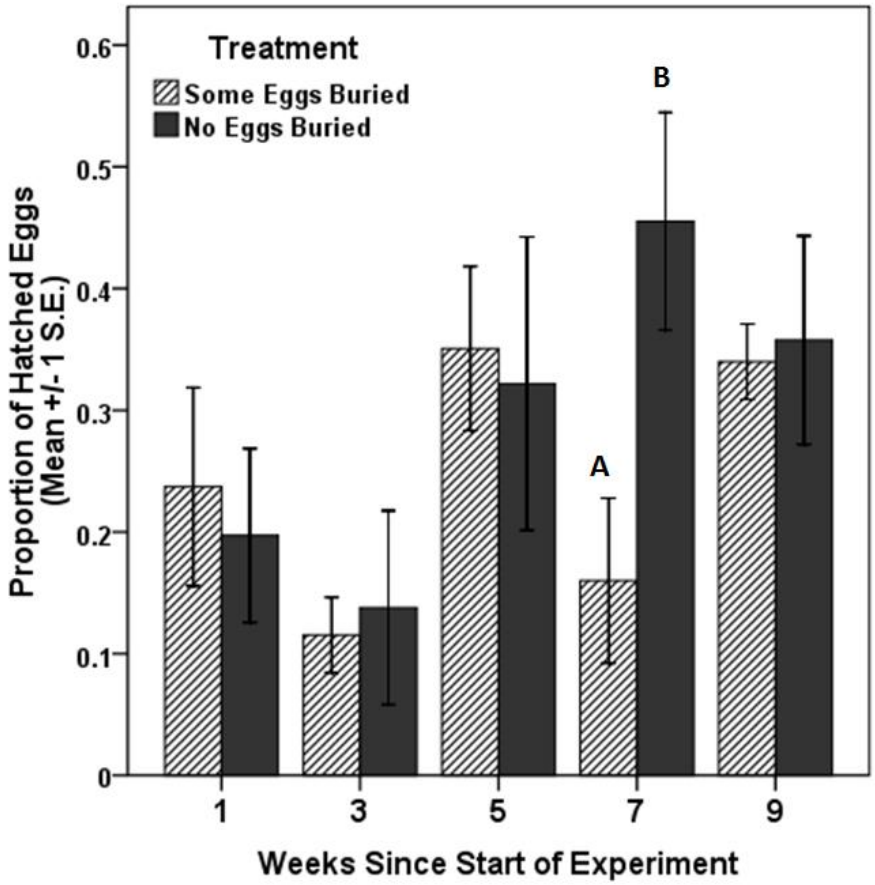

Figure 2.3: Mean (±SE) proportion of hatched eggs found in each treatment at each sampling time. The letters 'A' and 'B' represent significant differences between treatments at a single sampling time. 'Start of experiment' refers to the moment eggs were added to experimental tanks.

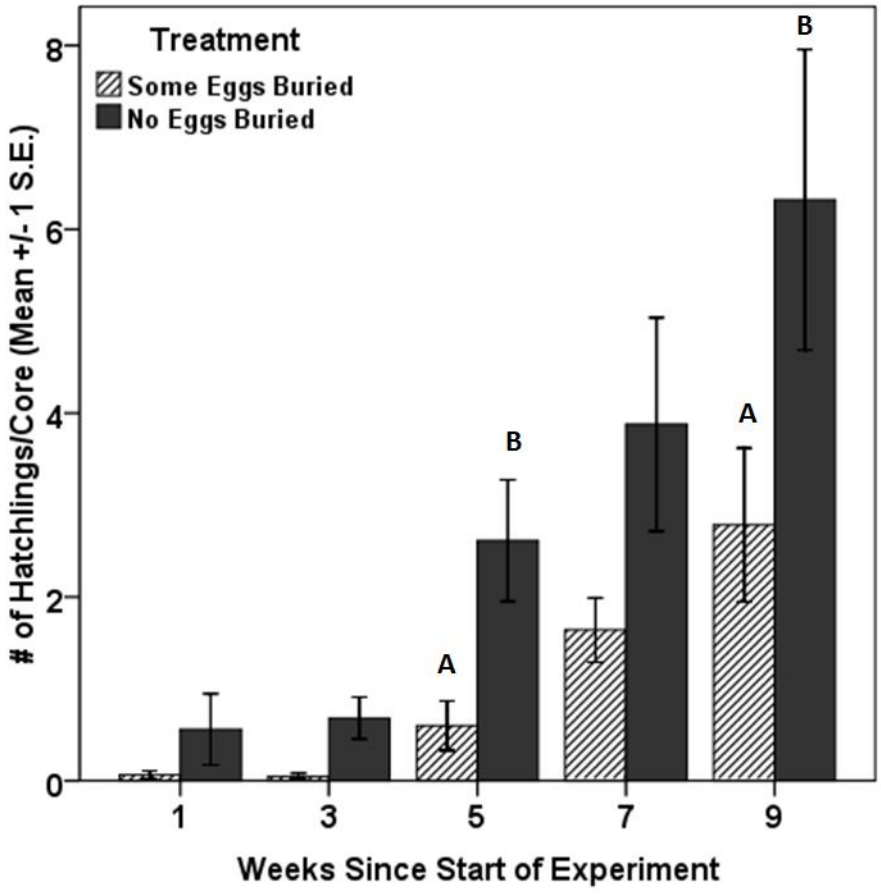

Figure 2.4: Mean (±SE) number of hatchlings found in each treatment at each sampling time. Each core represented an area of 7.07cm^2. The letters 'A' and 'B' represent significant differences between treatments at a single sampling time. 'Start of experiment' refers to the moment eggs were added to tanks.

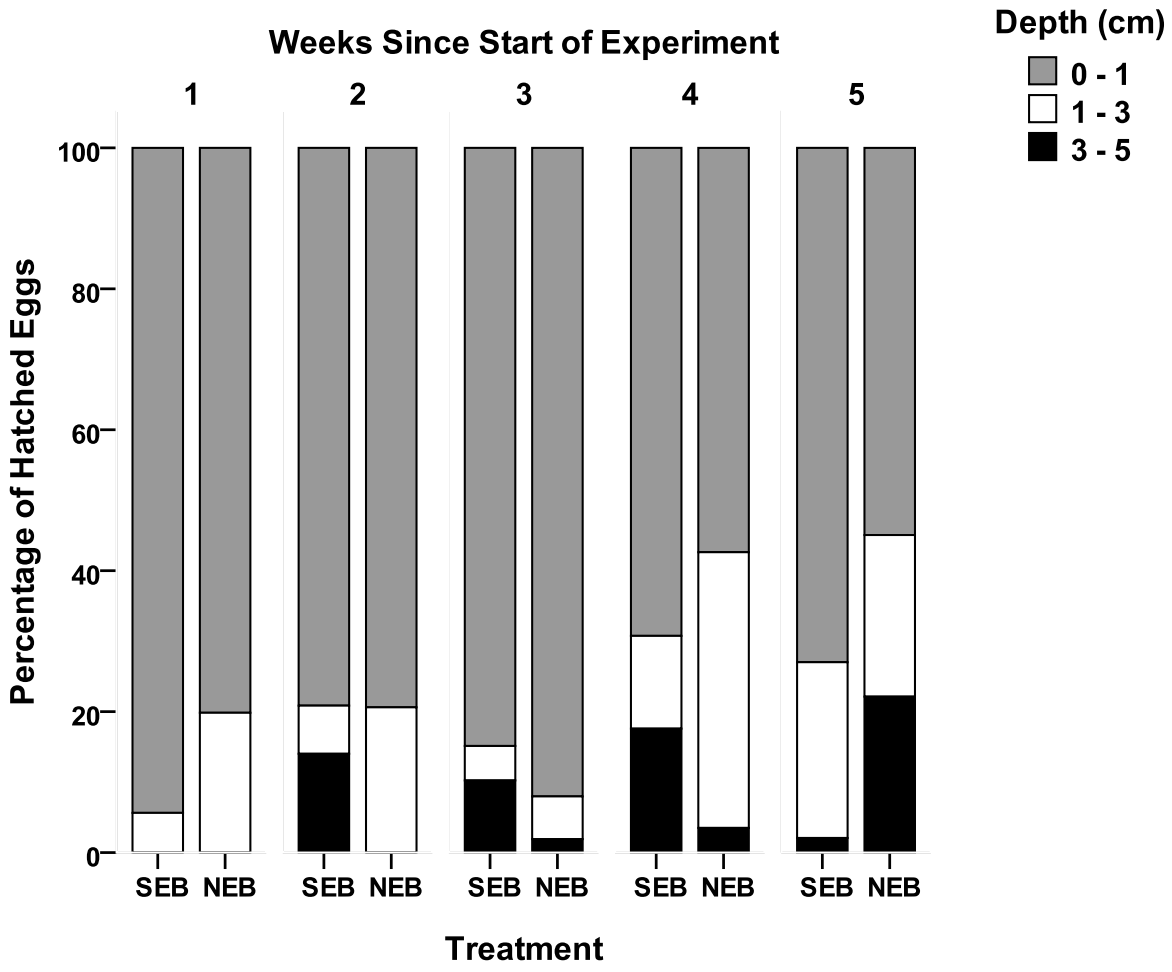

Figure 2.5: Relative proportions of eggs hatching at each depth between treatments at each sampling time. 'SEB' - 'some eggs buried'; 'NEB' - 'no eggs buried'. 'Start of experiment' refers to the moment eggs were added to experimental tanks.

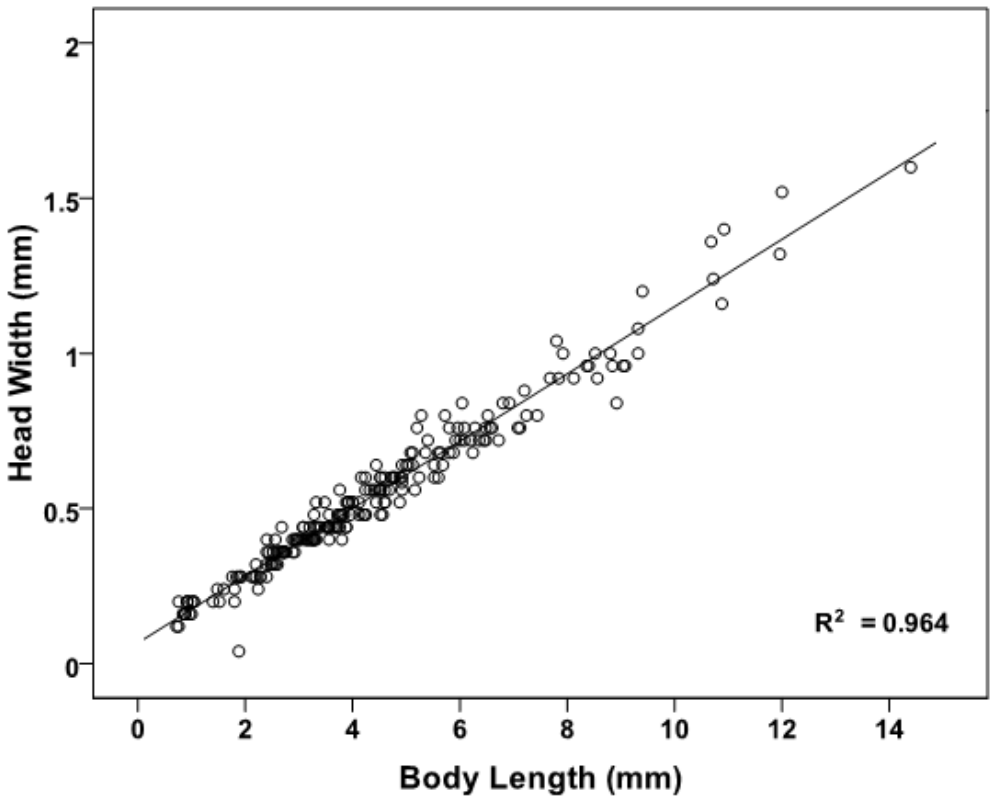

Figure 2.6: Relationship between hatchling head width (mm) and body length (mm) (n= 214). The two variables were highly and significantly correlated.

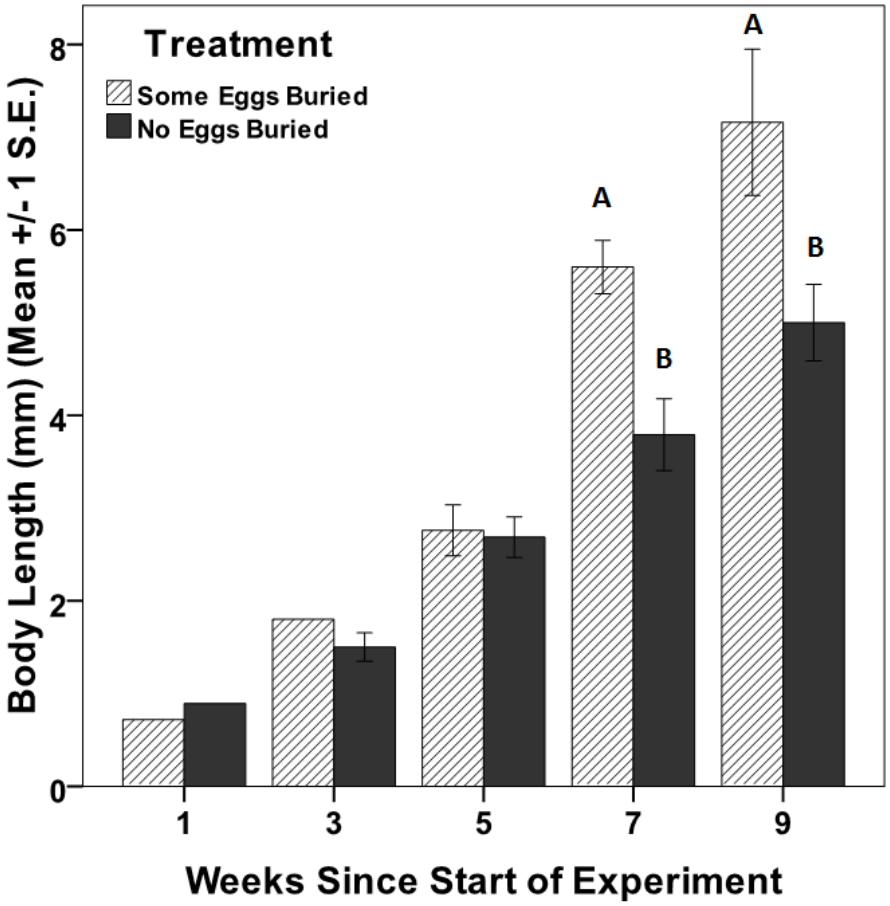

Figure 2.7: Mean (±SE) hatchling body length between treatments at each sampling event. The letters 'A' and 'B' represent significant differences in size between treatments. 'Start of experiments' refers to the moment eggs were added to tanks.

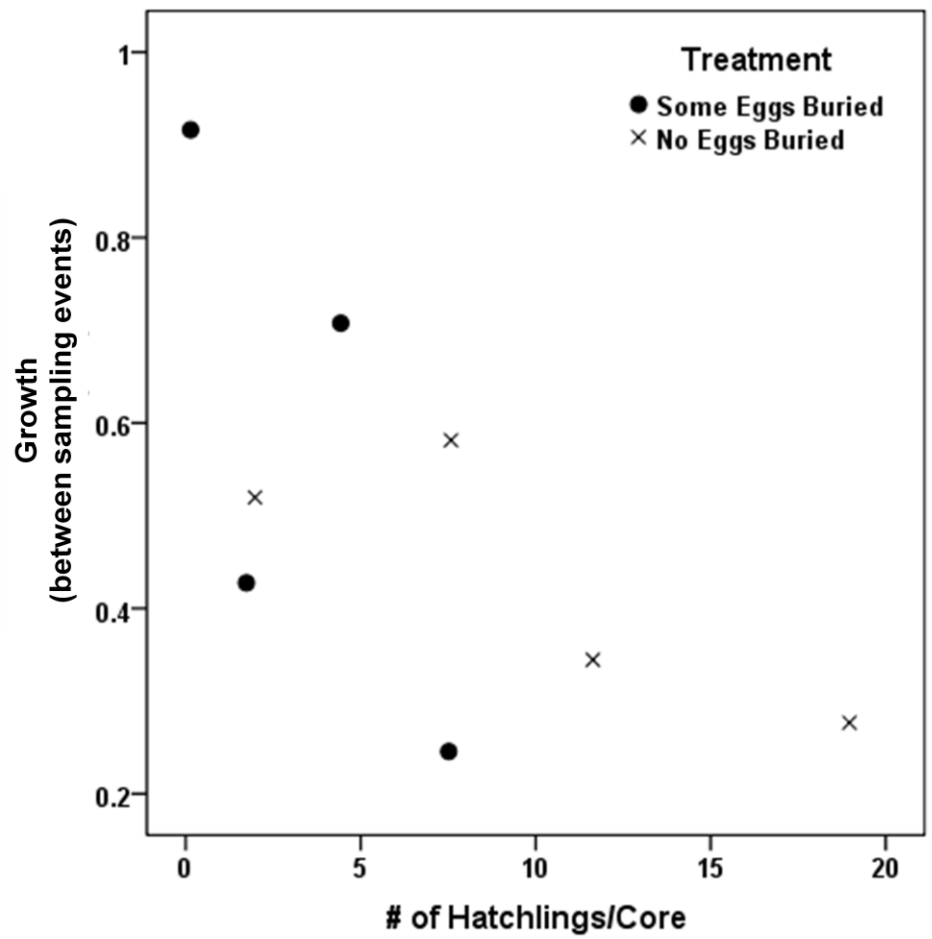

Figure 2.8: Relationship between mean hatchling growth (differences in hatchling size between sampling events) and hatchling density.

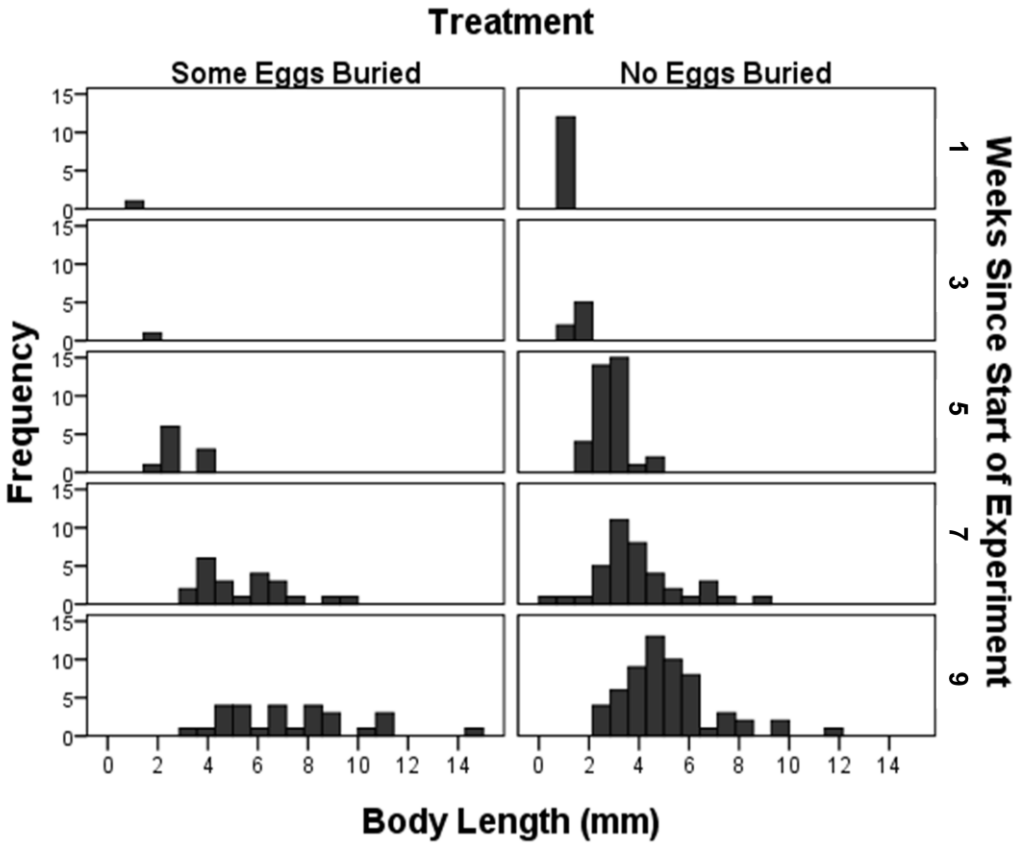

Figure 2.9: Size frequency distributions of hatchlings between treatments at each sampling time. 'Start of experiment' refers to the moment eggs were added to tanks.

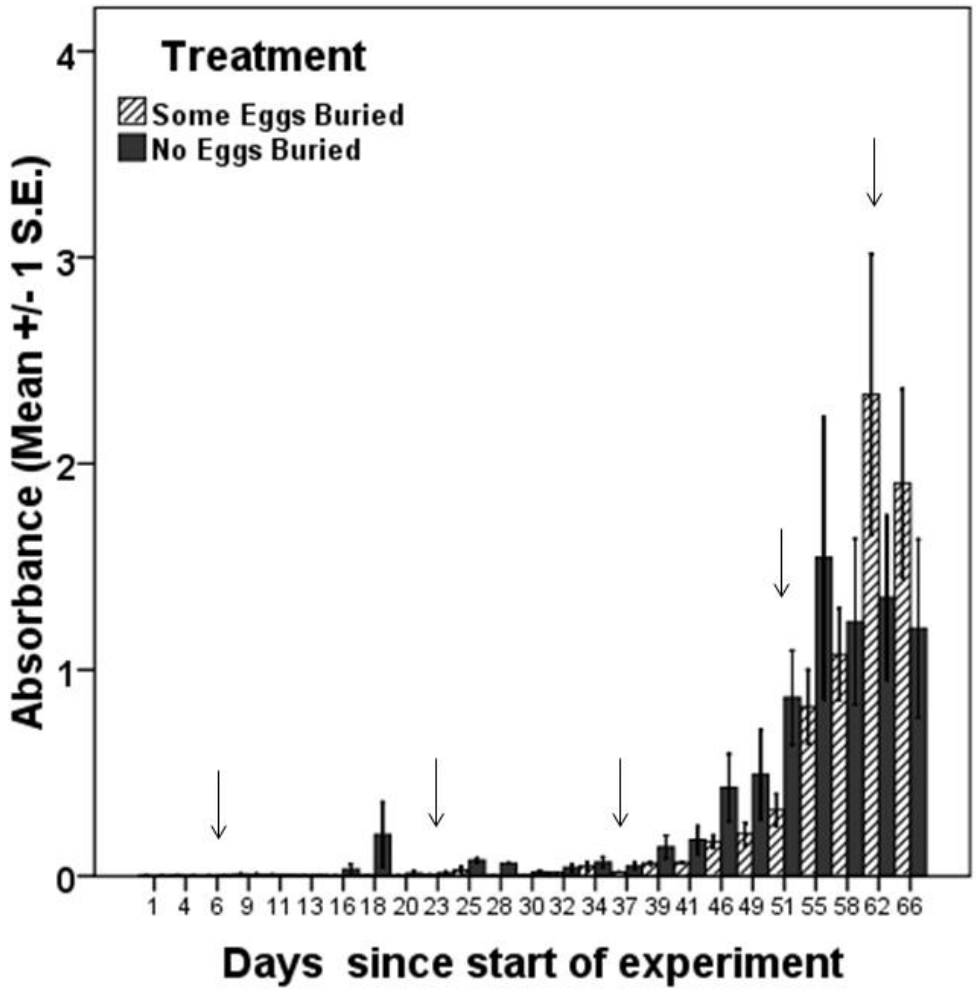

Figure 2.10: Mean (±SE) concentration of suspended sediments (measured in light absorbance) between treatments over the duration of the experiment. Arrows indicate days that were selected for statistical analysis and also correspond with core sampling events. 'Start of experiment' refers to the moment eggs were added to tanks.

CHAPTER 3: BIOTURBATION AS A SOURCE OF INTERCOHORT INTERACTIONS IN *HEXAGENIA* SPP. POPULATIONS

Introduction:

Benthic activity can have profound effects on aquatic systems. The burrowing, feeding and respiratory activities of benthic organisms, for example, can lead to bioturbation (McCall and Tevesz 1982; Krantzberg 1985; Meysman et al. 2006), which is the process of mixing and displacing sediment particles, thus altering the physical and chemical properties of the substrate (Fisher et al. 1980; Mermillod-Blondin and Rosenberg 2006). Bioturbation can contribute to sediment suspension in the water column (Matisoff and Wang 2000; Bachteram et al. 2005), which can make sediment-bound contaminants (Rosa 1985; Reible et al. 1996) and nutrients (Graneli 1979; Holdren and Armstrong 1980, Petterson 1998; Chaffin and Kane 2011) available to other organisms. Suspended sediments can also reduce light penetration into the water, consequently affecting primary production and oxygen concentrations (Luzier and Summerfelt 1997). Furthermore, suspended sediments can affect filtration rates in organisms (MacIsaac and Rocha 1995; Madon et al. 1998) and damage gill structures (Cordone and Kelley 1961).

Bioturbation can also play a role in egg bank dynamics. Egg banks refer to the natural storage of eggs whose development has been suspended due to unfavourable hatching conditions (Danks 1987; Bilton et al. 2001). Bioturbation can affect egg hatching by contributing to the distribution and dispersal of eggs in the sediment either through burial (Kearns et al. 1996; Gerlofsma 1999; Albertsson and Leonardsson 2000;

Meysman et al. 2006; Viitasalo 2007) or upward transport back to the surface (Marcus and Schmidt-Gengenbach 1986; Kearns et al. 1996; Meysman et al. 2006). Anoxia can occur as little as a few mm below the sediment surface (Jorgensen and Revsbech 1985), so burial can isolate eggs from the oxygenated layers of the sediment and consequently prevent development (Gerlofsma 1999; Meysman et al. 2006; Chapter 2). The upward transport of eggs back into the oxic layer, could allow development to resume (Marcus and Schmidt-Gengenbach 1986; Kearns et al. 1996; Meysman et al. 2006). This delay and staggering of hatching could also lead to a staggering in the development of subsequent stages, which could result in the formation of multiple cohorts in populations and even regulate seasonal reappearances.

The effect of egg burial in aquatic systems has been well documented in zooplankton. Copepod eggs, for example, can remain dormant in aquatic sediments for decades before hatching, contributing to the persistence of these populations (Marcus 1984; DeStasio 1989; Marcus et al. 1994; Hairston et al. 1995; Bilton et al. 2001). Eggs of several invertebrate species such as burrowing mayflies (Chapter 2; Hunt 1951), chironomids, and gastropods (Kefford et al. 2010), experience reductions or delays in hatching when buried in sediments. The eggs of many fish species, which are often highly sensitive to anoxia, exhibit reduced overall survival when buried (Ventling-Schwank and Livingstone 1994; Greig et al. 2005; Wyatt et al. 2010).

Bioturbation, as a mechanism of egg transport, has been demonstrated in several benthic species, and each species can contribute to egg bank dynamics in different ways. Some organisms, such as chironomids (Kearns et al. 1996), amphipods (Albertsson and Leonardsson 2000), and bivalves (Viitasalo 2007), facilitate the burial of eggs in

sediments through sedimentation and direct mixing of sediments. In contrast, tubificids cause the upward transport of eggs back to the surface through 'conveyor belt' feeding (Kearns et al 1996). They are oriented head-downwards in the sediment during feeding and defecate on the surface of sediments (Rhoads 1974; Marcus and Schmidt-Gengenbach 1986). Most studies that have examined the effects of bioturbation on egg transport, however, have focused on interactions among species. The above examples all refer to the transport of zooplankton eggs by benthic invertebrates. No studies to date have assessed the potential effects of such interactions among conspecifics. This form of intercohort interaction could profoundly influence the dynamics of populations.

Hexagenia are burrowing mayflies whose nymphs are found in soft sediments of shallow waters throughout North America (McCafferty 1975; Edmunds et al. 1976). The nymphs construct U-shaped burrows in the sediment that are on average 4.5 cm deep, although burrows as deep as 12.5 cm have been observed (Hunt 1953; Charbonneau et al. 1997; Charbonneau and Hare 1998). They use their forelegs to burrow into the sediment and once the burrows are constructed, they use their frontal process and abdominal gills to irrigate the burrows with oxygenated water (Needham et al. 1935; Lyman 1943; Eriksen 1963; Edmunds et al. 1976; Wang et al. 2001). Their burrowing activity contributes to the mixing of the upper layers of sediment as well as suspending sediment in the water column (Matisoff and Wang 2000; Bachteram et al. 2005), which ultimately settles, burying otherwise epibenthic materials, including eggs (Gerlofsma 1999; Chapter 2). If burial causes a delay in hatching for some eggs (Hunt 1951; Chapter 2), then the time of larval development might also be staggered (Gerlofsma, 1999). This, in turn,

could affect the size frequency distribution of nymphs and possibly account for the multiple cohorts that typify populations of these species (Heise et al. 1987).

Egg burial and oxygen penetration into sediments might be a function of nymph density. It has been suggested that many nymphs living in close association with another increase epibenthic circulation of oxygen and possibly flow through burrows (Hanes and Ciborowski 1992), therefore increasing the amount of oxygen available in sediments. However, increased transport of sediments at higher densities of nymphs (Bachteram et al. 2005) could facilitate egg burial to greater depths and isolate eggs from oxic layers.

The purpose of this study was to assess the effects of burrowing activity and nymphal density on egg hatching, egg burial and the subsequent development of early instar *Hexagenia* spp. nymphs. Egg hatching was observed in aquaria containing varying densities of burrowing nymphs. Suspended sediment concentrations were measured to quantify bioturbation as a surrogate for larval burrowing activity. I expected that the number of eggs buried in the sediment would depend on the density of nymphs; the higher the density, the greater the number of eggs that would become buried. Furthermore, I expected the transport of eggs into sediments to increase variation in hatching times and subsequently increase variation in hatchling development and size frequency distribution.

Methods:

Nymph Classifications:

Two classes of nymphs were used in this study: resident nymphs (also referred to as burrowing nymphs) and hatchlings. Resident nymphs ranged in length from 8-30 mm

at the start of the study, and were used to create burrowing activity (bioturbation) in study aquaria. Hatchlings were the nymphs that hatched from eggs placed in tanks during experimental trials. They typically range in size from 0.83-0.88 mm in length at the time of hatching (Hunt 1953). Hatchlings were collected and measured at various times through the experiment to quantify the effects of resident nymph activity on hatching, growth and development.

Experimental Design:

I conducted a lab experiment to examine the effects of different densities of resident (burrowing) nymphs on egg hatching and size of hatchlings over time. Eggs were placed in 8 L aquaria, each of which contained one of four densities of resident nymphs (5 replicate aquaria/treatment). Treatment densities were chosen to represent proportions of typical densities of individuals in western Lake Erie (350 nymphs/m^2; Schloesser et al. 2001). The four nymphal density treatments were 0X, 0.5X, 1.0X and 1.5X natural density (0, 8, 16 or 24 resident nymphs/aquarium respectively). Each aquarium was sampled repeatedly at 1, 3, 5, 7 and 9 weeks after the experiments began. Egg hatching and hatchling size were measured at each sampling event.

Egg Collection:

Hexagenia eggs were collected one hour after sunset from gravid female imagos (sexually mature adults) during peak emergence times in June 2010. Female imagos were found along the Detroit River in Windsor (N 42.33^o W 82.93^o) and Tecumseh (N 42.30^o W 82.86^o), ON and placed into 2 L polyethylene bags (50 females/bag) filled with

aerated, dechlorinated water, where they immediately deposited their eggs. Females were removed from bags the following day. Eggs were gradually induced into a dormant state (Friesen 1981) and stored in the collection bags at 8^{o}C until needed.

Nymph and Sediment Collection:

Resident nymphs and sediment were collected from the shore of the Chenal Ecart at the mouth of the St. Clair River in MacDonald Park (N 42.61^{o} W 82.47^{o}) in July 2010. The upper layers of sediment (~5 cm) were collected with D-frame dip nets at water depths of 0.5-1 m. Nymphs were retrieved from the sediment by sieving the sediment through 1 mm mesh box sieves and were then stored in coolers with lake water and plastic tubing (2 cm in length with an inner diameter of 0.5 cm), which provided a burrow-like structure for individual nymphs (Plant 2003). Additional sediment was collected and stored in plastic buckets with tight-fitting lids.

In the lab, sediment was frozen at -20^{o}C for 48 h, thawed and sieved through a 250 µm brass sieve to kill and remove any macroinvertebrates that may affect distribution of eggs in the sediment (Bachteram et al. 2005). The sediment was then kept in cold storage (~4^{o}C) until needed. These procedures are known not to affect sediment quality (Othoudt et al. 1991). Nymphs were placed into holding tanks with fresh sediment and aerated, dechlorinated water (~ 22^{o}C) until needed. The water was kept aerated with airstones placed 2 cm below the water surface.

Preparation of Aquaria:

Each tank (30 x 15 x 18.5 cm) was filled with 6 cm of sediment to which a depth of 12 cm of dechlorinated water (~22^{o}C) was added. Airstones were placed in the center of tanks approximately 2 cm below the water surface. Prior to the start of experiments, ammonia, pH and dissolved oxygen levels were monitored daily in each tank to insure water quality conditions appropriate for survival of eggs and nymphs once added to experimental tanks. A YSI 556 multi-probe meter was used to measure pH and oxygen and a salicylate-based aquarium testing kit was used for ammonia. Half the water was siphoned out of each tank and replaced with fresh water every other day until ammonia, pH and oxygen were at the desired levels (0mg/L, 7.0, and > 6.0mg/L respectively). The water in each tank was replaced approximately 5 times.

Transfer of Nymphs and Eggs into Experimental Aquaria:

Resident nymphs were removed from holding tanks by sieving sediment through 1 mm aperture brass sieves. Only visibly active nymphs were selected for use in experiments. Nymphs were subdivided into six length classes (0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 mm) and assigned to aquaria in stratified-random fashion to ensure that a similar size distribution was represented in each experimental tank. Before being put into an aquarium, each nymph was anesthetized using carbonated water and photographed on gridded plastic sheets (Winter 1994). The nymphs were then placed in aerated, dechlorinated water for 30-60 min to recover before being transferred into experimental tanks. Head widths and body lengths of resident nymph images were measured (to the nearest tenth of a mm) using IMAGE J software, to account for

variation in burrowing activity due to differences in size. The nymphs were also classified according to their stage of development based on the appearance of their mesonotum (Fig 3.1) (Clifford 1970). Stage 4 nymphs (the black wing pad stage) are ready to emerge. To minimize the likelihood of resident nymph emergence, only nymphs from stages 1-3 were used. Resident nymphs that died or emerged during experimental trials were replaced with a nymph of similar size from holding tanks.

Resident nymphs were placed in experimental aquaria one week prior to egg transfers. Eggs (n=1,500; ~33,300/m^2) were counted under a dissecting microscope (at very low light) and transferred into experimental tanks using a pipette just below the water surface to allow for even settling across the sediment (approximately 3.25 eggs/cm^2). Airstones were removed for 30 min to allow eggs to settle. Egg densities in the western basin of Lake Erie are approximately 14,890 (± 2,510)/m^2 (Plant et al. 2003). Higher densities of eggs were added to tanks so differences in the incidence of hatching were more easily distinguished.

Monitoring of Hatching

Egg hatching cannot be easily monitored in sediments. As a proxy for monitoring hatching rates in tanks, additional eggs were hatched in Petri dishes containing aerated dechlorinated water in parallel to the main experiment. Water in dishes was replenished daily to keep eggs well oxygenated. The number of hatched eggs in Petri dishes was monitored daily with a dissecting microscope. Experimental aquaria were first sampled on the day that at least 50% of eggs in Petri dishes had hatched.

Resident Nymph and Hatchling Feeding:

Food was added to tanks one day after resident nymphs were added to the experimental tanks (Appendix I). Nymphs were fed once a week with a dechlorinated water blended with cereal grass media and fish flakes (40 mL, 1.5 g, and 1.0 g respectively). No food was initially added to the tank without resident nymphs. In the tanks with 0.5X, 1.0X and 1.5X the natural density of nymphs, 2.5, 5 and 7.5 mL of food were added respectively. As soon as eggs began to hatch, 2 mL of the food solution was pipetted into each tank (in addition to the amount already added). This additional amount increased by 1 mL every consecutive week to a maximum increase of 6 mL/tank.

Sediment Suspension:

Sediment suspension (as an indicator of bioturbation) was measured using spectrophotometry. Two 5 mL samples were taken from the water column in each tank three times a week, poured into cuvettes and read at 750 nm in an SP1 Spectrometer 20+, using distilled water as the reference liquid (Bachteram et al. 2005). Absorbance (optical density) readings were recorded.

Sampling Procedure:

Once at least 50% of the eggs in the Petri dishes had hatched, two core samples (3 cm in diameter) were taken from the sediments of each tank. Additional pairs of cores were collected every two weeks until tanks had been sampled a total of five times. Only one of the core samples collected each time was processed and analyzed for this study. The second core sample collected in each tank was used as a reserve sample. Material

removed from tanks was replaced with sediment from a holding tank and sampled areas were recorded to avoid resampling. A total of ~424cm^3 of sediment was removed from tanks (~16% of the total sediment volume)

Each core sample was carefully pushed out of the collecting tube 1 cm at a time (Albertsson and Leonardsson 2000) and immediately preserved in a formal ethanol solution (5:2 95% ethanol: phosphate-buffered 100% formalin) diluted 1:1 with water. Several drops of lignin pink dye were added to each sample to stain eggs and hatchlings, allowing them to be easily recovered from sediments (Gerlofsma 1999).

Each sediment aliquot was sieved using a 90 µm brass sieve to remove fine particles without losing eggs (Hunt 1953; Gerlofsma 1999). Using a dissecting microscope, the number of eggs and hatchlings found in each sample were counted and recorded. Collected eggs were classified as either whole, hatched or damaged. Hatched eggs have a vertical slit extending about ¾ of the way down the side of the egg. It is from this slit that a nymph emerges (Hunt 1953). Damaged eggs are brittle and break when they are handled. Whole eggs, which were firm and cloudy/opaque, are assumed to be viable (Gerlofsma 1999). The head width and body length of hatchlings were measured to quantify development. Measurements were made with an ocular micrometer in the eyepiece of a dissecting microscope. Instar number was not identified since only a few are easily distinguishable (Hunt 1953) and the number of instars can vary across populations (Brittain 1982).

After enumeration of a sample, larger sediment particles that had not passed through the sieve were filtered through a Whatman Grade 1 filter paper. Filtered samples were then dried in an oven (Precision Scientific Thelco Lab oven: Model 15) at ~150^{o}C

for 24 h and dry mass was measured. The mean dry mass of all samples was calculated and the number of eggs and hatchlings found in each sample was expressed as standard numbers adjusted to the overall mean sediment dry mass (eggs or nymphs/1.706 g). This ensured that differences in the number of eggs and hatchlings found in each sample were not the result of differences in volume of sediment collected.

Additional core samples were also taken from tanks that contained only lake sediment to determine densities of eggs already present in sediments. However, no eggs were recovered from these cores.

Oxygen Penetration:

After experiments had concluded, oxygen penetration into sediments was determined by measuring the depth of the oxidized layer (characterized by a lighter brown colour; Aller and Aller 1986), visible through the sides of the aquarium. The minimum and maximum depth of the oxic layer was measured to the nearest mm using a ruler.

Statistical Analysis:

The difference in overall size of resident nymphs among treatments was analyzed using a Kruskal-Wallis test (a non-parametric equivalent of a 1-way ANOVA) to account for violations in normality and variance. Post-hoc comparisons between all possible pairs of treatments were performed using Mann-Whitney U-tests (Green and Salkind 2008) with Holm's correction for multiple testing (Holm 1979).

The number of hatched eggs found at each depth was summed to determine the total number of hatched eggs in each core sample. The same tabulation was performed for total eggs (whole, hatched and damaged) in each core. The number of hatched eggs was then expressed as a proportion of the total number of eggs to account for variations in egg retrieval among tanks. Differences in the proportions of hatched eggs among treatments were analyzed using a repeated measures ANOVA, with proportion of hatched eggs as the dependent variable, treatment as the independent variable (between-subjects variable) and sampling event as the repeated measure (within-subjects variable). The difference in proportions of hatched eggs at each individual sampling event was then analyzed using a series of univariate ANOVAs. The values used in both of these analyses were arcsine square root transformed to normalize the data.

The numbers of hatchlings at each depth were summed to provide a count of the total number of hatchlings in each core sample. A repeated measures ANOVA was performed to compare numbers of hatchlings among treatments. Number of hatchlings per core was the dependent variable, treatment was the independent variable and sampling event was the repeated measure. Prior to performing the ANOVA, an analysis of sphericity (Mauchly's sphericity test), revealed that the variances were not equal ($W = 0.253$, $p < 0.02$). All values were therefore square root transformed for the ANOVA. Differences in the number of hatchlings among treatments at each sampling event were then analyzed using a non parametric Kruskal-Wallis test.

To determine whether burrowing activity caused egg burial and whether there were differences in the depths to which eggs were buried among treatments, the total number of eggs found at each depth was recorded and compared. The difference in the

total number of eggs found in each treatment was first analyzed using a univariate ANOVA. Total number of eggs was the dependent variable and treatment was the independent/fixed variable. This test was used to determine whether differences in the total number of eggs found at each depth was a function of differences in egg burial among treatments or differences in sampling artefact, resulting in overall differences in the total number of eggs retrieved from tanks. The mean number of eggs found in each treatment was then determined for each depth and the proportion of eggs found at each depth was calculated.

To determine differences in hatching at each depth, the mean proportion of hatched eggs at each depth was determined for each treatment at each sampling event. The percentage of eggs hatching at each depth was then calculated.

Since head width and body length are highly correlated measures of size (chapter 2), only body length will be discussed in this chapter. A Kruskal-Wallis test was used to compare mean hatchling length among treatments for each of the five sampling periods. Mean body length was the dependent variable and treatment was the fixed variable. Post hoc comparisons were performed with Mann-Whitney U tests with Holm's correction for multiple testing. All hatchling length measurements were then pooled in each treatment and standard deviation and kurtosis values were calculated to compare differences in variation of hatchling size among treatments. Differences in nymphal growth between each sampling date (ln (mean $length_x$/mean $length_{x-1)}$ where x is the sampling date) were also calculated.

Variation in sediment suspension among tanks was analyzed using a nonparametric Kruskal-Wallis test with absorbance as the dependent variable and

treatment as the fixed variable. Although sediment measurements were taken three times a week, only the sampling time immediately prior to each core sampling event was analyzed for actual differences among treatments. This included days 6, 23, 37, 51 and 61 of sampling. Post-hoc comparisons between pairs of treatments were analyzed with a series of Mann-Whitney U tests with Holm's correction for multiple testing.

The difference in depths of oxygen penetration among treatments was determined by finding the mean minimum and mean maximum values of the oxic layer in each treatment.

Results:

The mean dry mass of all core samples collected was 1.706 ± 0.027 g (n=488). The number of eggs and hatchlings in each sample was adjusted so that the dry mass of each sample was equal to this value.

Resident Nymphs:

The mean length of resident nymphs differed significantly among treatments ($H_{(2)} = 10.328$, $p < 0.006$) with nymphs in the natural density treatment being significantly larger than resident nymphs in the other two treatments. Mean (±SE) lengths in the 3 treatments were 20.16±0.29 (1.0X), 13.27±0.77 (0.5X) and 12.17±0.26 (1.5X) mm.

Egg Hatching:

The repeated measures ANOVA revealed significant differences in the proportions of eggs hatching among treatments ($F_{(3,16)} = 3.804$, $p < 0.05$) (Fig 3.2). An

analysis of the proportions of hatched eggs at each sampling time revealed that differences among treatments were statistically significant in the first two sampling periods: week 1 ($F_{(3,16)} = 5.663$, $p < 0.008$) and week 3 ($F_{(3,16)} = 3.396$, $p < 0.04$), but not in the last three sampling periods: weeks 5 ($F_{(3,16)} = 0.111$, $p < 0.9$), 7 ($F_{(3,16)} = 0.644$, $p < 0.6$) and 9 ($F_{(3,16)} = 0.334$, $p < 0.8$).

After week 1, the greatest proportions of eggs had hatched in the 0.5X and 1.5X treatments. The lowest incidence of hatching occurred in aquaria with 0X and 1.0X natural densities of resident nymphs. At week 3, the 0.5X and 1.5X treatments again contained the highest proportions of hatched eggs. The proportion of hatched eggs at 1.0X natural density had risen by the time of the second sampling and was not significantly different from the 0.5X and 1.5X treatments. Lowest proportions of hatched eggs were found in the aquaria lacking resident nymphs, but the value did not differ significantly from proportions observed at 1.0X and 1.5X natural density.

No significant differences were found in the number of hatchlings among treatments (repeated measures ANOVA, $F_{(3,16)} = 1.494$, $p < 0.2$) (Fig 3.3). There were no significant differences among treatments when each sampling event was analyzed individually (week 1: $H_{(3)} = 5.046$, $p < 0.2$; week 3: $H_{(3)} = 2.717$, $p < 0.4$; week 5: $H_{(3)} = 3.114$, $p < 0.4$; week 7: $H_{(3)} = 6.337$, $p < 0.1$; week 9: $H_{(3)} = 5.469$, $p < 0.1$). By the end of the experiment, however, more hatchlings were found in the 0X and 0.5X treatments than in the 1.0X or 1.5X treatments

Egg Burial:

There was no significant difference in the number of eggs retrieved per core among tanks ($F_{(3,96)} = 1.344$, $p < 0.3$), so any differences in numbers of eggs found among depths are not likely related to sampling effort.

Large numbers of eggs were found buried in all treatments (Fig 3.4), even when the incidence of hatching was low and/or resident nymphs were absent (Fig 3.2 and 3.3). For example, in the first sampling period (week 1) more eggs were found buried in the 0X treatment than in any other treatment (Fig 3.4). By week 3, numbers of eggs buried were similar among all treatments.

In all treatments, highest percentages of hatched eggs occurred near the sediment surface for the duration of the experiment (Fig 3.5). The proportion of hatched eggs at deeper depths tended to increase through time for most treatments, but proportions varied greatly among sampling periods.

Hatchling Development:

There were no significant differences in the size of hatchlings among treatments (Fig 3.6). After 9 weeks, however, hatchlings collected from the 0X and 0.5X treatments were much smaller than hatchlings in the remaining two treatments. Growth of hatchlings was lower at higher densities of hatchlings in all treatments (Fig. 3.7) Standard deviations of hatchling size increased for all treatments through time (Table 3.1). Larger standard deviations in the first week were observed in the 0X and 1.0X densities of resident nymphs (0.079 and 0.080 mm, respectively) relative to hatchlings at 0.5X and 1.5X treatments (0.038 and 0.040 mm, respectively). By the end of the experiment, highest

standard deviations in hatchling size values were observed in treatments with higher resident nymph density (3.083 mm at 1.0X and 2.540 mm at 1.5X) when compared to lower density treatments (1.864 and 2.529 mm in 0X and 0.5X). Kurtosis values varied through time, but in the last two sampling periods they were lower at high densities of resident nymphs (0.591 and - 0.745 at 1.0X and - 0.652 and 0.912 at 1.5X natural density). Lower kurtosis values indicate stronger platykurtosis, meaning that hatchling sizes were more equally distributed around the mean (i.e. flatter distributions) at higher densities of resident nymphs (Fig 3.8).

Suspended Sediments:

Concentrations of suspended sediments in all treatments began to rapidly increase approximately 40 d after the start of the experiment (Fig 3.9). There were significant differences in concentrations of suspended sediments in the water column in the first three sampling periods (weeks 1, 3 and 5) ($H_{(3)} = 16.322$, $p < 0.001$; $H_{(3)} = 11.655$, $p < 0.009$; $H_{(3)} = 11.958$, $p < 0.008$). Post hoc analyses, however, revealed no significant differences among treatments in the third week. Suspended sediments were significantly lower in the 0X treatment than in remaining treatments in the first two weeks and 0.5X treatment was lower than 1.0X and 1.5X treatments in the first week (Fig 3.10). Suspended sediments in all treatments started to rapidly increase after ~40 days.

Oxygen Penetration:

Deepest oxygen penetration occurred at the highest (1.5X) density of resident nymphs, followed by the 1.0X treatment (Table 3.2). Lowest penetrations were observed in the 0X and 0.5X treatments.

Discussion:

Several studies have examined the effects of bioturbating organisms on egg transport and hatching in sediments (e.g., Marcus and Schmidt-Gengenbach 1986; Kearns et al 1996; Albertsson and Leonardsson 2000; Viitasalo 2007; Gyllstrom et al. 2008). Benthic activity can facilitate the vertical transport of eggs, causing the embryos to become isolated from the upper oxic layers of sediment. Exposure to anoxia can prevent development and hatching of eggs and can reduce overall viability (Gerlofsma 1999).

In this experiment, I postulated that higher densities of nymphs would promote egg burial, resulting in fewer eggs hatching overall. Bachteram et al. (2005) showed that concentrations of suspended sediments increased with increasing nymphal density. A higher density of nymphs means that more sediment will be transported through burrowing activity per unit time. Bachteram et al. (2005) also observed greater activity of individual nymphs at higher population densities, likely due to higher circulation of oxygen and food within the sediment (Hanes and Ciborowski 1992).

I also expected that higher densities of nymphs would increase oxygen penetration by increasing the mixing of upper layers of sediment. Nymphs use their abdominal gills as well as undulating abdominal movements to circulate oxygenated water through burrows (Needham et al. 1935; Lyman 1943; Eriksen 1963; Edmunds et al.

1976; Wang et al. 2001). Wang et al. (2001) showed that burrows of *Hexagenia limbata* can remain at 75-100 % oxygen saturation for almost a day (until new burrows are constructed). Furthermore, oxygen penetration diffuses up to 2 mm into the sediment surrounding burrow walls. Higher densities of burrowing nymphs would increase the number of oxic microenvironments available for eggs to hatch. Furthermore, a greater overall volume of oxygenated water would be circulated throughout the sediment (Eriksen 1963; Hanes and Ciborowski 1992), ultimately allowing eggs to hatch at deeper depths.

Both proportions of hatched eggs and number of hatchlings (Fig 3.2 and 3.3) exhibited similar patterns at weeks 1, 3 and 7, but differed at weeks 5 and 9. In Chapter 2, the possibility of hatched eggs being damaged or ingested by nymphs was discussed and it was found that approximately 40% of eggs were classified as damaged. The same percentage of eggs were classified as damaged in this chapter and similar to what was observed in Chapter 2, the proportion of hatched eggs found was highly variable (Fig 3.2). Furthermore, in the 0.5X treatment, there was a continuous decrease in the proportion of hatched eggs observed through time, suggesting a progressive loss of eggs, rather than a cessation of hatching. While hatchlings are unlikely to ingest larger particles like eggs, egg ingestion might be possible in the presence of larger conspecifics (i.e. resident nymphs) (Zimmerman and Wissing 1980). However, since the percentage of damaged eggs found in both chapters were very similar, it is unlikely that the presence of larger conspecifics contributes to the damaging of eggs.

In Chapter 2, I showed that the number of hatchlings continued to increase throughout the experiment, suggesting that number of hatchlings was a better indicator of

overall hatching in tanks, than the proportion of hatched eggs. In this chapter, however, there was greater variation in the number of hatchlings within treatments over time (Fig 3.3). While the number of hatchlings appeared to increase overall, I did occasionally see an apparent reduction in numbers, such as in the 0.5X resident density treatment in week 7. Variation in number of hatchlings may be due to the tendency of nymphs to aggregate in sediments. Hanes and Ciborowski (1992) observed clusters of nymph burrows in sediment while large areas were left unoccupied. Furthermore, Henry et al. (1986) observed *Hexagenia* nymphs sharing burrows. Living in close proximity to other nymphs, apparently increases overall survival because of greater oxygen and nutrient circulation within a smaller area (Hanes and Ciborowski 1992). In the present study, it is possible that hatchlings were clustered within tanks, thereby increasing the possibility of sampling areas with higher or lower densities of nymphs. When water was removed from tanks at the end of the experiment, burrows were visible in the sediment and seemed to be widely distributed throughout the tank. However, since burrows were examined only after the study was completed, it is possible that the increasing densities of hatchlings allowed for more widespread distribution throughout the tank.

Initially, the highest numbers of both hatched eggs and hatchlings were found in the 0.5X and 1.5X density treatments (Fig 3.2 and 3.3). If higher densities of resident nymphs cause egg burial, subsequently delaying or preventing hatching, then I would expect less hatching to occur at 1.0X and 1.5X natural densities of resident nymphs. This was observed in the 1.0X density, but didn't apply to the highest density of resident nymphs, where higher hatching occurred initially. Perhaps high densities of resident nymphs facilitate the circulation of oxygen throughout sediments (Eriksen 1963; Hanes

and Ciborowski 1992), allowing buried eggs to hatch. However, if this were the case, then I would most likely have recorded more hatching than was observed at natural density. On the other hand, the resident nymphs in the 1.0X treatment were significantly larger than nymphs in other treatments. Larger nymphs tend to create deeper burrows in the sediment (Hunt 1953), possibly facilitating deeper burial of eggs and preventing hatching from occurring.

In the absence of resident nymphs, I expected to find higher numbers of hatched eggs and hatchlings. Little or no burrowing activity should have occurred in this treatment until several of the eggs had hatched. Since fewer hatchlings and hatched eggs were initially observed in this treatment (especially when compared to 0.5X and 1.5X treatments; Fig 3.2 and 3.3), fewer eggs should have been buried. At week 1, however, more buried eggs (whole, hatched and damaged combined) were recovered from the 0X treatment and from the 1.0X treatment than the remaining two treatments (Fig. 3.4). While the burial of eggs at natural density could be explained by the greater size and density of resident nymphs (Bachteram et al. 2005), there should have been few eggs buried in the absence of resident nymphs, especially since I saw lower hatching at this time. Perhaps the burial of eggs in this treatment is a result of sampling methods. The edges of the coring tube may have pushed some eggs down into the sediment as samples were taken.

By the end of the experiment, the number of hatchlings and the proportion of hatched eggs yielded different results (Fig 3.2 and 3.3). There were few differences in the proportions of hatched eggs among treatments, although most hatching occurred at 1.5X the natural density of resident nymphs (Fig 3.2). In contrast, the 0X and 0.5X treatments

contained the greatest numbers of hatchlings, although these differences were not significant (Fig 3.3). This latter result would be expected if resident nymphs contributed to the burial of eggs in sediment.

Similar to what I observed in the second chapter, more hatching occurred at the surface of sediments in all treatments (Fig 3.5) than at greater depths. However, since all eggs were initially placed on the surface of sediments in this experiment, the overall distribution of eggs should be examined before claiming that burial affects hatching. Many eggs (whole, hatched and damaged combined) were found buried in the sediment as early as the first week of sampling, and up to 70% burial was observed in some treatments by the end of the experiment (Fig 3.4). Despite high levels of burial in treatments, highest percentages of eggs still hatched in the upper cm of sediments (Fig 3.5). For example, in week 5, almost 70% of the eggs recovered in the 0X treatment were buried, but almost 90% of hatching still occurred on the surface of sediments. The number of hatched eggs found at each depth varied within treatments, and hatched eggs at the deepest depths were found in both lower and higher densities of resident nymphs, once again suggesting that core sampling may have contributed to the burial of some eggs in tanks.

There was no significant difference in the size of nymphs among treatments. However, in the final (9^{th}) week of sampling, largest hatchlings occurred in treatments with high densities of resident nymphs (1.0X and 1.5X; Fig 3.6). The highest densities of hatchlings recovered in the 9^{th} week were in the two treatments with the lowest densities of resident nymphs (0X and 0.5X; Fig 3.3), suggesting that density-dependent growth has occurred among the hatchling nymphs, independently of the density of resident nymphs.

A comparison of *Hexagenia* growth and hatchling density in tanks showed that growth was reduced at higher densities of hatchlings (Fig. 3.7). Hanes and Ciborowski (1992) observed density-dependent growth in *Hexagenia* nymphs, a trend that also was observed in the second chapter of this thesis. While the density of resident nymphs in each tank might affect burial and hatching in tanks, it wouldn't likely affect density-dependent growth of hatchlings as much as would hatchling density. Highest resident nymph density was 24 nymphs/tank. However, in the absence of resident nymphs, highest hatchling density was almost 20 hatchlings per core sample (3 cm in diameter), which is well over 1000 nymphs/tank (Fig 3.3).

Hatchling size provides support for using number of hatchlings instead of proportion of hatched eggs as an indicator of overall hatching in tanks. More hatchlings were found in tanks where the average size of hatchlings was smaller (Fig 3.2 and 3.6). Previous studies (Hanes and Ciborowski 1992; Chapter 2) as well as the results of this experiment (Fig 3.7) have shown that density-dependent growth occurs in *Hexagenia*, suggesting that the size of hatchlings should be smaller at higher densities. Proportion of hatched eggs, however, showed more eggs hatching in tanks where the average size of hatchlings was larger. Therefore, it is more likely that the number of hatchlings, rather than proportion of hatched eggs, is a better indicator of hatching trends.

Variation in hatchling size differed over time. Size frequency distributions of hatchlings were relatively narrow during the first three sampling events (weeks 1, 3 and 5; high kurtosis) (Fig 3.8). By week seven, size distribution of hatchlings became more platykurtic, especially at higher densities of resident nymphs (1.0X and 1.5X treatments). The largest standard deviations were initially found in the absence of resident nymphs

and at natural density, but standard deviations became proportionately larger at the 1.0X and 1.5X resident densities in later weeks (Table 3.1). The burial of eggs and subsequent return to the surface may produce staggered hatching and cause the formation of multiple cohorts within populations. Natural populations of nymphs have multiple cohorts as evidenced by broad size frequency distributions (Heise et al. 1987), and this is reflected in the extended emergence periods of subimagos (sexually immature adults), which are a consequence of offsets in the development of individuals (Schloesser and Hiltunen 1984; Heise et al. 1987; Giberson and Rosenberg 1994).

As expected, highest concentrations of sediment suspension occurred at higher densities of resident nymphs in all sampling periods that were analyzed (Fig 3.10). At higher densities of nymphs, higher volumes of sediment would be excavated during burrowing (Bachteram et al. 2005). Suspended sediment concentrations in the 0X treatment remained lower than the other treatments until week 7, at which time it increased dramatically. By week 7, there were more hatchlings in the 0X treatment aquaria than in 0.5X or 1.0X treatments (Fig 3.3) and by week 9, there were more hatchlings in the 0X treatment aquaria than in all other treatments. This would have contributed to the increases in suspended sediment observed in the 0X density treatment. Sediment suspension concentrations remained higher at 1.0X and 1.5X treatments, most likely due to the large size of resident nymphs in these treatments (Fig 3.10). Even though the number of hatchlings found in 0X treatment was higher than in the other treatments, the hatchlings were also significantly smaller (Fig 3.3 and Fig 3.6) and concentrations of suspended sediments are proportional to both nymph size and density (Bachteram et al. 2005).

Deepest oxygen penetration in sediments occurred in the 1.5X treatment (Table 3.2). Since it has been suggested that increased densities of nymphs increase oxygen circulation throughout sediment (Eriksen 1963; Hanes and Ciborowski 1992), I would expect oxygen penetration to be deeper at higher densities of nymphs. Although the highest densities of hatchlings occurred in the 0X and 0.5X treatments, (Fig 3.3), the observed oxygen penetrations were much lower in these treatments (Table 3.2). Reduced penetration in these treatments may be relative to the size of hatchlings, which were significantly smaller than the hatchlings that developed in the other treatments. Smaller nymphs tend to burrow near the sediment-water interface, whereas larger nymphs create burrows deeper in the sediment (Hunt 1953). The greater oxygen penetration at highest densities of resident nymphs could explain the initial hatching success observed in this treatment when compared to other treatments (Fig 3.2 and 3.3). However, increased burrowing activity could also have eventually caused eggs to become buried deeper in the sediment, isolating them within the anoxic layer. Since oxygen penetration was measured solely by the appearance of the sediment, it is impossible to determine the actual concentrations of dissolved oxygen present within these layers. Available oxygen concentrations in these layers may still be low enough to cause delays in hatching and development (Gerlofsma 1999).

Conclusion:

More eggs hatched on the surface of sediments than at any other depth in all treatments. Most hatching occurred in treatments containing no or low densities of resident nymphs (according to number of hatchlings found). This suggests that higher

densities of resident burrowing nymphs bury significant numbers of eggs in sediments and consequently delay hatching. However, many eggs were initially found buried even in the treatment lacking resident nymphs and when little hatching had occurred, indicating that the method of sampling tanks likely contributed to egg burial as well.

Differences in the size of hatchlings among treatments suggested that growth was dependent on the density of other hatchlings but independent of densities of resident nymphs. By the end of the experiments, the greatest variations in hatchling length were observed at highest densities of resident burrowing nymphs, indicating that resident nymphs induce a staggering in hatching, either through delays in hatching caused by egg burial at greater depths or through the burial and subsequent movement of eggs back to the sediment surface. The staggering of egg hatching could lead to the formation of multiple cohorts within populations.

Highest concentrations of sediment suspension occurred in two treatments with the higher densities of resident nymphs, reflecting the degree of resident nymph burrowing activity. Greatest oxygen penetrations into sediments also occurred in treatments with high densities of resident nymphs, showing that an increase in burrowing activity also increases the amount of oxygenated water that is circulated throughout sediments.

The overall lower incidence of egg hatching that occurs at higher densities of resident burrowing nymphs, together with the higher variation found in hatchling size, suggests that a density-dependent mechanism is present in *Hexagenia* spp. - larger conspecifics at later stages of development apparently have the ability to regulate both the mean size and size frequency distribution of future cohorts. This suggests that intercohort

interactions are occurring. The offset of development in hatchling nymphs could potentially reduce overall competitive pressure for resources and space relative to direct interactions that might occur if all nymphs developed simultaneously.

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Table 3.1: Descriptive statistics of lengths of hatchlings retrieved from experimental tanks for each of the four density treatments. 'ND' refers to 'natural density' of resident nymphs.

Sampling Event	***Treatment (# of resident nymphs)***	***# of hatchlings***	***Mean Body Length (mm)***	***Std. Deviation***	***Kurtosis***
1	None	12	0.89	0.079	- 0.267
(week 1)	0.5 x ND	10	0.80	0.038	1.185
	1.0 x ND	6	0.88	0.080	0.925
	1.5 x ND	28	0.80	0.049	- 0.682
2	None	7	1.54	0.292	0.533
(week 3)	0.5 x ND	20	1.55	0.252	- 0.308
	1.0 x ND	6	1.87	0.219	2.422
	1.5 x ND	9	1.37	0.167	-1.097
3	None	36	2.86	0.658	0.587
(week 5)	0.5 x ND	39	2.73	0.780	0.099
	1.0 x ND	22	3.34	0.764	- 0.252
	1.5 x ND	19	2.88	1.025	0.678
4	None	39	4.01	1.746	1.164
(week 7)	0.5 x ND	20	4.73	1.599	2.266
	1.0 x ND	24	5.42	1.537	0.591
	1.5 x ND	29	5.12	2.045	- 0.652
5	None	59	5.12	1.864	2.460
(week 9)	0.5 x ND	46	5.69	2.529	4.325
	1.0 x ND	24	6.80	3.083	- 0.745
	1.5 x ND	28	7.48	2.540	0.912

Table 3.2: The minimum and maximum depths of the oxygenated layer in tanks at the end of experiments in the four density treatments. 'ND' refers to 'natural density' of nymphs.

Treatment (# of resident nymphs)	Mean Min Depth (mm)	Standard Error	Mean Max Depth (mm)	Standard Error
None	5.6	0.358	10.2	0.769
0.5 x ND	5.8	0.438	10.2	1.035
1.0 x ND	5.8	0.334	12.2	0.955
1.5 x ND	11.8	1.035	17	1.442

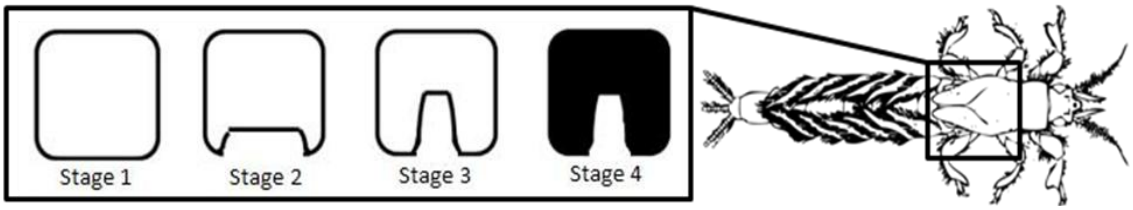

Figure 3.1: Appearance of the mayfly mesonotum at various stages of development. At stage 4, nymphs are ready to emerge as subimagos. Nymph illustration modified from Needham (1996).

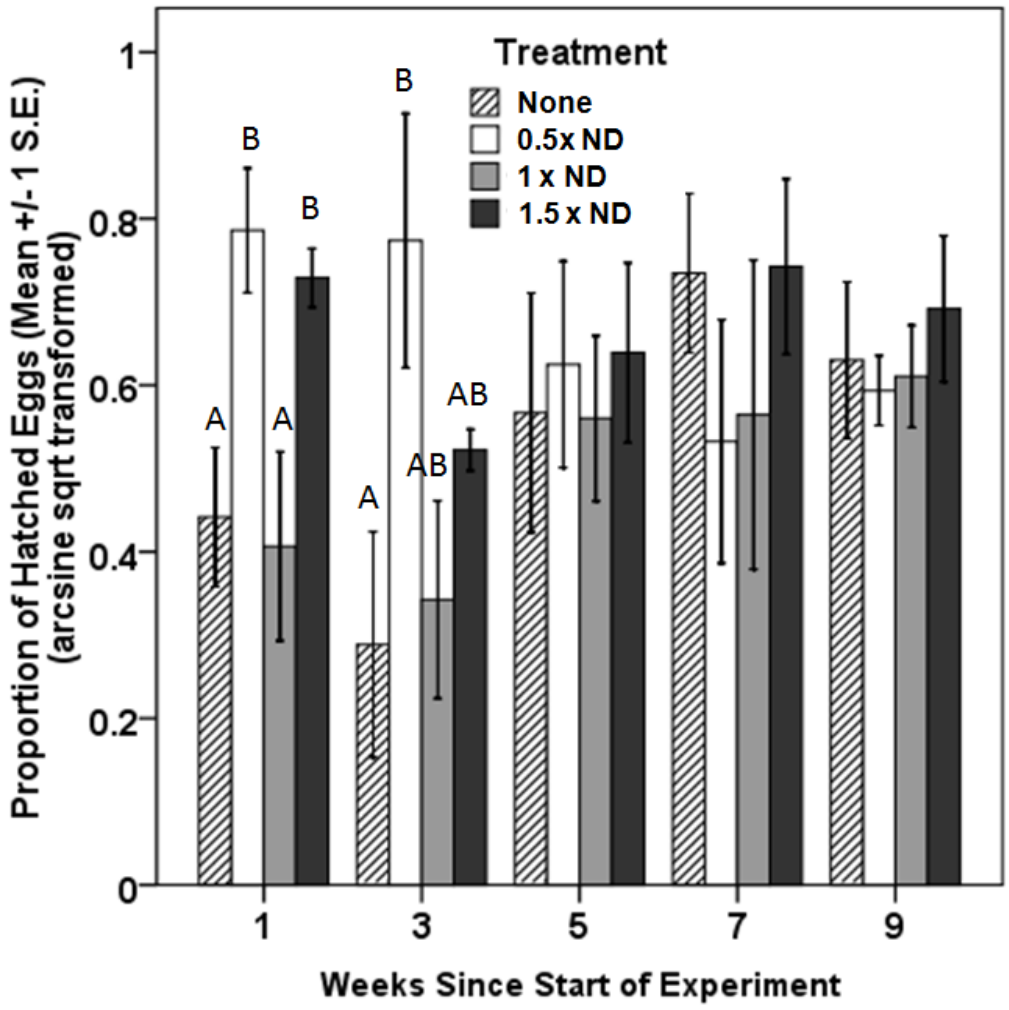

Figure 3.2: Mean (±SE) proportion of hatched eggs found in each treatment at each sampling time. The letters 'A' and 'B' represent significant differences among treatments at the time of sampling. 'ND' refers to natural density of resident nymphs. 'Start of experiment' refers to the moment when eggs were transferred into tanks.

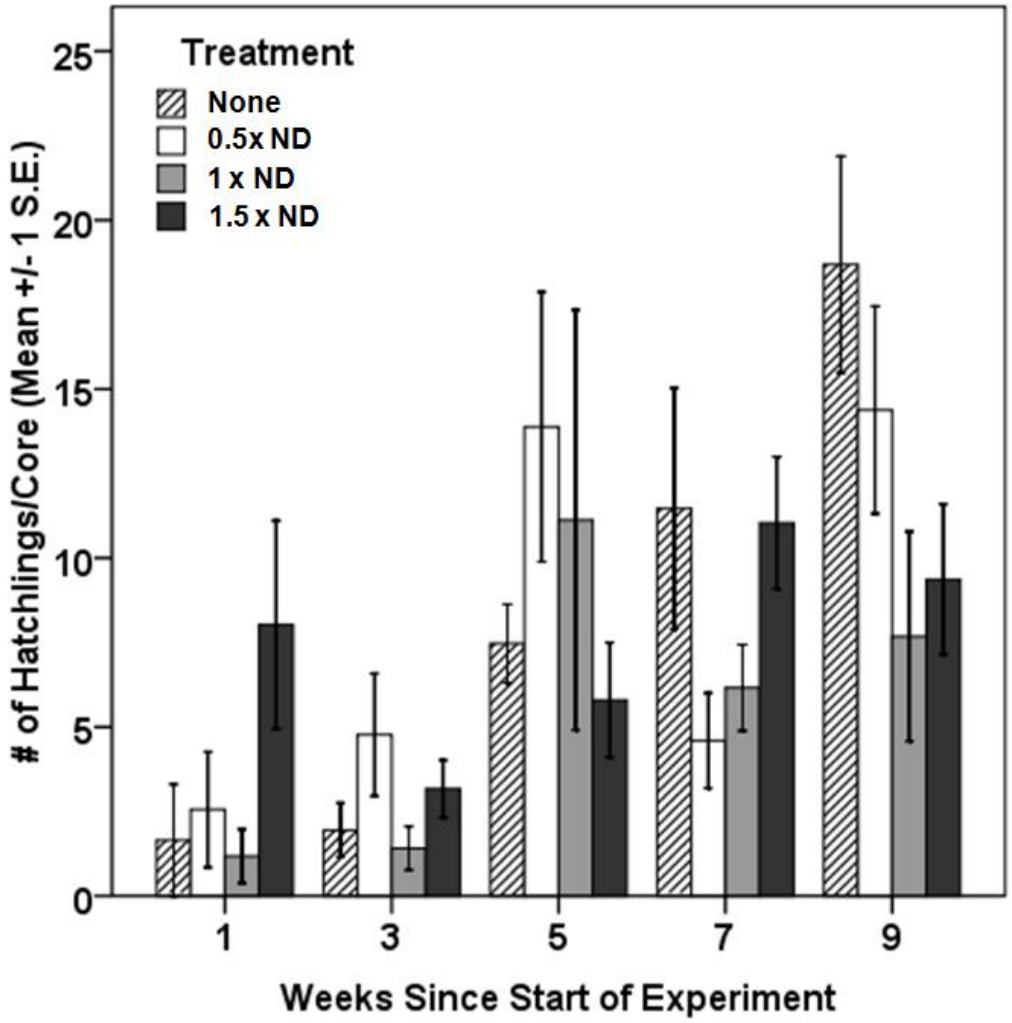

Figure 3.3: Mean (±SE) hatchling density (nymphs/core) found in each treatment at each sampling time. Each core represented an area of 7.07cm^2. 'ND' refers to natural density of resident nymphs. 'Start of experiment' refers to the moment when eggs were transferred into tanks.

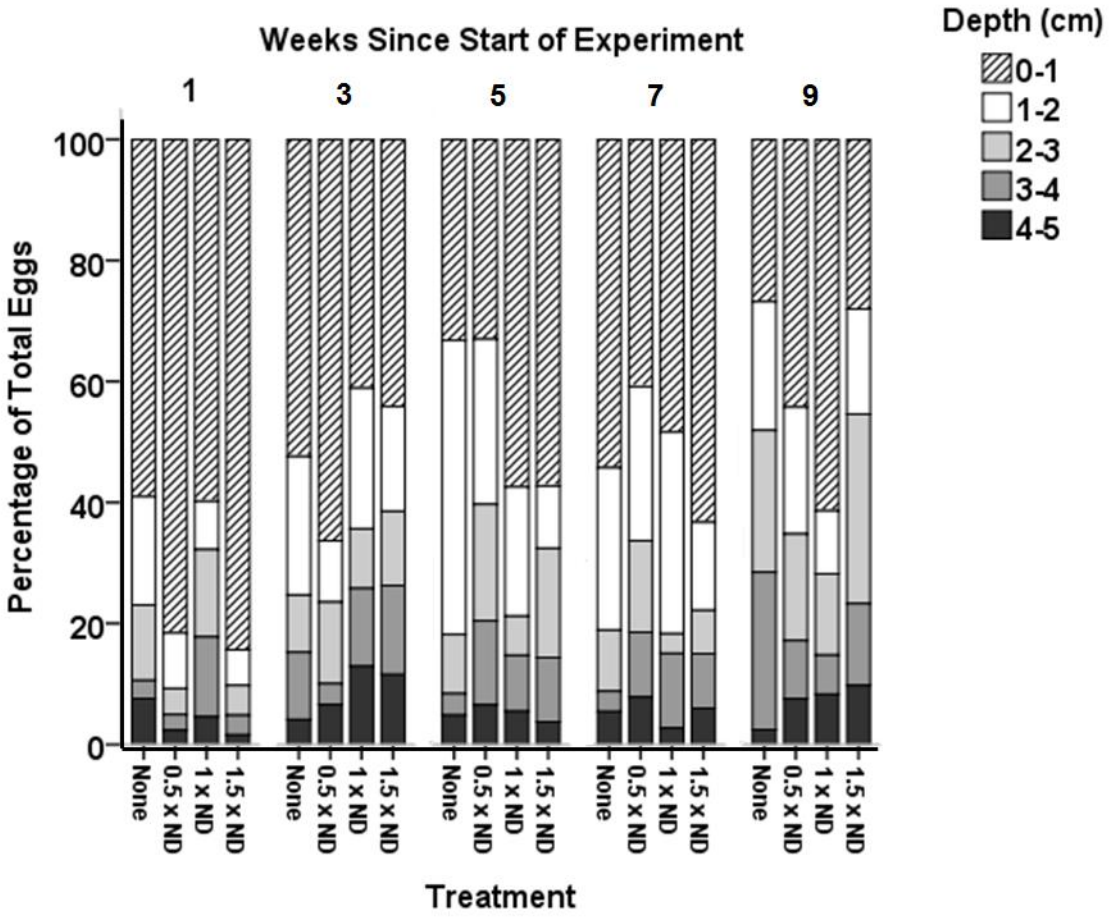

Figure 3.4: Depth distribution of eggs (whole, hatched and damaged combined) retrieved from sediment cores among treatments. 'ND' refers to 'natural density' of burrowing nymphs. 'Start of experiment' refers to the moment eggs were transferred into tanks.

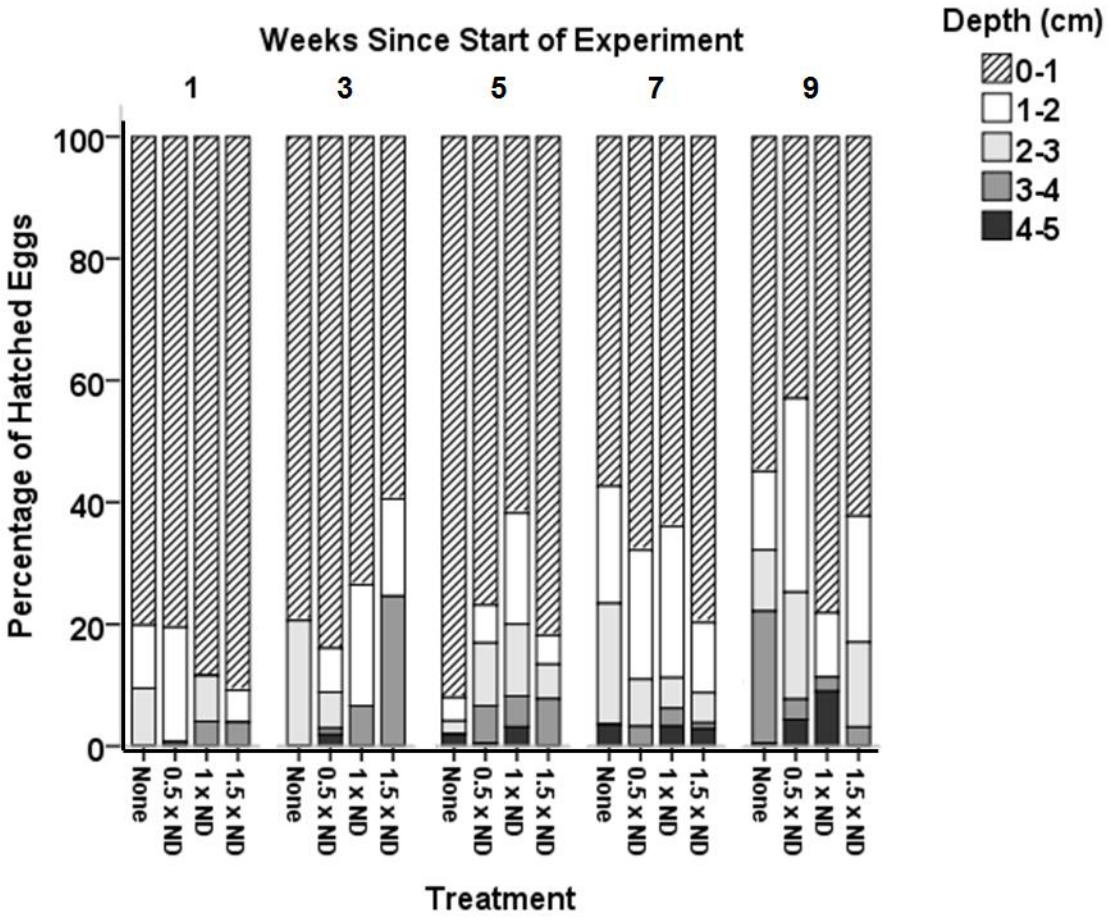

Figure 3.5: Depth distribution of hatched eggs retrieved from sediment cores among treatments. 'ND' refers to 'natural density' of burrowing nymphs. 'Start of experiment' refers to the moment eggs were transferred into tanks.

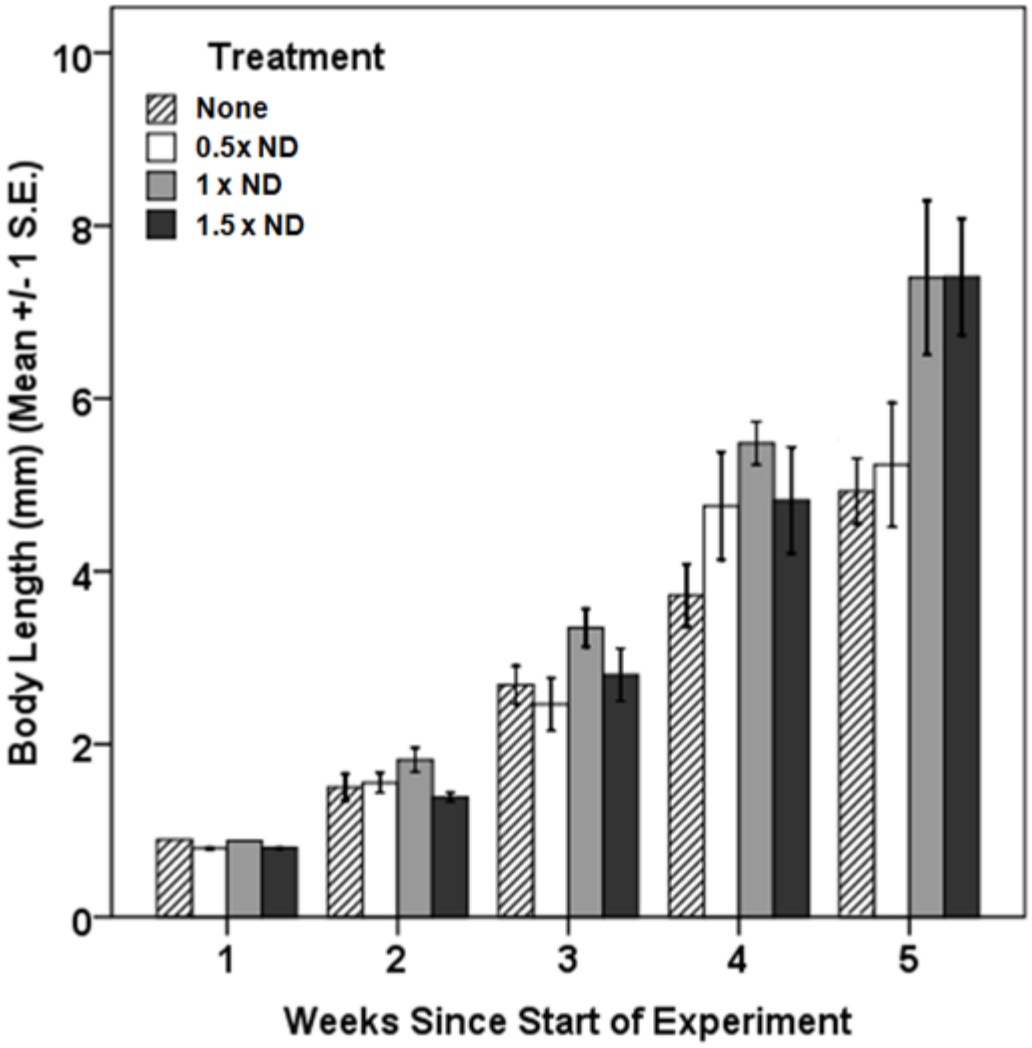

Figure 3.6: Mean (±SE) hatchling body length (mm) among treatments at each sampling event. 'Start of experiment' refers to the moment eggs were transferred into tanks.

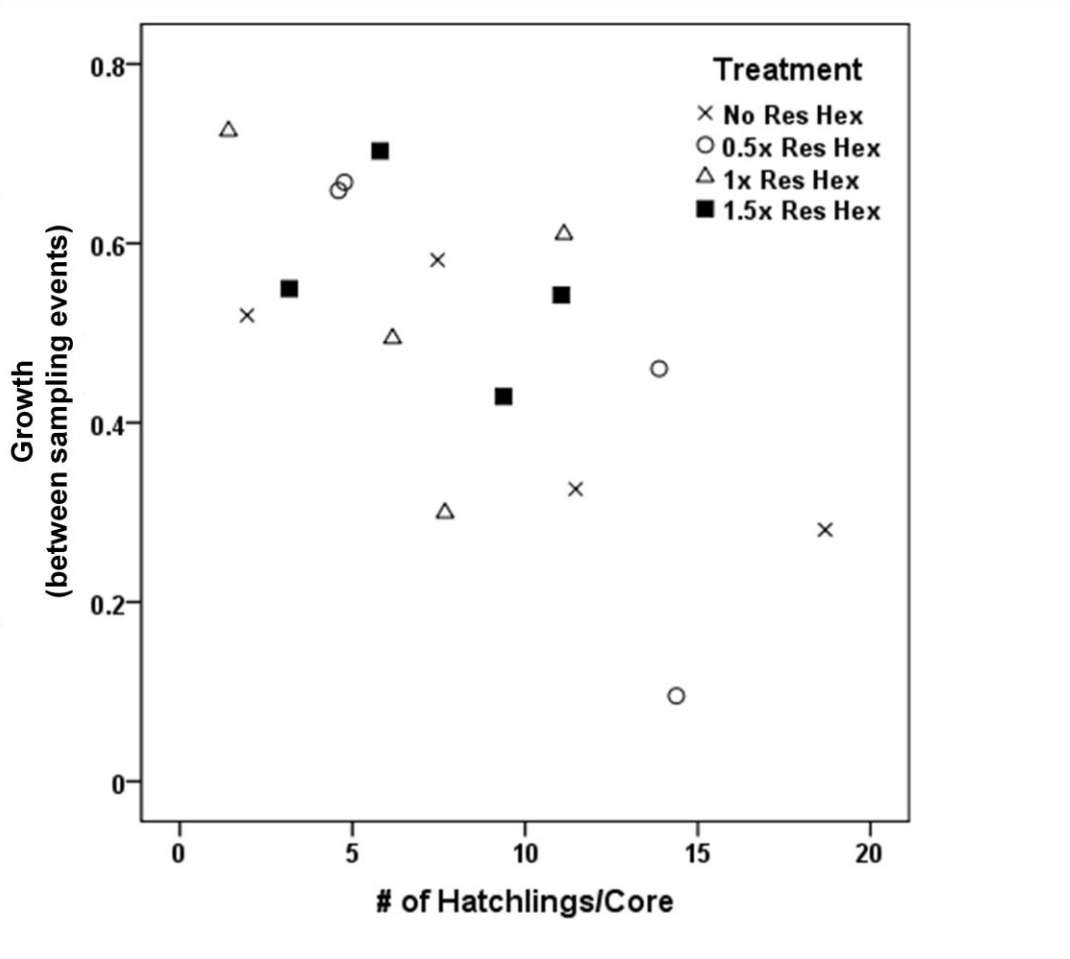

Figure 3.7: Relationship between mean hatchling growth (differences in hatchling length between sampling events) and hatchling density in the four density treatments. 'ND' refers to natural density of resident nymphs.

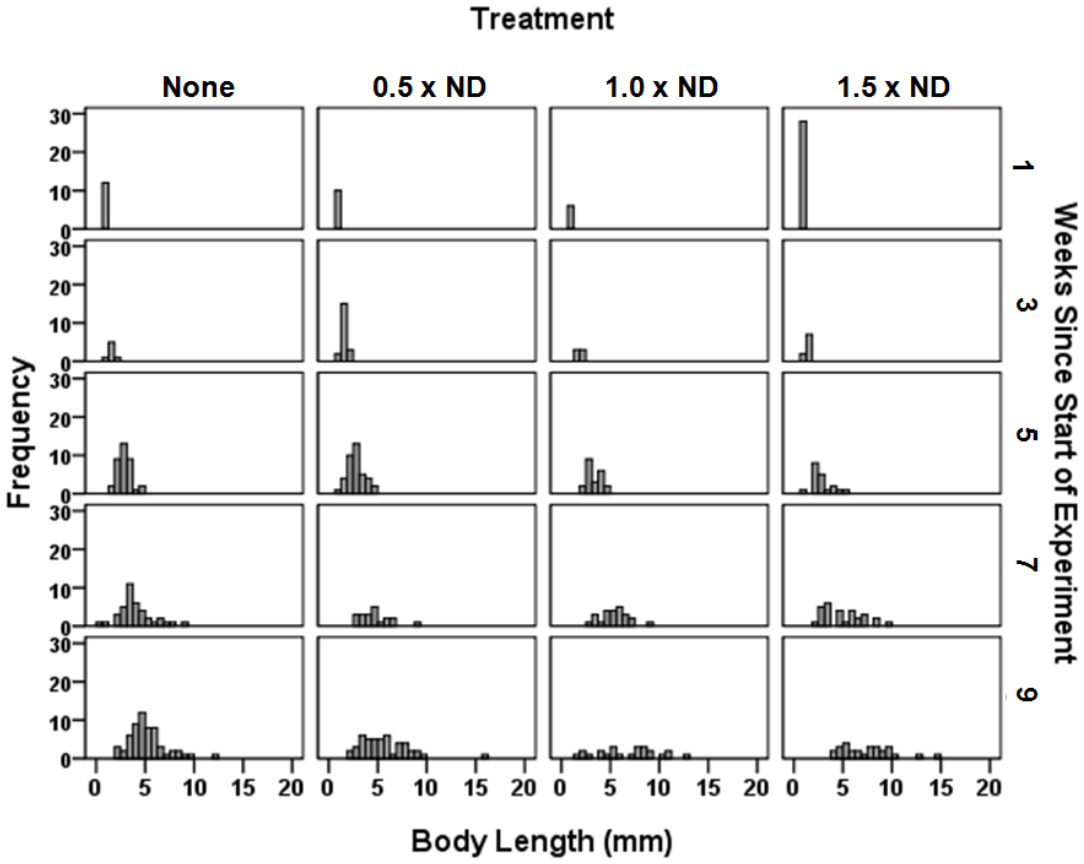

Figure 3.8: Size frequency distributions of hatchlings among treatments at each sampling time. 'ND' refers to natural density of resident nymphs. 'Start of experiment' refers to the moment when eggs were transferred into tanks.

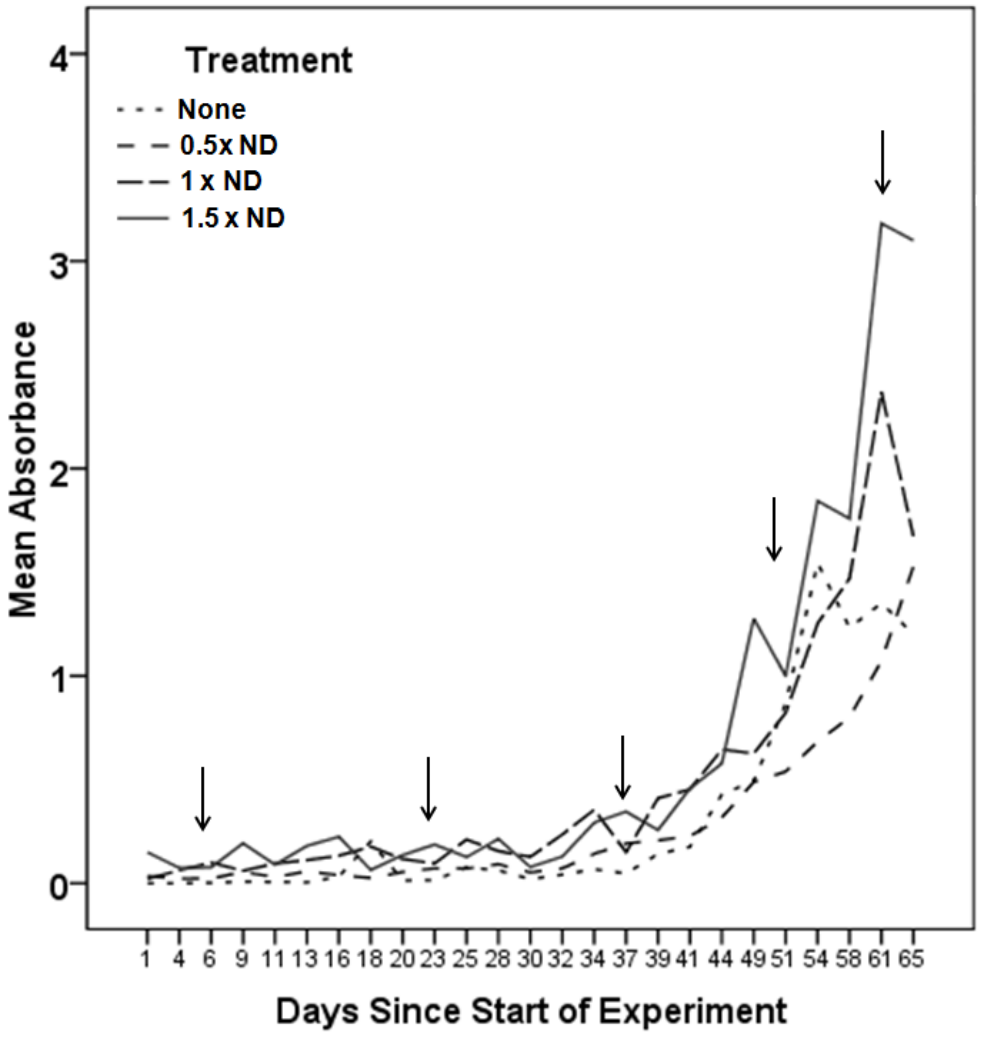

Figure 3.9: Mean concentrations of suspended sediments (measured in light absorbance) among treatments over the duration of the experiment. Arrows indicate days that were selected for statistical analysis. Chosen dates correspond with core sampling events. 'Start of experiment' refers to the moment egg were transferred into tanks.

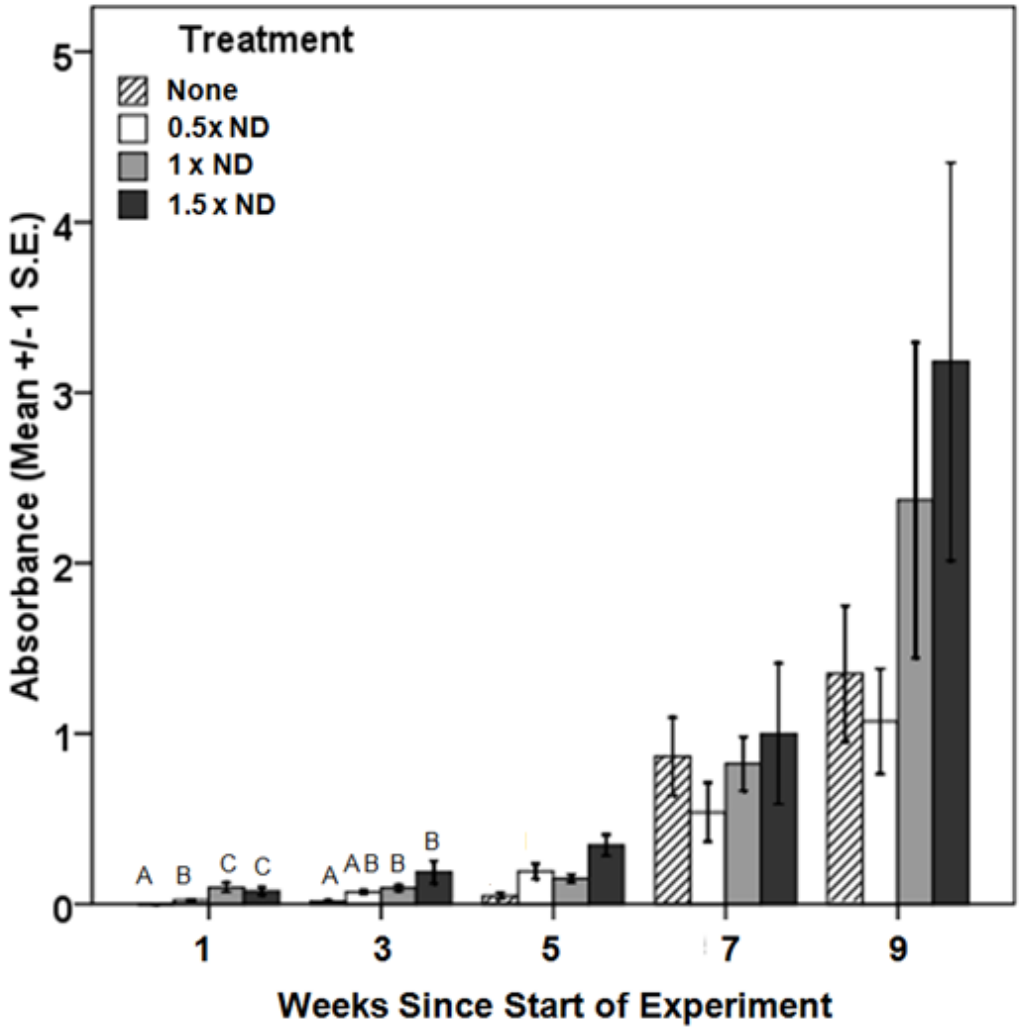

Figure 3.10: Mean (±SE) concentrations of suspended sediments (measured in light absorbance) among treatments on five different days. Dates correspond with core sampling events. 'A', 'B' and 'C' indicate significant differences in treatments at each specific sampling time. 'ND' refers to natural density of resident nymphs and 'start of experiment' refers to the moment egg were transferred into tanks.

CHAPTER 4: GENERAL DISCUSSION

Overview of Results:

Egg transport by benthic organisms has been well documented, especially in zooplankton embryos (Marcus 1984; DeStasio 1989; Marcus et al. 1994; Hairston et al. 1995; Bilton et al. 2001). The burial of eggs in sediments isolates them from oxygenated layers and subsequently prevents development (Kearns et al. 1996; Albertsson and Leonardsson 2000; Viitasalo 2007), while the subsequent return of these eggs to the surface allows development to resume (Marcus and Schmidt-Gengenbach 1986; Kearns et al. 1996). The redistribution of eggs in the sediment can stagger hatching and development of subsequent stages, forming multiple cohorts within populations. The depth at which eggs are buried and can hatch may depend on the density of bioturbators. While increased benthic activity may facilitate egg burial (Gerlofsma 1999; Bachteram et al. 2005), it could also increase the depths to which oxygen can penetrate sediments allowing eggs to hatch at deeper levels (Wang et al. 2001).

I expected that the burial of *Hexagenia* eggs in the sediment would prevent hatching from occurring. I also expected nymphal burrowing to contribute to egg burial within sediments, with more eggs being buried at higher densities of burrowing nymphs. Finally, I expected that the delays in hatching would stagger the development of subsequent stages, therefore causing multiple cohorts to form within populations.

My studies showed that burial of *Hexagenia* eggs in sediments suspends egg development; more eggs hatched on the surface of sediments than at any other depth. Furthermore, hatching was lower in the presence of *Hexagenia* burrowing activity

suggesting that nymphs contribute to the redistribution of eggs throughout sediments. Although oxygen penetration of up to 2 cm below the sediment surface was observed, eggs were found at much deeper depths. Eggs that had been isolated from oxygenated layers would have been prevented from hatching.

Highest concentrations of suspended sediments were observed at higher densities of burrowing nymphs, indicating that an increase in nymph density also increases the volume of sediment being transported in a particular area per unit time. Furthermore, oxygen penetration into sediments increased in the presence of high densities of burrowing nymphs, suggesting that an increase in burrowing activity also increases the amount of oxygen that is circulated throughout sediments.

Highest variation in hatchling size occurred when eggs were buried and when eggs were in the presence of higher densities of burrowing nymphs, suggesting that bioturbation contributes to egg burial, consequently delaying and staggering hatching and forming multiple cohorts within populations.

Hatched Eggs vs. Hatchlings as Indicators of Egg Hatching Patterns:

In this study, differences in hatching trends were observed between hatched eggs and hatchlings. In the second chapter, the proportion of hatched eggs showed high variation over time, whereas the number of hatchlings increased continuously. In the third chapter, there was high variation among both hatched eggs and hatchlings, but the number of hatchlings reflected density-dependent expectations of hatchling size. In other words, treatments that showed higher densities of hatchlings also showed smaller

hatchling sizes, which is consistent with patterns of density-dependent growth patterns observed in these mayflies (Hanes and Ciborowski 1992)

Both proportion of hatched eggs and number of hatchlings, have their advantages and disadvantages as indicators of hatching. Eggs are more brightly stained than hatchlings with lignin pink, allowing them to be more easily distinguished from sediments (Gerlofsma 1999; personal observation). It is possible, however, that eggs could have become damaged in sediments, resulting in them being misclassified. An average of 40% of the eggs retrieved from experiments were classified as damaged (in both chapters 2 and 3). I also suggested that eggs could have been ingested by nymphs during feeding activities. *Hexagenia* are deposit feeders that are generally thought to ingest fine organic matter (Needham et al. 1935; Hunt 1953; Zimmerman and Wissing 1980). *Hexagenia* eggs fall into the range of ingested particle sizes observed in the gut of mayfly nymphs, but only in larger nymphs (> 8mm) (Zimmerman and Wissing 1980). It is highly unlikely that hatchlings would be able to ingest eggs during feeding activities. Furthermore, since there was very little difference in the percentage of hatched eggs found in chapters 2 and 3, it is unlikely that larger conspecifics significantly contributed to egg damage.

The disadvantage of using number of hatchlings as an indicator of hatching is that they tend to aggregate in sediments. Hanes and Ciborowski (1992) suggested that tightly-clustered communities of nymphs could create higher circulation of oxygen in sediments, increasing overall survival. The aggregation of nymphs would increase the likelihood of sampling areas with high differences in nymph density. Observations of burrows after

experiments had concluded, however, showed that burrows were widely distributed throughout tanks.

Because nymphs decompose at a higher rate in sediments than eggs, their mortality would be difficult to measure. Any nymphs that had died during experimental trials were immediately removed and exchanged with nymphs of a similar size from holding tanks. However, high levels of turbidity in many of the tanks only allowed for nymphs floating near the surface of tanks to be replaced.

Due to the large number of damaged eggs found in tanks, lower variation in number of hatchlings, and trends consistent with density-dependent growth, it is more likely that hatchlings are a better indicator of overall hatching than proportion of hatched eggs.

Effect of Egg Burial on Hatching:

In both chapter 2 and 3, I found that a higher percentage of eggs hatched on the surface than at any other depth within the sediment, indicating that burial can prevent or delay embryonic development. This has been demonstrated in other invertebrates such as zooplankton (Marcus 1984; DeStasio 1989; Marcus et al. 1994; Hairston et al. 1995; Bilton et al. 2001), chironomids and gastropods (Kefford et al. 2010), as well as in embryos of many species of fish including rainbow smelt (Wyatt et al. 2010), Atlantic salmon (Greig et al. 2005) and whitefish (Ventling-Schwank and Livingstone 1994). Gerlofsma (1999) found that *Hexagenia* eggs are highly dependent on oxygen for development. Increased exposure to anoxia, as well as exposure at later stages of development, decreases hatching and overall viability. Oxygen penetration into sediments

is commonly restricted to the upper layers of sediment; the oxic layer is usually no more than a couple of millimeters thick (Jorgensen and Revsbech 1985). Thus, the burial of eggs would isolate them from oxygenated layers, consequently preventing development. However, *Hexagenia* continually circulate aerated water throughout their burrows (Needham et al. 1935; Lyman 1943; Eriksen 1963; Edmunds et al. 1976) and studies have shown that nymphs can maintain 75-100% air saturation in their burrows (Wang et al. 2001). Furthermore, oxygen penetrates approximately 2 mm into the sediment surrounding these burrows (Wang et al. 2001).

In my study, oxygen penetration was found no more than 2 cm below the sediment surface, with deepest layers found in higher densities of burrowing (resident) nymphs. Since oxygen concentrations within the oxic layer were not measured, we cannot conclude that eggs successfully hatch at all locations within this layer. Therefore, hatched eggs found at depths below the oxic layer were likely either hatched within the sediment surrounding nymph burrows or were hatched at the surface and subsequently buried through burrowing activity or core sampling. The latter is the more plausible explanation since the data from chapter 2 showed more hatched eggs buried when all eggs were placed on the surface of sediments than when eggs were manually buried in sediments.

Effect of Egg Burial on Hatchling Development:

Size frequency distributions of nymphs in Chapter 2 showed that higher variation in hatchling size occurred when eggs were buried, suggesting that burial of eggs delays and staggers hatching. This is consistent with previous studies on these species. Hunt

(1951) showed that Hexagenia eggs buried 5-6.5 cm under stream silt were delayed in hatching by 2-3 weeks compared to unburied eggs. *Hexagenia* exhibit high size variation in natural conditions and have emergence periods that can last over several weeks (Corkum 2010). Variation in hatching and development could be responsible for the multiple cohorts commonly observed in *Hexagenia* (Heise et al. 1987).

Highest variation in nymph development also occurred at higher densities of resident nymphs (Chapter 3). Bachteram et al. (2005) observed that concentrations of suspended sediments were proportional to nymph density. Highest levels of bioturbation in this study were observed at highest densities of resident nymphs, indicating an increase in burrowing activity. This could have lead to the continual movement of eggs in the sediment, consequently varying hatching times. If hatching did occur at deeper depths, then it was likely delayed (Hunt 1951), therefore introducing smaller individuals into the population and increasing variation in hatchling size.

Hatchling size and growth was lower at higher densities of hatchlings, indicating density-dependent growth. These findings are consistent with previous observations of these species (Hanes and Ciborowski 1992). Variation in hatchling size was higher when eggs were manually buried in sediments and when they were exposed to higher densities of resident (burrowing) nymphs. In these cases, there was a lower density of hatchlings by the end of experiments. Hanes and Ciborowski (1992) showed that at high densities of *Hexagenia* nymphs, variation in size of nymphs increased, a trend that has been observed in a number of other insect species including caddisflies (Hart 1987) and stoneflies (Peckarsky and Cowan 1991). This variation in mayfly nymph size was consistently observed, regardless of food level, indicating the occurrence of interference competition

in nymphs (Hanes and Ciborowski 1992). Since higher variation was observed at lower densities of hatchlings in this study, it is likely that variation occurred as a result of differential hatching times, rather than interference competition.

Effect of Burrowing Activity on Egg Burial:

More eggs hatched at lower densities of burrowing (resident) nymphs (according to number of hatchlings). Since it was shown that egg burial prevents hatching, these results suggest that increased burrowing activity facilitated the burial of eggs in sediments. Egg redistribution in sediments has commonly been observed in the presence of other bioturbating organisms (Marcus and Schmidt-Gengenbach 1986; Kearns et al 1996; Albertsson and Leonardsson 2000; Viitasalo 2007). However, in my study, eggs were found buried at greater depths in all treatments, even in the absence of burrowing nymphs and/or large numbers of hatchlings. It is possible that core sampling contributed to egg burial in sediments, making it difficult to observe how the presence of bioturbating nymphs affects egg distribution. Therefore, we cannot exclusively conclude from this study that nymphs facilitate burial of eggs or that eggs will be distributed to deeper depths in the presence of high densities of nymphs. Trends observed from hatching and nymphal development, however, reflect what we would have expected if this were occurring. If eggs were buried through core sampling, it would have been restricted to sampled areas only. Eggs in the rest of the tank would have maintained their original distributions and would have been responsible for the patterns in hatching and development that I observed.

Recommendations for Similar Experiments:

This study made the assumption that oxygen penetration into the sediment decreases with increasing depth. This assumption was based on previous studies that have shown that anoxia commonly occurs a few mm below the sediment surface (Jorgensen and Revsbech 1985). Oxygenated layers in experimental aquaria (characterized by the lighter color of sediment) were observed up to approximately 2 cm below the sediment surface. Eggs can hatch normally at oxygen concentrations above 6.5 mg/L (Gerlofsma 1999). However, the exact concentrations of oxygen available in these sediments were not determined and therefore, it is unknown at which particular depths eggs are still able to hatch. In future studies, use of oxygen microprobes would provide a better understanding of how burrowing activity affects oxygen penetration into sediments and exactly how far eggs can be buried in sediments before development becomes completely suspended due to anoxia.

Furthermore, as eggs hatch, early-appearing hatchlings will begin to cause bioturbation, affecting egg distribution in sediment. We found that by the end of the experiments, more eggs had hatched in lower-density treatments of resident nymphs than at higher densities. The greater number of hatchlings would increase overall levels of bioturbation in tanks and possibly increase egg movement within sediments. This makes it difficult to assess how certain densities of bioturbating organisms cause egg burial. Some studies have used artificial eggs to study egg transport in sediments. For example, to study the effects of bioturbation by chironomids and tubificids on copepod egg movement in sediments, Kearns et al. (1996) used polystyrene beads, which were the same size and specific gravity as copepod eggs.

In this study, the movement of eggs in sediment, especially in the absence of bioturbating individuals, suggests that core sampling could have contributed to egg burial in tanks. This made it difficult to ascribe patterns of egg burial solely to the actions of bioturbating resident nymphs. Instead of repeatedly sampling a single aquarium, it might be better to set up a series of smaller aquaria each of which could be completely sampled, and each of which could be harvested at different time periods. In other words, instead of setting up five tanks that are sampled repeatedly, one could set up 25 significantly smaller tanks (with removable bottoms) at the same time and process a subset of the aquaria at each of the five sampling events. Since the entire aquarium would be processed, there would be minimal opportunity for transport of eggs due to prior sampling activity.

Finally, high variation occurred in all treatments within this experiment, which could in part be explained by the small sample sizes. In addition to the suggestions made above, it would be beneficial to repeat these experiments or include more replicates to reduce overall variation within the experiment.

Future Studies:

This study focused on the delay or prevention of hatching by nymphal bioturbation. However, in natural conditions, where eggs are likely to become buried by additional means such as storm events or sediment loadings from such sources as tributaries and shoreline erosion, nymphal burrowing activity may actually facilitate hatching. My experiments showed that burrowing nymphs can increase oxygen penetration into the sediment, possibly allowing eggs to hatch at deeper depths. Further

experimentation, in which resident nymphs are added to tanks which contain only buried eggs, may reveal that eggs benefit from the burrowing activities of nymphs.

Significance:

Although, limitations in the sampling method made it difficult to draw definitive conclusions about the effect of burrowing activity on egg transport within the sediment, slowed or reduced incidence of egg hatching and greater variation in hatchling size in the presence of resident burrowing nymphs suggest that resident nymphs contribute to egg burial and consequently delay and stagger hatching. High size variation and extended emergence periods are common in *Hexagenia* (Heise et al. 1987; Schloesser and Hiltunen 1984; Giberson and Rosenberg 1994; Corkum 2010). Variation in hatching and development could be responsible for the multiple cohorts commonly observed in burrowing mayflies.

The suppression of egg hatching by established individuals could be an evolutionary beneficial strategy. Early hatchers can suppress development of possible competitors and consequently have higher survival. Furthermore, in cases where nymph populations are diminished due to anoxic events, previously suppressed eggs can provide a new cohort and reestablish populations. This is the first study to examine bioturbation and ecosystem engineering as a mechanism of intercohort interactions within populations. It contributes to our understanding of how the activities of conspecifics affect life history patterns and population structures as well as the nature of interactions between individuals.

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APPENDIX I: FEEDING PROTOCOL

The following protocol is based on Standard Operating Procedure: *Hexagenia spp.* Culturing (SOP HX1.v2) from the Ontario Ministry of the Environment Laboratory Services Branch, Aquatic Toxicology Unit.

Recipe:

1.5 g cereal grass media* (alfalfa powder may also be used as a substitute)

1.0 g commercial fish food flakes (i.e. Spirulina)**

40 mL dechlorinated water

Ingredients were blended (on high) for 5 minutes in an electric blender.

* Cereal grass media was purchased from Ward's Natural Sciences, St. Catherines, Ontario.

** Spirulina (fish food flakes) was obtained from Tetra Conditioning Food®, available at most pet stores

Storage of Food:

Food was prepared weekly. Any extra food was stored at 4^{o}C (±2) and always used within two weeks. Prepared food was not used if colour had changed to a light brown.

Feeding Regime:

Resident Nymphs

In tanks containing resident nymphs, food was added one day after transfer of nymphs into tanks. Nymphs were fed once a week by pipetting the prepared food just beneath the water surface; airstones were removed for 30 minutes to allow food to settle. Approximately 0.3mL of food was added per resident nymph.

Hatchlings

Once at least 50% of the eggs that had been transferred into tanks had hatched (monitored with eggs placed in Petri Dishes), additional volumes of food were added to tanks on a weekly basis and increased over time according to the following schedule:

After 50% of eggs hatched	Volume/tank
Week 1	2 mL
Week 2	3 mL
Week 3	4 mL
Week 4	5 mL
Week >5	6 mL

In tanks containing resident nymphs, the above volumes of food were added in addition to the food added for resident nymphs. In tanks without resident nymphs, only the volumes of food outlined in the above table were added to tanks.

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