INFLUENCE OF SEDIMENT ACIDIFICATION ON THE BURROWING BEHAVIOUR, POST-SETTLEMENT DISPERSAL, AND RECRUITMENT OF JUVENILE SOFT-SHELL CLAMS (*MYA ARENARIA* L.)

by

Jeff C. Clements

Bachelor of Science (Honours, Biology), Cape Breton University, 2011

Bachelor of Arts (Psychology concentration), Cape Breton University, 2011

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in the Graduate Academic Unit of Biology

Supervisor:	Heather L. Hunt
Examining Board:	Jim Kieffer (Biology GAU) Raouf Kilada (Biology GAU) Keith Dewar (Business GAU)
External examiner:	Christopher Harley (Department of Zoology, UBC)

This dissertation is accepted by the
Dean of Graduate Studies

THE UNIVERSITY OF NEW BRUNSWICK
August, 2016
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"There were times over the years when life was not easy, but if you're working a few hours a day and you've got a good book to read, and you can go outside to the beach and dig for clams, you're okay."

Mary Oliver

Abstract

Ocean acidification (OA) is expected to elicit biological impacts in the near future by impacting the behaviour and physiology of marine animals, potentially leading to decreased fitness and survival. While acidification in the water column has received much attention, sediment acidification has received relatively little, although conditions in sediment porewater can already reach conditions far beyond those of near-future OA projections. This dissertation assesses some impacts of porewater acidification on the burrowing behaviour, post-settlement dispersal, and recruitment of juvenile soft-shell clams (*Mya arenaria*), and attempts to elucidate the biological mechanism for observed burrowing responses. In addition, this thesis attempts to assess the potential impact of sediment acidification on burrowing behaviour in the context of another ocean climate driver – ocean warming. In laboratory and field experiments, burrowing was depressed and post-settlement dispersal was increased by sediment acidification (low sediment pH) comparable to conditions often experienced by clams in the Bay of Fundy. In the lab, increasing temperature appeared to partially alleviate the effect of sediment acidification on burrowing, and $GABA_A$-like neurotransmitter interference under low pH conditions was found to be the mechanism for hindered burrowing responses. In a field study, air temperature and sediment pH were good predictors of juvenile clam abundance, explaining 68% of the variability in clam abundance, although other factors such as rainfall appeared important. Coupled with previous studies, this thesis suggests that bivalve burrowing and recruitment will likely be impacted by near-future OA in areas where sediments will become more acidic as overlying seawater pH drops.

Acknowledgements

First and foremost I want to thank my supervisor, Dr. Heather Hunt, for her ongoing support and dedication to this project; she has been a source of unparalleled intellectual and mental support. I'm grateful to my dissertation committee, Drs. Bruce MacDonald and Chris Gray, for their guidance and support. I want to thank my girlfriend, Jenna MacQuarrie, along with family and friends for their continued support. Thanks to my undergraduate supervisor, Dr. Tim Rawlings (CBU), for his mentorship and guidance, which have been critical in getting me where I am today, and for access to his lab and resources at CBU during various "vacations" to Cape Breton. I'd also like to express my gratitude to the people who have assisted me throughout my PhD project including mentors (Dr. Mark Green, Dr. Rémy Rochette, Dr. Jim Kieffer, Dr. Anton Feicht, Dr. Trevor Hamilton, Dr. Kate Frego, and Dr. George Waldbusser and his lab group at OSU), lab and field assistants (Krystal Woodard, Adam Downie, Melanie Bishop, Kristin Dinning, Brent Wilson, Guðjón Sigurdsson, Betsy Barber, Marie-Joseé Abgrall, Brady Quinn, Kurt Simmons, Kristen Legault, Johan Jaenisch, Andrea Castillejos, Mathieu Desjardins, Jacob Boddy, Dr. Patricia Vázquez and Dr. Marcial Arellano, Erin Miller, Feng Tang, Tim Barton), lab technicians at UNBSJ (MJ Maltais, Don Scott, Alison McAslan, Kelly Cummings-Martell, Samantha Mahon, Kimberly Osepchook, Faith Penney), and others critical in the fruition of this project (George Protopopescu and the team at Downeast Institute, and Kevin Ferris and team at Ferris Chemicals). I also wish to thank NSERC, UNB, and NBIF for providing funding. Finally, I want to thank coffee and beer, without whom I never could have made it even close to this far.

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Chapter 1

General introduction: Marine animal behaviour in a high CO_2 ocean

This chapter (with the exception of section 1.8.4) is published in Marine Ecology Progress Series:

Clements JC, Hunt HL (2015) Marine animal behaviour in a high CO_2 ocean. Mar Ecol Prog Ser 536:259-279

Abstract

Recently, the impacts of ocean acidification (OA) on marine animal behaviour have garnered considerable attention, as they can impact biological interactions and, in turn, ecosystem structure and functioning. Here, I review the literature and synthesize current understanding of how a high CO_2 ocean may impact animal behaviour, elucidate critical unknowns, and provide suggestions for future research by reviewing current published literature surrounding OA and marine behaviour. Although studies have focused equally on vertebrates and invertebrates, vertebrate studies have primarily focused on coral reef fishes, in contrast to the broader diversity of invertebrate taxa studied. A quantitative synthesis of the direction and magnitude of change in behaviours from current conditions under OA scenarios suggests primarily negative impacts that vary depending on species, ecosystem, and behaviour. The interactive effects of co-occurring environmental parameters with CO_2 elicit different effects than those observed under elevated CO_2 alone, though only 12% of the 69 studies assessed have incorporated

multiple factors; only one study has examined the effects of carbonate system variability on the behaviour of a marine animal. Altered $GABA_A$ receptor functioning under elevated CO_2 appears responsible for many behavioural responses; however, this mechanism is unlikely to be universal. I recommend a new focus on determining the behavioural effects of elevated CO_2 in the context of multiple environmental drivers and future carbonate system variability, and gaining a better mechanistic understanding of the association between acid-base regulation and $GABA_A$ receptor functioning. This knowledge could explain observed species-specificity in behavioural responses to OA and lend to a unifying theory of OA effects on marine animal behaviour.

1.1 Introduction

Animal behaviour contributes significantly to understanding the overall welfare and status of a particular species or population (Gonyou 1994, Sih et al. 2004) and can indicate environmental conditions and animal welfare in both laboratory and natural settings (e.g. Mench 1998). Animal behaviour also has potential evolutionary and ecological consequences. For example, predator avoidance by prey and other associated behaviours (e.g. locomotion and learning) can influence prey species survival (e.g. Brodie Jr. et al. 1991). Shifts in feeding behaviour can also change predator survival and persistence (e.g. Persons et al. 2001). Behaviours such as feeding and predator avoidance can change population and community structure, and ultimately ecosystem functioning. For example, environmental contamination can suppress feeding behaviour and prey avoidance by fishes and can change predator-prey interactions, having implications for contaminant transfer through a food web (Weis et al. 2001). Hindered

prey detection could also alter optimal foraging in a wide range of species (e.g. Malmros 2012).

Many factors can influence animal behaviour. Internal physiological and biochemical processes primarily drive some behaviours, while external environmental processes drive others (e.g. Hughes 1988), such as thermoregulation in birds (e.g. Luskick et al. 1978) and predator avoidance in fish (e.g. Gregory 1993). The interactive influence of both internal and external processes, however, influences most behaviours because external environmental conditions can trigger internal sensory processes and lead to a particular behaviour (Mench 1998, Breed & Sanchez 2010). These examples illustrate the importance of knowing the internal and external processes that drive animal behaviour and understanding how changes in such processes influence behaviour.

Climate change has attracted increasing attention as an external physiological and behavioural driver. In marine systems, climate change, predominantly driven by increased anthropogenic CO_2 in the atmosphere, can affect animal behaviour through two primary environmental changes: 1) ocean warming on an increasingly warming planet, and 2) increasing oceanic CO_2 concentrations and a resultant shift in pH to more acidic conditions. Though scientists have long recognized global warming and its potential impact on animal behaviour (e.g. Walther et al. 2001, Walther et al. 2002, Doney et al. 2012), the latter process, known as ocean acidification (OA), has only recently been identified as a global concern. Since the onset of the Industrial Revolution (~1760-1840), increasing atmospheric CO_2 concentrations have raised CO_2 concentrations in the surface of the open ocean and decreased seawater pH by approximately 0.1 units over the subsequent 250 years, with an expected further drop of

0.2-0.3 units by the end of this century (RCP8.5; Hoegh-Guldberg et al. 2014). Furthermore, coastal systems experience an array of acidic sources that the open ocean does not, such as terrestrial and freshwater runoff, coastal upwelling, and changes to watershed dynamics (Duarte et al. 2013), which can increase carbonate system variability and create conditions more acidic than future open ocean projections (e.g. Reum et al. 2014, Wallace et al. 2014). Studies in both open ocean and coastal ecosystems link behavioural impacts in various marine taxa to OA (Munday et al. 2009, Briffa et al. 2012).

Until recently, few studies have addressed potential impacts of OA on marine animal behaviour because most acidification studies have focused primarily on physiology (e.g. Pörtner et al. 2004, Pörtner 2008), calcification (e.g. Ries et al. 2009), and fitness and survivorship (e.g. Kurihara 2008) of larval and juvenile animals. However, increasing evidence shows that OA can influence the behaviour of many marine organisms (Briffa et al. 2012). The recent increase in studies on OA and marine animal behaviour (Figure 1.1) warrants a new review to synthesize current knowledge, highlight knowledge gaps, and elucidate areas of research that deserve particular focus. Here I provide a comprehensive overview of the current scientific literature surrounding behavioural impacts of OA on marine animals. I also present a quantitative synthesis, calculating the direction and magnitude of behavioural changes from current conditions under various OA scenarios, providing a quantitative synthesis of OA effects on marine animal behaviour. Finally, I provide suggestions for future research and highlight key questions requiring attention.

1.2 Literature search and quantitative synthesis

1.2.1 Literature search

I conducted an online search for papers through ISI Web of Science and Google Scholar using the keywords “ocean acidification” or “acidification” or “carbon dioxide” or “CO_2”, combined with “behaviour” or “animal behaviour” within the text of the article. I then limited the search to original research articles that included some measure of behaviour in their analyses, regardless of date published. I then carefully checked the reference list of each paper obtained in the online search to find any papers the online literature search may have missed. Because OA is a hot topic and literature on the subject grows almost daily, additional literature searches using both search methods outlined above were conducted bi-weekly after the initial literature search to ensure that new publications were not missed. The combination of these methods provided extensive coverage of the published literature assessing the effects of OA on marine animal behaviour, yielding a total of 69 relevant papers (Figure 1.1).

Data extracted from each paper included general bibliographical information, species studied, life stage tested, near-future CO_2 partial pressure (pCO_2; pressure exerted by CO_2 in a mixture of other gases) levels employed (pH or saturation state for studies not reporting pCO_2; near-future is defined as conditions projected for 2050 or 2100 as defined by the IPCC – pCO_2 ~ 700-1200, pH ~ 7.8-7.5 – and far beyond refers to conditions substantially exceeding these projections), behaviour(s) or proxy of behaviour(s) measured (i.e., behavioural endpoint(s)), and the absolute % change in a behavioural endpoint from control (see “Quantitative synthesis” below). In comparison

to ambient conditions, I also determined the overall behavioural effect of each OA level, as either positive (a statistically significant increase in a measured behaviour under elevated CO_2), negative (a statistically significant decrease in a measured behaviour under elevated CO_2), no effect (no statistically discernable change in a measured behaviour under elevated CO_2) or a mixed effect (effects in a measured behaviour under elevated CO_2 differing between elevated CO_2 levels). Importantly, I applied these definitions to the overall directionality of effect and they do not reflect the generalized outcome of the behavioural change. For example, I considered increased clownfish activity under elevated CO_2 a positive effect, although increased activity could theoretically decrease survival.

1.2.2 Quantitative synthesis

I manually extracted raw data from applicable publications (defined as peer-reviewed publications comparing a directly-measured behavioural endpoint under elevated CO_2 conditions to ambient conditions) obtained from the literature search. I then calculated the absolute % change in behaviour (defined as the overall % change in behaviour without incorporating directionality of change) from ambient conditions for each OA scenario tested in each publication. The absolute % changes in behavioural endpoints were calculated as:

$$\frac{Elevated\ CO_2\ endpoint - ambient\ CO_2\ endpoint}{Ambient\ endpoint} \times 100\%$$

where “elevated endpoint” is the average measured behaviour under a given OA scenario and “ambient endpoint” is the average measured behaviour under present-day conditions.

To encompass all potential OA scenarios, data include magnitudes for behaviour measured under OA conditions in isolation, in the context of other environmental parameters, in the context of pH variability, and under a variety of experimental time frames (e.g. some studies examined behaviour in the same CO_2 treatments at different time periods; the analysis included all time periods). However, I excluded experimental treatments including a neurotransmitter agonist or antagonist to explore $GABA_A$ receptor functioning as a mechanism for a particular behavioural change under OA conditions. Furthermore, for assessing the impacts of transgenerational acclimation and adaptation, I only compared treatments that reared parents and offspring under the same CO_2 conditions. For example, only OA parent-OA offspring treatments were compared to ambient parent-ambient offspring treatments, given that parents and offspring most likely experience similar CO_2 levels at a given time.

Of course, my approach has limitations. For example, values visually estimated from published figures may include some error. Additionally, positive changes can exceed 100% while negative changes cannot, adding further complication. Finally, the quantitative synthesis included only studies that compared a behavioural endpoint across various acidification groups (e.g. ANOVA-type designs; 20 papers using regressions and/or other non-applicable analyses were excluded) and, as such, does not incorporate every study from the literature review. However, despite these limitations, the data provide valuable information on the overall magnitude of change, which is important in

determining the degree to which future oceanic CO_2 conditions will impact particular organisms.

1.3 Impacts of ocean acidification on marine animal behaviour

1.3.1 General findings

Our review of the literature indicated that OA influences the behaviour of both vertebrates and invertebrates in multiple ways (Table 1.1, 1.2). Experimental conditions in the vast majority of published studies employed ambient (control) and CO_2-enriched (experimental) seawater treatments based on mid- and end-of-century OA projections, with the exception of a few studies employing conditions well beyond near-future projections (Table 1.1, 1.2). In all studies, CO_2 injections was used to alter CO_2 concentrations and pH conditions (i.e., higher CO_2 results in lower pH) without altering alkalinity conditions. Studies typically rear animals in such conditions for a given period of time and then expose them to applicable experiments to measure differences in behaviour between animals from the different rearing conditions. Other studies reared animals in ambient CO_2 water and simply introduced them to experimentally acidified environments (Table 1.1, 1.2). As such, all of the studies assessing the impacts of OA on marine animal behaviour were experimental in nature. Although most employed laboratory experiments, some studies (7 out of 69) employed field experiments by rearing animals in the laboratory under elevated CO_2 conditions and then introducing them to a natural environment, with the exception of Green et al. (2009, 2013), who manipulated sediment pH and aragonite saturation with crushed shell hash in the field and quantified natural clam settlement, and Munday et al. (2014), who collected fishes

from sites naturally mimicking present-day and near-future CO_2 conditions and used them in laboratory-based behavioural experiments (Table 1.1, 1.2). The data synthesis found an average change of 233% for vertebrates and 41% for invertebrates in behavioural endpoints (i.e., the measured behaviour in a given experiment) between present-day conditions and end-of-century projections. The smaller set of studies that tested CO_2 conditions projected for 2050 and 2300 found a mean % change in vertebrate behaviour between present day and mid-century and present day and 2300 of 21% and 4% (likely reflective of low sample size), respectively, in contrast to a mean 84% change in invertebrate behaviour between present day and 2300. However, the direction (positive, negative, no effect) and magnitude (% change in behaviour from ambient conditions) of these impacts varied considerably (Figure 1.2, Table 1.3), with apparently greater impact on vertebrates (Figure 1.2). The following sections synthesize the current literature outlining the influence of OA on the behaviour of vertebrate and invertebrate marine taxa in detail.

1.3.2 Vertebrates

Although studies have assessed teleost and elasmobranch fishes, the vast majority focus on teleost coral reef fishes (Figure 1.3, Table 1.1). Despite the narrow range of vertebrate taxa assessed, studies suggest a wide array of effects of near-future OA on marine fish behaviour (Table 1.3).

Laboratory studies clearly define the effects of near-future CO_2 conditions on the predator-prey interactions of fishes, particularly in coral reefs (Tables 1.1, 1.3). Dixson et al. (2010) first reported that, in contrast to fish raised under ambient CO_2 conditions,

settlement-stage clownfish larvae (*Amphiprion percula*; 11 d post-hatching) raised under elevated CO_2 conditions could not distinguish predator olfactory cues from those of non-predators and spent more time in the presence of other fish cues regardless of species; however, no such behavioural shift occurred under elevated CO_2 in newly hatched larvae. Similarly, Munday et al. (2010) reported negative impacts on predator avoidance responses (time spent in water containing a predator cue) in settlement-stage clownfish (*A. percula*) and damselfish (*Pomacentrus wardi*), and these negative behavioural impacts directly affected field survival of laboratory-reared *P. wardi*. Munday et al. (2014) also reported reduced predator avoidance in two damselfish and two cardinalfish species residing in coral reefs with naturally-elevated CO_2 (CO_2 seeps) in comparison to fishes residing in ambient CO_2 reefs. Attraction to predators (rather than avoidance) under elevated CO_2 was also reported in coral trout (*Plectropomus leopardus*) (Munday et al. 2013) and goldsinny wrasse (*Ctenolabrus rupestris*) (Sundin & Jutfelt 2015). Conversely, OA had no effect on avoidance of seabird predation (as measured by sheltering response) by marine three-spined sticklebacks (G*asterosteus aculeatus*).

Other responses of fish prey to predators may also be hindered under elevated CO_2 conditions (Table 1.1). For example, Ferrari et al. (2012a) reported that juvenile damselfish (*Pomacentrus amboinensis*) did not respond as well to visual predator cues (i.e., seeing the predator) under elevated CO_2 as compared to ambient CO_2 levels. A suite of other damselfishes are also reported to suffer 30-95% decreases in antipredator responses to other predator cues under elevated CO_2 (Ferrari et al. 2011a). While juvenile clownfish (*A. percula*) reared under ambient CO_2 conditions avoided recordings of predator-rich reef conditions, those reared in elevated CO_2 conditions could not, suggesting that OA can diminish auditory response in reef fishes (Simpson et al. 2011).

Rearing under elevated CO_2 hindered escape behaviour (time to locate an exit point and successfully exit an enclosure) of three-spined sticklebacks (*G. aculeatus*) (Jutfelt et al. 2013), and responses (apparent looming, reaction, and escape distances) of damselfish to predatory, ambient-CO_2 dottybacks (effect diminished when predators also raised under elevated CO_2) (Allan et al. 2013).

Cognitive functioning in marine fishes can also be impacted by OA. Chivers et al. (2014) and Ferrari et al. (2012b) both reported that juvenile damselfish (*P. amboinensis*) reared under elevated CO_2 were unable to learn the identity of their predators and consequently suffered reduced behavioural defences. Likewise, Jutfelt et al. (2013) reported diminished learning of escape behaviours in three-spined sticklebacks (*G. aculeatus*) reared under high CO_2. Impaired behavioural lateralization – the preference for moving right or left – has also been reported in coral reef fishes (*Neopomacentrus azysron*: Domenici et al. 2012; *P. wardi*: Domenici et al. 2014) and a temperate fish (*G. aculeatus*: Jutfelt et al. 2013, Lai et al. 2015, Näslund et al. 2015), but juvenile cod (*Gadus morhua*) (Jutfelt & Hedgärde 2015) and wrasse (*C. rupestris*) (Sundin & Jutfelt 2015) appear unaffected, and small-spotted catsharks (*Scyliorhinus canicula*) exhibit increased lateralization (Green & Jutfelt 2014), suggesting differences in OA-induced behavioural responses between taxonomic groups and/or ecosystems. Transgenerational acclimation, in which parental environmental experiences influence offspring's "normal" reaction under similar conditions, is unlikely to alleviate OA effects on behavioural lateralization in coral reef fishes (Welch et al. 2014), but increased temperature may (Domenici et al. 2014).

Reduced antipredator responses can lead to increased predation under elevated CO_2 conditions. For example, dottyback predation on several species of damselfishes has been reported to increase under elevated CO_2 due to hindered escape responses (Ferrari et al. 2011b). However, predatory behaviour can also be negatively impacted by elevated CO_2. Cripps et al. (2011) reported increased activity and reduced attraction to prey odour in elevated CO_2-reared dottybacks (*Pseudochromis fucus*), while Dixson et al. (2014) found decreased attraction to prey odour in predatory dogfish (*Mustelus canis*). Conversely, juvenile wrasse (*C. rupestris*) activity (Sundin & Jutfelt 2015), and epaulette shark (*Hemiscyllium ocellatum*) foraging (Heinrich et al. 2015) appear unaffected by elevated CO_2. Nowicki et al. (2012) reported increased feeding rates of juvenile anemonefish (*Amphiprion melanopus*) under elevated CO_2 and temperature but no significant effects of OA at present-day temperatures. While Ferrari et al. (2011b) reported increased predation rates and reduced prey selectivity in brown dottybacks reared under elevated CO_2, Ferrari et al. (2015) demonstrated that elevated temperatures reversed the effects of increased CO_2 on prey selectivity and amplified predation rates, resulting in abnormally high predation rates.

Various other aspects of fish behaviour can be altered by elevated CO_2. Under elevated CO_2, Jutfelt et al. (2013) reported increased shyness in three-spined sticklebacks (*G. aculeatus*) and Hamilton et al. (2014) reported increased anxiety (i.e., more time spent in dark) in Californian rockfish (*Sebastes diploproa*), the latter resulting from hindered $GABA_A$ receptor functioning. Conversely, four species of damselfishes exhibited increased boldness in the presence of predator cues under elevated CO_2 (Ferrari et al. 2011a). Munday et al. (2014) observed increased boldness but contrasting

effects on activity levels for damselfishes and cardinalfishes exposed to naturally elevated CO_2. Similarly, McCormick et al. (2013) reported increased activity in *P. amboinensis* and decreased activity in *Pomacentrus mollucensis* under elevated CO_2, which reversed the relative aggressiveness and habitat-specific competitive dominance of these two species.

Elevated CO_2 can also affect homing ability and settlement in marine fishes. When raised under elevated CO_2 conditions, adult cardinalfish (*Cheilodipterus quinquelineatus*) could not distinguish between home- and foreign-site odours (Devine et al. 2012a). Similarly, orange clownfish larvae (*A. percula*) reared under elevated CO_2 conditions were more attracted to settlement stimuli that ambient-reared larvae avoided and, unlike ambient-reared larvae, were unable to distinguish their parents from other conspecific adults (Munday et al. 2009). Devine et al. (2012b) reported that the larvae of two damselfishes (*P. amboinensis* and *P. moluccensis*) lost the acute ability to discriminate between the odours of three habitat types (hard-bottom, soft-bottom, or coral rubble) while *P. chrysurus* was unaffected by elevated CO_2; however, within 24 h, all three species were able to settle in their preferred habitat, possibly by using secondary settlement cues (e.g. visual) under OA conditions. Similarly, Devine & Munday (2013) reported that the coral gobies *Paragobiodon xanthosomus* and *Gobiodon histrio* were unable to identify and choose their habitat (the coral *Seriatopora hystrix*) when reared under elevated CO_2.

Near-future OA has been reported to impact fish swimming behaviour, albeit only in a single species. Swimming duration in the small-spotted catshark (*S. canicula*) was longer in elevated CO_2-reared sharks (Green & Jutfelt 2014). In contrast, juvenile and

larval Atlantic cod (*G. morhua*) swimming is highly resilient to OA, as swimming speed, duration, distance, turn angles, and resting time are reportedly unaffected by elevated CO_2 levels far beyond those projected for 2100 (see Table 1; Melzner et al. 2009, Maneja et al. 2013; Jutfelt & Hedgärde 2015).

1.3.3 Invertebrates

Compared to vertebrates, OA effects on invertebrate behaviour have been explored in a wider variety of taxa (Figure 1.3, Table 1.2). Similarly, however, studies suggest a range of negative, positive, and absence of near-future OA effects on marine invertebrate behaviour (Table 1.3).

1.3.3.1 Molluscs

Like coral reef fishes, predator-prey interactions among molluscs are likely to be influenced by near-future OA, although reported effects vary across species. Watson et al. (2014) reported hindered conch snail (*Gibberulus gibberulus gibbosus*) predator avoidance resulting from $GABA_A$ receptor interference, with the number of snails jumping, the number of jumps per snail, and the jumping distance in the presence of a predator reduced under elevated CO_2. In contrast, Quierós et al. (2015) demonstrated that *Nucella lapillus* foraging distance and time increased under more acidic conditions, likely enhancing feeding but potentially increasing susceptibility to predators. While some measures of king scallop (*Pecten maximus*) escape performance (i.e., clapping force) were negatively affected by elevated CO_2, others (i.e., number of claps) were not (Schalkhausser et al. 2012). Manríquez et al. (2014) reported that predator (crab, *Acanthocyclus hassleri*) avoidance by larval and newly-settled muricid snails

(*Concholepas concholepas*) was negatively impacted by elevated CO_2, but the ability of both stages of *C. concholepas* to detect prey (mussels, *Perumitylus purpuratus*) was undiminished. Spady et al. (2014) observed that pygmy squid (*Idiosepius pygmaeus*) switched modes of defense under elevated CO_2, but reported no effects on frequency of defense. Sanford et al. (2014) found that total number of oysters (*Ostrea lurida*) drilled by invasive predatory gastropods (*Urosalpinx cinerea*) increased when oysters were reared under elevated CO_2, regardless of predator CO_2 rearing conditions. Elevated CO_2 has also been reported to induce positive effects on predator avoidance in juvenile *C. concholepas*, as self-righting (ability to reattach foot to substrate following dislodgement) increased under elevated CO_2 (Manríquez et al. 2013). Although conditions were well beyond those of near-future OA projections, Bibby et al. (2007) reported increased predator (*Carcinus maenas*) avoidance behaviour in intertidal gastropods (*Littorina littorea*) under elevated CO_2, which they attributed to reduced physiological defences (shell thickness and oxygen consumption). In contrast, Landes & Zimmer (2012) reported that near-future OA did not alter the *C. maenas* and *L. littorea* predator-prey interaction, although individual physiological effects were observed for each species. Feeding behaviour and efficiency in gastropods (*C. choncolepas*) (Vargas et al. 2013, 2014) and bivalves (*Perumytilus purpuratus*) (Vargas et al. 2013) feeding on plankton has also been reported to decrease under elevated CO_2.

Elevated CO_2 can also influence the movement and activity of marine molluscs. Ellis et al. (2009) found that intertidal gastropod (*Littorina obtusata*) embryos raised under elevated CO_2 spent more time stationary and less time swimming and crawling compared to ambient CO_2-reared larvae. Further, the spinning rate of embryonic snails

was lower and periodization – the average length of time that the embryos spent between periods of movement and non-movement – was greater in elevated CO_2 embryos than those reared in ambient CO_2 (Ellis et al. 2009). Similarly, movement speed in adult *N. lapillus* decreased under elevated CO_2, while foraging time was unaffected, and foraging distance and prey handling time increased; warming negated the OA effects on speed and foraging distance (Queirós et al. 2015). In contrast, activity and movement of pygmy squid (*I. pygmaeus*) increased under both moderate and severe near-future projections of elevated CO_2 (Spady et al. 2014).

In addition to water column acidification, sediment porewater acidification (i.e., acidification of the water residing in the space between grains of sediment below the sediment-water interface) can influence the behaviour of infaunal molluscs. Green et al. (2013) reported that settling hard clams (*Mercenaria mercenaria*) rejected and did not burrow into more acidic sediments, while Green et al. (2009) reported negative effects of more acidified sediment on soft-shell clam (*Mya arenaria*) settlement. Although this study used sediment geochemical conditions beyond near future surface ocean projections, conditions fell within the range currently observed in surface-sediment porewater along the northwest Atlantic coast. While the behavioural responses to sediment acidification have been attributed to lower carbonate saturation state within bottom sediments, the specific biological mechanism(s) underpinning bivalve burrowing and dispersal responses to sediment acidification remain unknown.

1.3.3.2 Arthropods

Marine arthropods are reported to experience a variety of behavioural OA effects. For example, Dissanayake & Ishimatsu (2011) reported decreased swimming speed in prawns (*Metapenaeus joyneri*) reared under pH conditions far beyond those projected for the end of this century (pH 6.8), although elevated temperature attenuated the impacts of low pH. Although stable low pH conditions did not alter the swimming speed or time spent swimming, crawling, conglobating (curling into a ball), or resting of intertidal isopods (*Paradella dianae*) subjected to predator harassment, variable low pH conditions decreased swimming speed, swimming time, and crawling time (Alenius & Munguia 2012).

Arthropod feeding and foraging behaviour in a more acidic ocean have also been studied. Feeding rates of Antarctic krill (*Euphausia superba*) were higher under elevated CO_2 conditions; however, metabolic activity and nutrient excretion also increased, suggesting that negative shifts in physiological processes could result in reduced growth and reproduction despite positive shifts in feeding behaviour (Saba et al. 2012). Conversely, feeding rates (amount of food consumed per unit time) and clearance rates (volume of water cleared of food particles per unit time) of copepods (*Centropages tenuiremis*) declined after 24 h of elevated CO_2 exposure, but increased dramatically after 36 and 90 h (Li & Gao 2012). Appelhans et al. (2012) reported that green crab (*Carcinus maenas*) feeding rates were reduced by a 10 week exposure to pH conditions beyond those expected by the end of this century (pH 7.38), but not by near-future conditions (pH 7.8). de la Haye et al. (2012) also reported that foraging behaviour (time spent in contact with a food cue, time spent moving, and antennular flicking rate) in

hermit crabs (*Pagurus bernhardus*) was reduced under CO_2 conditions beyond those predicted for 2100 (CO_2 ~12000 ppm, pH 6.8).

Predator escape behaviour of marine arthropods under elevated CO_2 has also been assessed. Zittier et al. (2012) reported that the righting response of adult spider crabs (*Hyas araneus*) was unaffected by elevated CO_2 alone, but reduced when both CO_2 and temperature were elevated, suggesting that spider crabs may be more vulnerable to predators in a warm, high CO_2 ocean. In addition, de la Haye et al. (2011) reported that assessment and choice behaviour (time required to change shells, movement time, and antennular flicking) of hermit crabs (*P. bernhardus*) was negatively impacted under elevated CO_2 conditions beyond end-of-century projections.

1.3.3.3 Echinoderms

The impacts of OA on echinoderm behaviour are less studied than molluscs and arthropods. Elevated CO_2 conditions (slightly higher than end-of-century projections) increased sea urchin (*Amblypneustes pallidus*) grazing, although this effect was offset by increased eutrophication (Burnell et al. 2013). Barry et al. (2014) found that foraging time of the deep-sea urchin *Strongylocentrotus fragilis* was increased under elevated CO_2 conditions, but foraging speed was unaffected. Although not directly measured, the feeding performance of larval sand dollars (*Dendraster excentricus*) has been suggested to decline under elevated CO_2, as the stomachs and bodies of sand dollars reared in elevated CO_2 were smaller than ambient CO_2-reared conspecifics (Chan et al. 2011). Appelhans et al. (2012, 2014) also reported that prey consumption in sea stars (*Asterias*

rubens) feeding on bivalves (*Mytilus edulis*) was lower in sea stars exposed to elevated CO_2, although righting response was not impacted.

The effects of OA on swimming performance and settling behaviour of echinoderms have been examined. Chan et al. (2011) reported larval sand dollar (*D. excentricus*) feeding performance was negatively impacted by OA, but elevated CO_2 did not affect the speed, trajectory, or direction of larval swimming. Likewise, Chan et al. (2015) observed that decreased pH had no impact on swimming behaviour of larval green sea urchins (*Strongylocentrotus droebachiensis*). In contrast, Uthicke et al. (2013) reported that settlement success of larval sea stars on crustose coralline algal (CCA) was lower in sea stars exposed to elevated CO_2, but only when the CCA substrate was also exposed to elevated CO_2 for 85 days.

1.3.3.4 Other taxa

Other invertebrates known to experience behavioural changes in response to OA include corals and polychaete worms (Widdicombe & Needham 2007, Albright et al. 2010, Doropoulos et al. 2012, Webster et al. 2013). The settling behaviour, metamorphosis, and recruitment of coral larvae has been reported to decrease under elevated CO_2, a likely consequence of altered interactions between corals and their symbiotic zooxanthellae (Albright et al. 2010, Doropoulos et al. 2012, Webster et al. 2013). In addition, the burrowing activity of *Nereis virens* (Polychaeta) was reported to be unaffected in more acidified sediments (Widdicombe & Needham 2007). Given the severe lack of research addressing the behavioural impacts of elevated CO_2 on these and other understudied taxa, a comprehensive understanding of how OA will impact the

behaviour of these animals (and other unexplored taxa) is not yet possible and more research involving such taxonomic groups is warranted. Although molluscs, arthropods, and echinoderms are among the more diverse and abundant invertebrates, understanding OA effects on behaviours of lesser-studied animals is necessary to fully understand how their populations and associated communities and ecosystems may be impacted by OA.

1.4 The role of GABA

GABA (gamma-aminobutyric acid) is an inhibitory neurotransmitter found in the central and peripheral nervous systems of vertebrates and in the peripheral nervous system of some invertebrates (Jessen et al. 1979), which opens ion channels and promotes the flow of ions in and out of cells (Nilsson et al. 2012). There are two receptors associated with GABA: $GABA_A$ and $GABA_B$. Of these, the $GABA_A$ receptor is of particular concern with OA because of its conductance for chloride (Cl^-) and bicarbonate (HCO_3^-) – the two ions most likely to be impacted by OA. Mechanistically, under ambient CO_2 conditions, extracellular Cl^- and HCO_3^- concentrations are slightly higher than intracellular concentrations, maintaining the equilibrium potential near resting membrane potential (Nilsson et al. 2012). When $GABA_A$ receptors open, Cl^- and HCO_3^- flow into the cell, preventing depolarization, maintaining a negative membrane potential, and reducing neural activity (Nilsson et al. 2012). However, under elevated CO_2, animals excrete Cl^- and accumulate HCO_3^- from the external environment (i.e., seawater) to prevent acidosis (e.g. Heuer & Grosell 2014). This alters the ion gradient across the neural membrane and can potentially result in membrane depolarization, neural pathway excitation, and altered behaviour (Figure 1.4).

Studies have suggested $GABA_A$ receptor disruption as a mechanism by which behaviour is impaired by OA (Nilsson et al. 2012, Hamilton et al. 2014, Watson et al. 2014, Chivers et al. 2014, Lai et al. 2015). Since the $GABA_A$ receptor is particularly vulnerable to OA, studies have focused on elucidating this pathway as a mechanism for behavioural changes under elevated CO_2. For example, treating fishes exposed to elevated CO_2 with gabazine – a $GABA_A$ antagonist – can alleviate negative behavioural effects of OA (Nilsson et al. 2012, Hamilton et al. 2014, Chivers et al. 2014, Lai et al. 2015). Similar results have also been reported for a marine gastropod (*G. gibberulus gibbosus*) (Watson et al. 2014), although more work is needed pertaining to $GABA_A$'s role in invertebrate behaviour under elevated CO_2. Given that GABA is more predominant in vertebrates than invertebrates and that vertebrates heavily rely on GABA for motor and sensory function, the influence of OA on $GABA_A$ may explain why fishes appear more susceptible to the behavioural effects of OA than invertebrates. However, this explanation may not fully explain all behavioural changes associated with OA. Other physiological functions (see Table 1 in Briffa et al. 2012) can act independently or synergistically with $GABA_A$ to alleviate or amplify OA effects on some behaviours (e.g. swimming behaviour or predator escape). For example, although predator escape behaviour in coral reef fishes under elevated CO_2 can be hindered via $GABA_A$ interference, physical changes to structures involved in sensory functions such as otoliths (e.g. Bignami et al. 2013) may act to amplify or negate negative behavioural changes. Such physiological changes in response to OA can be variable among species (e.g. Munday et al. 2011) and warrant more research.

Although thought to be important in marine animal behaviour (both vertebrates and invertebrates), the role of GABA in decision making for many animals, particularly invertebrates, is still poorly understood. It is known that GABA receptors are found in the pedal ganglia of bivalves (e.g. Vitellaro-Zuccarello & De Biasi 1988, Karhunen et al. 1993, Welsh et al. 2014) and GABA has been suggested to increase settlement in some bivalve species (Garcia-Lavandeira et al. 2005, Mesías-Gansbiller et al. 2008). Although it is plausible that $GABA_A$ interference could be responsible for bivalve burrowing responses and is likely to be responsible in other cephalized (nervous tissue concentrated toward one end – head – of the animal) invertebrates, the specific mechanism(s) involved in bivalve burrowing behaviour are not well defined and studies definitively elucidating this mechanism in bivalves and other invertebrate taxa are needed.

Ultimately, the behavioural effects of OA are likely to be driven by a variety of changes (positive or negative) that may act synergistically or independently to alter behaviour. Studies should work toward an understanding of how multiple OA effects act to change animal behaviour and relate such findings to other important ecological endpoints (e.g. survival, ecosystem functioning, biodiversity). Furthermore, a better understanding of the link between acid-base regulation and $GABA_A$ receptor functioning under elevated CO_2 may explain species specific effects and provide a holistic understanding of OA and marine animal behaviour.

1.5 Interactive effects of multiple environmental parameters

Multiple environmental drivers (e.g. temperature, salinity, eutrophication) can act synergistically, antagonistically, or independently of OA to impact various biological

processes (e.g. Denman et al. 2011, Bopp et al. 2013). For example, increasing temperature can attenuate, amplify, or have no impact on the direction and magnitude of biological changes observed under elevated CO_2 alone (Table 1.4). As such, understanding how other environmental drivers may interact with elevated CO_2 is critical to understanding how OA will impact animal behaviour.

Although studies have started to assess how other environmental drivers may interact with OA to yield biological effects, knowledge is limited. With respect to animal behaviour, only 8 studies have assessed OA in the context of other drivers, suggesting contrasting outcomes for different species and behaviours (Table 1.4). With the exception of a single study (Burnell et al. 2014), only temperature-CO_2 interactions have been assessed (Table 1.4), resulting in different effects than those imposed by OA alone. Nowicki et al. (2012) reported that, under elevated CO_2 and temperature, anemonefish (*A. melanopus*) food consumption increased, whereas elevated CO_2 alone had no effect. Conversely, Domenici et al. (2014) reported that negative impacts of OA on behavioural lateralization in *P. wardi* were attenuated under higher temperatures. Ferrari et al. (2015) found higher temperatures amplified negative effects of elevated CO_2 on dottyback (*P. fuscus*) predation rates, but acted antagonistically to attenuate the negative impact of OA on prey selectivity. Similarly to OA alone, elevated temperature and OA had no effect on the predator-prey interaction between *C. maenas* and *L. littorea* (Landes & Zimmer 2012). Increased temperature attenuated the OA effect on prawn (*M. joyneri*) swimming behaviour (Dissanayake & Ishimatsu 2011) and negatively impacted activity capacity in spider crabs (*H. araneus*) (Zittier et al. 2012). Increased temperature also amplified the

OA effect on sea urchin grazing capacity (*A. pallidus*) (Burnell et al. 2013), although this was partially attenuated under eutrophic conditions (Burnell et al. 2013).

It is clear that OA effects on marine animal behaviour will be influenced by co-occurring environmental changes. The complex interactions between multiple environmental drivers highlight the importance of assessing OA in synergy with other factors. Although elevated temperature appears to predominantly alleviate the impacts of elevated CO_2 (Table 1.4), studies should focus on understanding how OA will impact behaviour in association with other co-occurring environmental changes, including hypoxia, salinity, and eutrophication.

1.6 High CO_2 behaviour in the context of environmental variability

When assessing the impacts of OA on marine species, biological responses are typically measured under relatively stable carbonate system conditions mimicking end-of-century projections. Although this may be reasonable for the buffered open ocean, present-day oceanic CO_2 concentrations are known to vary spatially and temporally, particularly in coastal regions (e.g. Duarte et al. 2013, Waldbusser & Salisbury 2014), driven by a variety of biotic and abiotic factors (e.g. Hinga 2002, Blackford & Gilbert 2005, Doney et al. 2007, Dore et al. 2009). However, the ways in which carbonate system variability will respond to a changing climate are still unknown (e.g. Helmuth et al. 2014). Given the highly complex spatial and temporal variability in the marine carbonate system and the uncertainty of how climatic variability will respond to changing conditions, it is imperative that studies assess the behavioural impacts of

carbonate system variability on marine species. Since such variability can modulate an organism's duration of exposure to conditions above, at, or below those that may elicit a biological effect, as well as increase or decrease the frequency and magnitude of extremes that an organism experiences (e.g. Shaw et al. 2013), variability can offset or amplify OA effects on behaviour. For example, Alenius & Munguia (2012) found stable low pH conditions (7.60 ± 0.01 (SE); approx. range 7.5-7.7) had no impact on isopod (*P. dianae*) swimming behaviour and harassment response, but increased variability around the low pH mean (7.60 ± 0.03 (SE); approx. range 7.3-8.0) had a negative impact on both behaviours.

Because OA effects on behaviour have primarily been determined under relatively stable CO_2 conditions, it is unclear how well results will allow us to predict effects in more variable coastal systems. Although the reported results for larval reef fishes are likely accurate since they reside in the well-buffered open ocean, coastal species are likely to experience more variable conditions (Duarte et al. 2013, Waldbusser & Salisbury 2014). Because I cannot yet predict environmental variability in the future, it is difficult to apply such parameters accurately to experimental designs (e.g. Helmuth et al. 2014). As a result, research programs should be developed to predict carbonate system variability under projected future means to accurately determine the behavioural effects of coastal OA, although such a task proves difficult. Moreover, carbonate system variability should be coupled with variability in other environmental drivers. Although difficult and highly complex, such studies would provide much more predictive power for understanding OA effects on behaviour and would advance understanding toward

much needed unifying theory for large scale predications regarding the biological impacts of OA.

1.7 Acclimation and adaptation potential

While most studies assessing OA effects on behaviour employ only one life history stage, some have addressed the potential for transgenerational and temporal acclimation and adaptation to alleviate single-generation effects. While the negative effects of OA on the escape performance of juvenile reef fish were partially alleviated by parental exposure to elevated CO_2 (Allan et al. 2014), transgenerational acclimation and adaptation had no impact on the negative effects of OA on predator avoidance and lateralization in juvenile damselfish (*Acanthochromis polyacanthus*; Welch et al. 2014). Appelhans et al. (2014) reported that juvenile sea star (*A. rubens*) feeding behaviour and righting response did not display acclimation potential over a 6 week period. Similar effects in the laboratory and field for fishes residing in naturally elevated-CO_2 environments also suggest that temporal acclimation and adaptation are insufficient to offset OA effects on reef fish behaviour (Munday et al. 2014).

Populations can also adapt to OA through genetic adaptation, where the offspring of successfully reproducing individuals in an OA-exposed population inherit successful traits from parents to tolerate elevated CO_2 (Shaw & Etterson 2012). Although genetic adaptation has been tested for physiological endpoints (e.g. Schlegel et al. 2012, Schlegel et al. 2015), the role of genetic heritability in alleviating OA effects on behaviour remains untested. However, approaches to such experiments have been proposed (Sunday et al. 2014) and provide a template for expanding OA-behaviour

research into this realm. Ultimately, at present, acclimation and adaptation do not appear sufficient in reducing OA effects on behaviour, but more research is certainly warranted.

1.8 Generalizations for future research and dissertation objectives

1.8.1 General conclusions

Near-future OA is likely to impact marine animal behaviour in a myriad of ways. While invertebrates appear more vulnerable to OA physiologically, fishes appear to be more affected behaviourally, with the direction and magnitude of OA effects likely to vary across species, ecosystems, and behaviours. Not all studies have used realistic OA scenarios and behavioural responses appear unpredictable beyond pCO_2 conditions of ~1000 µatm; however, such studies should not be considered in the context of near-future OA. Furthermore, behavioural changes do not always result in negative outcomes, but can elicit positive and/or negative impacts. For example, the overall outcome of bivalves rejecting more acidic sediments can positively reduce "death by dissolution" (Green et al. 2009, 2013), but increase vulnerability to other mortality factors (Hunt & Scheibling 1997). As such, more research exploring an array of species, systems, and behaviours is necessary to understand how OA affects behaviour and how this translates to overall outcomes. The interactive effects of multiple environmental drivers and their associated variability require immediate attention, along with the potential of transgenerational acclimation and adaptation, and a mechanistic understanding of the link between acid-base regulation and $GABA_A$ receptor functioning under elevated CO_2. Detailed suggestions for future OA-behaviour research for vertebrates and invertebrates are highlighted below.

1.8.2 Vertebrates

Predator-prey interactions, homing ability, choice and discriminatory behaviour, auditory response, learning, foraging, exploratory behaviour, and behavioural lateralization have all been reported to be affected by OA in marine fishes, while other behaviours such as swimming behaviour appear relatively unaffected. Although the impacts of OA on marine fishes are well documented, studies have predominantly focused on coral reef fishes. Given the specialized nature of coral reef ecosystems and the high degree of biodiversity in comparison to most other systems, it is important to expand OA-fish behaviour studies to more taxa residing outside of coral reefs. Furthermore, among the studies that have been conducted, contrasting results for fish species within coral reef systems suggest that OA effects on fish behaviour are species specific. It is thus necessary to better understand the mechanistic association between acid-base regulation and $GABA_A$ receptor functioning, as this could reconcile species specific effects and lead to an overarching theory of how OA affects behaviour. Although co-occurring environmental drivers (e.g. temperature, salinity, oxygen, eutrophication) will interact with OA to alleviate or amplify the effects of elevated CO_2 on marine fish behaviour, studies incorporating multiple drivers are limited (Table 1.2). Furthermore, environmental variability is neglected in OA-fish behaviour studies. As such, research is needed to determine how changes in multiple environmental drivers will interact with OA to impact marine fish behaviour and how variability associated with these drivers will influence behaviour in coastal fish species. Finally, although some studies have marked and observed laboratory-reared fish in the wild (e.g. Ferrari et al. 2011a, Devine et al. 2012a), most studies rely on laboratory experiments. More field

studies of fish behaviour in areas of naturally elevated CO_2 should be conducted to broaden the current understanding of how OA will or potentially already has impacted the behaviour of marine fishes.

1.8.3 Invertebrates

Although OA will impact the behaviour of marine invertebrates, the impacts will likely be variable across species, ecosystems, and behaviours. For example, the direction and magnitude of OA effects on predator-prey relationships will depend on the dynamics of the system and species involved, with different behaviours being impacted in different ways. OA effects on invertebrate behaviour may differ across developmental stages for an individual species as well. Given the contrasting results within and between species and systems, coupled with the lack of OA-induced behavioural research for some groups of organisms (e.g. corals, polychaetes, and a myriad of other invertebrate taxa), research employing different systems and taxonomic groups is warranted. Although $GABA_A$ receptor interference seems to be a widely applicable mechanism for OA impacts on behaviour, a mechanistic understanding of OA effects on invertebrate behaviour is rudimentary and requires more research. As with fishes, understanding of the link between acid-base regulation and $GABA_A$ receptor functioning could account for observed species-specificity and help to develop a unifying theory of OA effects on invertebrate behaviour.

1.8.4 Dissertation objectives

The broad intent of my dissertation is to build on the work pertaining to the impacts of OA on bivalve burrowing behaviour by exploring the effects of sediment acidification

on juvenile soft-shell clams (*Mya arenaria*). It is well known that increases in anthropogenic CO_2 will increase oceanic CO_2 concentrations and reduce surface ocean pH in a fairly predictable fashion over the coming centuries. However, primarily due to the bacterial decomposition of organic matter, surface sediment porewater already experiences pH conditions far beyond future ocean acidification projections, often experiencing truly acidic conditions (pH < 7.0), with such conditions potentially becoming worse under future ocean conditions (e.g. Widdicombe et al. 2009). While studies suggest that settlement-stage bivalves can be impacted by sediment acidification (Green et al. 2013), data pertaining to other life history stages of these animals are lacking. As such, Chapters 2 and 3 of my dissertation use laboratory and field approaches, respectively, to determine if sediment acidification can influence the burrowing behaviour of juvenile (0.5-3 mm) *M. arenaria*. Furthermore, Chapters 2 and 3 also test whether or not sediment acidification can influence the post-settlement dispersal (i.e., movement via water currents from one patch of sediment to another) of juvenile *M. arenaria*. Chapter 3 introduces a novel method for stabilizing sediment pH over a 24-48 h period, allowing short-term field experiments. Together Chapters 2 and 3 examine whether or not this life history stage of marine infaunal bivalves can be impacted by sediment acidification. Next, in Chapter 4, I experimentally test whether or not $GABA_A$- like neurotransmitter interference serves as the physiological mechanism by which juvenile *M. arenaria* burrowing behaviour is impeded under more acidic sediments, and also test whether or not seawater temperature can influence the burrowing response of juvenile clams to low-pH sediments. Finally, in Chapter 5, I present the results of a field study to determine whether or not sediment acidification

and other environmental variables can influence juvenile *M. arenaria* abundance (i.e., clam recruitment) along the northern shore of the Bay of Fundy.

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Table 1.1. Summary of the impacts of elevated CO_2 on marine fish behaviour. Studies are organized chronologically within Teleostei and Elasmobranchii. Effects reported are for treatments looking at the impacts of CO_2 in isolation and are reported as no effect (=), negative (–), or positive (+). Asterisks indicate studies that employed field experiments. Bolded references indicate studies incorporating multiple environmental factors (see Table 1.4 for alternative effects).

Reference	**Species**	**Life stage**	**Behaviour**	***p*CO_2**	**Effect**
Teleostei					
Melzner et al. (2009)	*Gadus morhua*	Juvenile	Swimming	5792 ppm	=
				3080 ppm	=
Munday et al. (2009)	*Amphiprion percula*	Larvae	Olfactory discrimination	1050 ppm	–
				1710 ppm	–
			Homing	1050 ppm	–
				1710 ppm	–
Dixson et al. (2010)	*Amphiprion percula*	Hatched	Predator detection	1000 ppm	=
		Settling	Predator detection	1000 ppm	–

Munday et al. (2010)*	*Amphiprion percula*	Settling	Predator response	550 ppm	=
				700 ppm	–
				850 ppm	–
	Pomacentrus wardi	Settling	Predator response	550 ppm	=
				700 ppm	–
				850 ppm	–
Cripps et al. (2011)	*Pseudochromis fuscus*	Adult	Olfactory preference	600 µatm	–
				950 µatm	–
			Activity	600 µatm	=
				950 µatm	+
			Feeding	600 µatm	–
				950 µatm	=
Ferrari et al. (2011a)*	*Pomacentrus chrysurus*	Juvenile	Predator response	700 ppm	–
				850 ppm	–
	P. moluccensis	Juvenile	Predator response	700 ppm	–
				850 ppm	–

	P. amboiensis	Juvenile	Predator response	700 ppm	–
				850 ppm	–
	P. nagasakiensis	Juvenile	Predator response	700 ppm	–
				850 ppm	–
Ferrari et al. (2011b)	*Pseudochromis fuscus*	Adult	Predation rate (small prey)	700 µatm	=
			Predation rate (large prey)	700 µatm	=
			Prey selectivity (small prey)	700 µatm	=
			Prey selectivity (large prey)	700 µatm	–
Simpson et al. (2011)	*Amphiprion percula*	Juvenile	Auditory pred. avoidance	600 µatm	–
				700 µatm	–
				900 µatm	–
Devine et al. (2012a)*	*Cheliodipterus quinquelineatus*	Adult	Homing ability	550 ppm	–
				700 ppm	–
				950 ppm	–

Devine et al. (2012b)	*Pomacentrus chrysurus*	Settling	Habitat preference	700 ppm	=
				850 ppm	–
			Settlement rate	700 ppm	=
				850 ppm	=
	P. moluccensis	Settling	Habitat preference	700 ppm	–
				850 ppm	–
			Settlement rate	700 ppm	=
				850 ppm	–
	P. amboiensis	Settling	Habitat preference	700 ppm	=
				850 ppm	=
			Settlement rate	700 ppm	–
				850 ppm	=
Ferrari et al. (2012a)	*Pomacentrus amboiensis*	Juvenile	Visual risk assessment	550 µatm	=
				700 µatm	=
				850 µatm	–
Ferrari et al. (2012b)	*Pomacentrus amboiensis*	Pre-settling	Predator response	850 µatm	–
			Learning	700 µatm	–

Domenici et al. (2012)	*Neopomacentrus azysron*	Settling	Lateralization	880 µatm	–
Nilsson et al.(2012)	*Amphiprion percula*	Larvae	Olfactory preference	900 µatm	–
	Neopomacentrus azysron	Settling	Lateralization	900 µatm	–
Nowicki et al. (2012)	*Amphipriom melanopus*	Juvenile	Food consumption	530 µatm	=
				960 µatm	=
			Activity	530 µatm	=
				960 µatm	=
Allan et al. (2013)	*Pseudochromis fuscus*	Adult	Predation success	880 µatm	=
			Predation rate	880 µatm	=
			Attack distance	880 µatm	=
	Pomacentrus amboiensis	Juvenile	Reaction distance	880 µatm	=
			Looming threshold	880 µatm	=
			Escape distance	880 µatm	–
Devine et al. (2013)*	*Paragobiodon xanthosomus*	Adult	Habitat preference	880 µatm	–

	Gobiodon histrio	Adult	Habitat preference	880 µatm	–
Jutfelt et al. (2013)	*Gasterosteus aculeatus*	Adult	Boldness	990 µatm	–
			Exploratory	990 µatm	–
			Lateralization	990 µatm	–
			Learning	990 µatm	–
Hamilton et al. (2014)	*Sebastes diploroa*	Juvenile	L/D preference (anxiety)	1125 µatm	–
Maneja et al. (2013)	*Gadus morhua*	Larvae	Swimming	1400 µatm	=
				4200 µatm	=
McCormick et al. (2013)	*P. moluccensis*	Settling	Activity	945 µatm	=
			Aggressiveness	945 µatm	+
	P. amboiensis	Settling	Activity	945 µatm	+
			Aggressiveness	945 µatm	–
Munday et al. (2013)	*Plectopomus leopardus*	Juvenile	Activity	570 µatm	=
				700 µatm	–
				960 µatm	–

Allan et al. (2014)	*Amphiprion melanopus*	Juvenile	Predator avoidance (hiding)	570 µatm	=
				700 µatm	–
Chivers et al. (2014)	*Pomacentrus amboiensis*	Juvenile	Predator escape	960 µatm	–
			Response to predators	1087 µatm	=
				987 µatm	–
Domenici et al. (2014)	*Pomacentrus wardi*	Juvenile	Lateralization	921 µatm	–
Ferrari et al. (2014)	*Pseudochromis fuscus*	Adult	Predation rate	995 µatm	=
			Prey selectivity	995 µatm	–
Munday et al. (2014)	*Dascyllus aruanus*	Juvenile	Olfactory discrimination	441-998 µatm	–
			Predator avoidance	441-998 µatm	–
			Activity	441-998 µatm	–
			Boldness	441-998 µatm	+

Pomacentrus moluccensis	Juvenile	Olfactory discrimination	441-998 µatm	–
		Predator avoidance	441-998 µatm	–
		Activity	441-998 µatm	–
		Boldness	441-998 µatm	+
Apogon cyanosoma	Juvenile	Olfactory discrimination	441-998 µatm	–
		Predator avoidance	441-998 µatm	–
		Activity	441-998 µatm	+
		Boldness	441-998 µatm	+
Cheliodipterus quinquelineatus	Juvenile	Olfactory discrimination	441-998 µatm	–
		Predator avoidance	441-998 µatm	–
		Activity	441-998 µatm	+

			Boldness	441-998 µatm	+
Welch et al. (2014)	*Acanthochromis polyacanthus*	Juvenile	Predator avoidance	656 µatm	–
				912 µatm	–
			Lateralization	656 µatm	=
				912 µatm	–
			Olfactory prey tracking	741 µatm	=
				1064 µatm	–
Jutfelt & Hedgärde (2015)	*Gadus morhua*	Juvenile	Boldness	1000 µatm	=
			Lateralization	1000 µatm	=
Lai et al. (2015)	*Gasterosteus aculeatus*	Adult	Lateralization	992 µatm	–
Näslund et al. (2015)	*Gasterosteus aculeatus*	Adult	Predator avoidance	1000 µatm	=
			Lateralization	1000 µatm	–

Sundin & Jutfelt (2015)	*Ctenolabrus rupestris*	Juvenile	Predator avoidance	995 µatm	–
			Lateralization	995 µatm	=
			Activity	995 µatm	=
Elasmobranchii					
Dixson et al. (2014)	*Mustelus canis*	Adult	Activity	741 µatm	=
				1064 µatm	=
			Attacking prey	741 µatm	–
				1064 µatm	–
Green & Jutfelt (2014)	*Scyliorhinus canicula*	Adult	Swimming	900 µatm	–
			Lateralization	900 µatm	–
Heinrich et al. (2015)	*Hemiscyllium ocellatum*	Adult	Foraging behaviour	615 µatm	=
				910 µatm	=

Table 1.2. Summary of the impacts of elevated CO_2 on marine invertebrate behaviour. Studies are organized chronologically within their respective phylum. Effects reported are for treatments looking at the impacts of CO_2 in isolation and are reported as no effect (=), negative (–), or positive (+). Asterisks indicate studies that employed field experiments. Bolded references indicate studies incorporating multiple environmental factors (see Table 1.4 for alternative effects). NR = pCO_2 not reported.

Reference	**Species**	**Life stage**	**Behaviour**	***p*CO$_2$**	**Effect**
Cnidaria					
Albright et al. (2010)	*Acropora palmata*	Larvae	Settlement	~650 µatm	–
				~880 µatm	–
Doropoulos et al. (2012)	*Acropora millepora*	Larvae	Settlement	807 µatm	–
				1299 µatm	–
Annelida					
Widdicombe & Needham (2007)	*Nereis virens*	Adult	Burrowing	NR	
				pH: 7.3	=
				pH: 6.5	=
				pH: 5.6	=

Mollusca

Bibby et al. (2007)	*Littorina littorea*	Adult	Predator avoidance	NR	
				pH: 6.63	+
Ellis et al. (2009)	*Littorina obtusata*	Embryonic	Spinning time	1093 ppm	
			Spinning rate	1093 ppm	=
			Crawling	1093 ppm	–
			Periodization	1093 ppm	–
Green et al. (2009)*	*Mya arenaria*	Settling	Settlement	NR	
				pH: 7.32	–
Schalkhausser et al. (2012)	*Pecten maximus*	Adult	Clapping amount	1135 µatm	=
			Clapping force	1135 µatm	–
Green et al. (2013)*	*Mercenaria mercenaria*	Plantigrade	Burrowing	NR	
				Ω: 0.68	–
				Ω: 0.05-1.05	–

Manriquez et al. (2013)	*Concholepas concholepas*	Juvenile	Self-righting	716 µatm	+
				1036 µatm	+
Vargas et al. (2013)	*Concholepas concholepas*	Larvae	Feeding	700 ppm	–
				1000 ppm	–
			Food selectivity	700 ppm	–
				1000 ppm	–
Watson et al. (2013)	*Gibberulus gibberulus gibbosus*	Adult (jumping)	Predator escape	961 µatm	–
		Adult (non-jumping)	Predator escape	961 µatm	+
Clements & Hunt (2014)	*Mya arenaria*	Juvenile	Burrowing	NR	
				Ω: 0.05-1.05	–
			Dispersal	NR	
				Ω: 0.05-1.05	+

Manriquez et al. (2014)	*Concholepas concholepas*	Larvae	Prey detection	700 µatm	=
				1000 µatm	=
			Predator response	700 µatm	=
				1000 µatm	–
		Juveniles	Prey detection	700 µatm	=
				1000 µatm	=
			Predator response	700 µatm	=
				1000 µatm	–
Sanford et al. (2014)	*Urosalpinx cinerea*	Juvenile	Drilling predation	1000 µatm	=
Spady et al. (2014)	*Idiosepius pygmaeus*	N/A	Activity	626 µatm	+
				956 µatm	+
Vargas et al. (2014)	*Concholepas concholepas*	Larvae	Feeding rate	700 µatm	–
				1000 µatm	–
	Perumytilus	Juvenile	Feeding	700 µatm	–

	purpuratus				
				1000 µatm	–
Queirós et al. (2015)	*Nucella lapillus*	Adults	Activity (speed)	750 ppm	–
				1000ppm	–
			Foraging time	750 ppm	=
				1000ppm	=
			Foraging distance	750 ppm	=
				1000ppm	–
			Prey handling time	750 ppm	=
				1000ppm	–
Arthropoda					
de la Haye et al. (2011)	*Pagurus bernhardous*	Adult	Shell detection	12191 µatm	=
			Shell selection	12191 µatm	–
			Antennular flicking	12191 µatm	–

			Movement	12191 µatm	–
Dissanayake & Ishimatsu (2011)	*Metapenaeus joyneri*	Adult	Swimming	11054 µatm	–
Alenius & Munguia (2012)	*Paradella dianae*	Adult	Activity	NR	
				pH 7.60 stable	=
				pH 7.60 variable	–
Appelhans et al. (2012)	*Carcinus maenas*	Adult	Feeding	1120 µatm	=
				4000 µatm	–
de la Haye et al. (2012)	*Pagurus bernhardus*	Adult	Foraging	12061 µatm	–
			Movement	12061 µatm	–
			Antennular flicking	12061 µatm	–
Li & Gao (2012)	*Centropages tenuiremis*	Planktonic	Sensitivity	1000 µatm	=
				>1700 µatm	–
			Feeding rates	1000 µatm	+

Landes & Zimmer (2012)	*Carcinus maenas*	Small adult	Prey handling time	377-539 µatm	=
			Prey selectivity	377-539 µatm	=
		Large adult	Prey handling time	377-539 µatm	–
			Prey selectivity	377-539 µatm	=
Saba et al. (2012)	*Euphausia superba*	Adult	Feeding rate	672 ppm	–
Zittier et al. (2012)	*Hyas araneus*	Adult	Self-righting	750 µatm	+
				1120 µatm	+
				3000 µatm	+
Echinodermata					
Chan et al. (2011)	*Dendraster excentricus*	Larvae	Swimming	1000 ppm	=
Appelhans et al. (2012)	*Asterias rubens*	Adult	Feeding	1250 µatm	=
				3500 µatm	–

Burnell et al. (2013)	*Amblypneustes pallidus*	Juvenile	Grazing	640 µatm	+
Uthicke et al. (2013)	*Acanthaster planci*	Larvae	Settlement	877 µatm	–
Appelhans et al. (2014)	*Asterias rubens*	Juvenile	Feeding	1120 µatm	–
				4000 µatm	–
			Self-righting	1120 µatm	=
				4000 µatm	=
Barry et al. (2014)*	*Strongylocentrotus fragilis*	Adult	Movement	3255 ppm	=
			Foraging	3255 ppm	–
Chan et al. (2015)	*Strongylocentrotus droebachiensis*	Larvae	Swimming	NR	
				pH: 7.3	=
				pH: 6.5	=

Table 1.3. The relative number of behaviours (as displayed in Tables 1.1 and 1.2; each label under the column "behaviour" was treated as a single data point) exhibiting a positive effect, negative effect, no effect, or mixed effect (any combination of the previous 3; dependent upon different CO_2 levels tested) to ocean acidification for vertebrates and invertebrates.

	Positive	Negative	No effect	Mixed	Total
Vertebrates					
Teleosti	8	47	23	13	91
Reef fishes	8	42	21	13	84
Other	0	5	2	0	7
Elasmobranchii	0	3	2	0	5
TOTAL	**8**	**49**	**26**	**13**	**96**
Invertebrates					
Mollusca	5	13	6	4	28
Arthropoda	2	9	5	2	18
Echinodermata	1	3	4	1	9
Porifera	0	3	0	0	3
Polychaeta	0	0	1	0	1
TOTAL	**8**	**28**	**16**	**7**	**59**

Table 1.4. Summary of studies assessing the impacts of OA on marine animal behaviour in the context of co-occurring environmental parameters. Impacts are classified as positive (+), negative (–), or no effect (=)

Species	Additional factor(s)		Observed effect		Reference
			OA only	OA + additional factor(s)	
FISHES					
Amphiprion melanopus	Elevated temp (31.5°C)	Foraging	=	Synergistic + effect of elevated temp & pCO_2; – effect of elevated temp at ambient & moderate pCO_2	Nowicki et al. (2012)
Pomacentrus wardi	Elevated temp (31°C)	Behavioural lateralization	–	Increased temp attenuated elevated pCO_2 effect	Domenici et al. (2014)
Pomacentrus amboinensis and *P. nagasakiensis*	Elevated temp (31°C)	Predation	=	Synergistic + effect of elevated temp & pCO_2	Ferrari et al. (2015)

		Prey selectivity	=	Synergistic – effect of elevated temp & pCO_2	
INVERTS					
Metapenaeus joyneri	Elevated temp (25°C)	Swimming	–	Increased temp attenuated elevated pCO_2 effect	Dissanayake & Ishimatsu (2011)*
Carcinus maenas	Elevated temp (8-18°C)	Prey handling time	=	No effect	Landes & Zimmer (2012)
Hyas araneus	Elevated temp (4-12°C)	Activity capacity	=	Synergistic – effect of elevated temp & pCO_2	Zittier et al. (2012)
Amblypneustes pollidus	Elevated temp (20°C) and eutrophication	Grazing	+	Synergistic + effect of elevated temp & pCO_2; eutroph. partially attenuated synergistic effect	Burnell et al. (2014)

Nucella lapillus	Elevated temp (+2°C)	Activity	–	Increased tempattenuated elevated pCO_2 effect	Queirós et al. (2015)
		Foraging	–	Increased temp attenuated elevated pCO_2 effect	

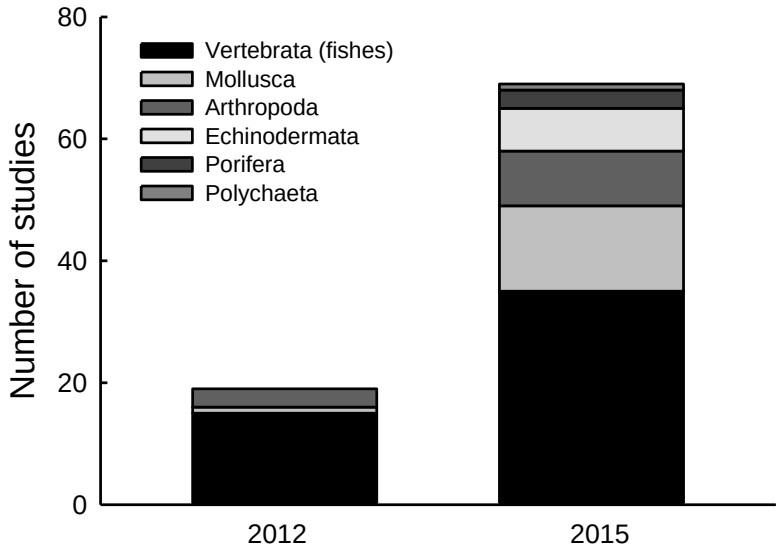

Figure 1.1. The total number of studies assessing the impacts of near-future ocean acidification on marine animal behaviour reported in Briffa et al. (2012) (n = 19; only included fishes, molluscs, and arthropods) and in this review (n = 69).

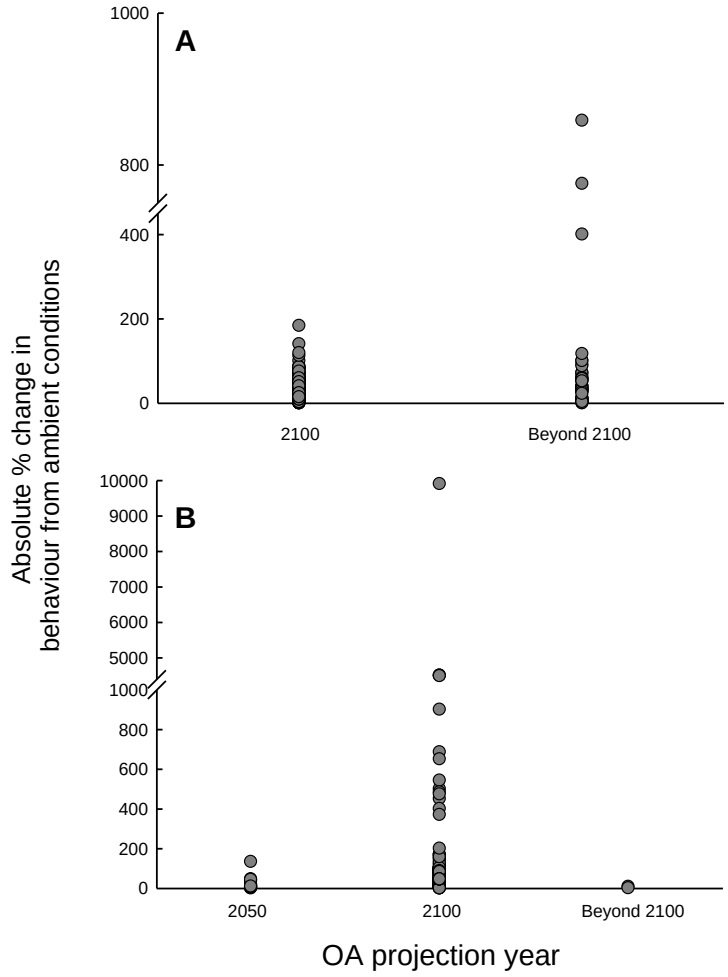

Figure 1.2. Scatterplot depicting reported impact magnitudes (absolute % change in all endpoints from ambient conditions) of near-future OA on marine invertebrate (A) and vertebrate (B) behaviour for mid-century, end-century, and beyond end-century OA scenarios.

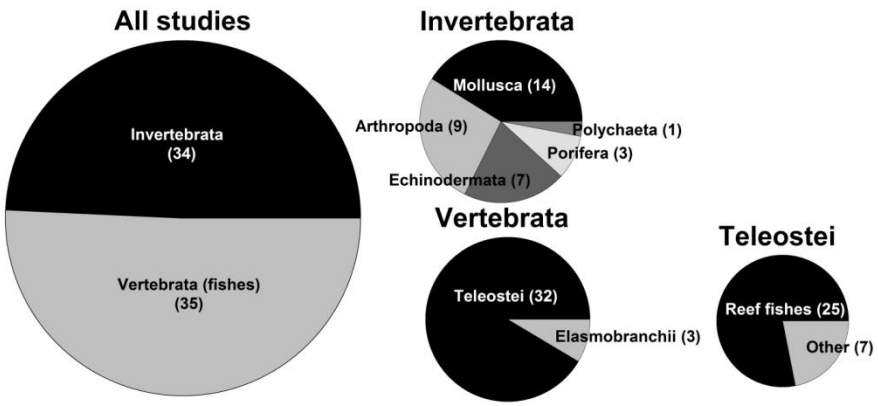

Figure 1.3. Taxonomic distribution of studies assessing the impact of ocean acidification on the behaviour of marine animals. Pie slice areas depict the relative percentages of the corresponding taxonomic group. Values in parentheses indicate the total number of publications incorporating the corresponding taxonomic group into their study (n = 69). Totals of lower taxonomic levels do not necessarily add to those of higher levels due to the incorporation of >1 taxonomic group in some studies.

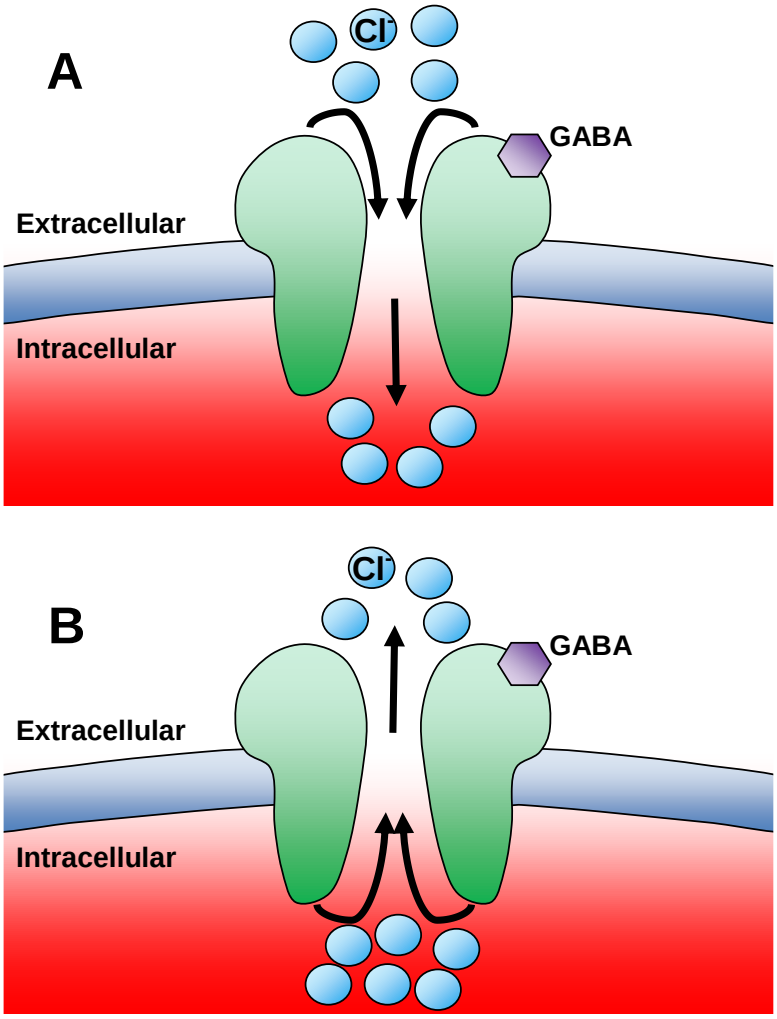

Figure 1.4. Visual representation of $GABA_A$-receptor functioning via ion gradients under ambient (A) and elevated (B) CO_2 conditions. Under ambient conditions, extracellular [Cl^-] and [HCO_3^-] is slightly higher than intracellular [Cl^-] and [HCO_3^-], maintaining the equilibrium potential near the resting membrane potential. Under elevated CO_2, acidosis is counteracted through the excretion of Cl^- and the accumulation of HCO_3^-, altering the ion gradient across the neural membrane and potentially resulting in membrane depolarization, neural pathway excitation, and altered behaviour. $GABA_A$ receptor functioning may be potentiated or reversed, depending on the magnitude of Cl^- and HCO_3^- changes in acid-base regulation. Adapted from Hamilton et al. (2014).

Chapter 2

Influence of sediment acidification and water flow on sediment acceptance and dispersal of juvenile soft-shell clams (*Mya arenaria* L.)

This chapter is published in the Journal of Experimental Marine Biology and Ecology:

Clements JC, Hunt HL (2014) Influence of sediment acidification and water flow on sediment acceptance and dispersal of juvenile soft-shell clams (*Mya arenaria* L.) J Exp Mar Biol Ecol 453:62-69

Abstract

Although ocean acidification is expected to reduce carbonate saturation and yield negative impacts on open-ocean calcifying organisms in the near future, acidification in coastal ecosystems may already be affecting these organisms. Few studies have addressed the effects of sedimentary saturation state on benthic invertebrates. Here, I investigate whether sedimentary aragonite saturation ($\Omega_{aragonite}$; because *M. arenaria* shells are almost completely comprised of aragonite) and proton concentration ($[H^+]$) affect burrowing and dispersal rates of juvenile soft-shell clams (*Mya arenaria*) in a laboratory flume experiment. Two size classes of juvenile clams (0.5-1.5 mm and 1.51-2 mm) were subjected to a range of sediment $\Omega_{aragonite}$ and [H+] conditions within the range of typical estuarine sediments ($\Omega_{aragonite}$ 0.21–1.87; pH 6.8–7.8; $[H^+]$ 1.58×10^{-8}–1.51×10^{-7}) by the addition of varying amounts of CO_2, while overlying water pH was kept constant ~7.8 ($\Omega_{aragonite}$ ~1.97). There was a significant positive relationship

between the percent of juvenile clams burrowed in still water and $\Omega_{aragonite}$ and a significant negative relationship between burrowing and $[H^+]$. Clams were subsequently exposed to one of two different flow conditions (flume; 11cm s^{-1} and 23 cm s^{-1}) and there was a significant negative relationship between $\Omega_{aragonite}$ and dispersal, regardless of clam size class and flow speed. No apparent relationship was evident between dispersal and $[H^+]$. The results of this study suggest that sediment acidification may play an important role in soft-shell clam recruitment and dispersal. When assessing the impacts of open-ocean and coastal acidification on infaunal organisms, future studies should address the effects of sediment acidification to adequately understand how calcifying organisms may be affected by shifting pH conditions.

2.1 Introduction

As a result of increasing anthropogenic CO_2 emissions, ocean acidification is expected to impose unfavorable conditions on marine-dwelling organisms in many parts of the ocean by the end of this century (IPCC 2007, Orr et al. 2005). However, because they are subjected to an array of acidifying sources not present in the open ocean, many coastal areas already experience lower pH conditions than future open-ocean predictions (Duarte et al. 2013). Although coastal organisms are already subjected to sub-optimal conditions (with respect to pH relative to future ocean acidification scenarios), we are only beginning to understand the potential ecosystem impacts and biological effects of coastal acidification, which appears highly variable both temporally and spatially.

It is well established that acidification can inflict a multitude of negative impacts on marine molluscs (Guinotte & Fabry 2008, Gazeau et al. 2013) and other organisms with

calcium carbonate structures (Orr et al., 2005). However, the consequences of ocean acidification on the growth and calcification of adults and juveniles across, and even within species are highly variable, with the exception of pteropods (Gazeau et al. 2013). Furthermore, the calcium carbonate shell is not the only endpoint impacted by ocean acidification. Internal blood pH levels can be lowered with increasing acidity, ultimately impacting a variety of physiological processes and potentially leading to long-term increases in mortality (e.g. Dickinson et al. 2012, Gazeau et al. 2013). Development can also be impacted by acidification, with longer development times, increased larval abnormalities, and decreases in larval survival and size all consistent with increasing acidification (e.g. Gazeau et al. 2013). Increased acidification may also affect organismal behaviour. For instance, Bibby et al. (2007) found that increasing CO_2 hindered shell production of the intertidal gastropod *Littorina littorea* and subsequently increased avoidance behaviour in the presence of predators, resulting in the potential for altered biological interactions, while Green et al. (2013) suggested that increasing sediment acidification can negatively affect the burrowing behaviour of infaunal bivalves.

Although it has been demonstrated that changes acidity and carbonate saturation (Ω) in the water column can affect various organisms, few studies have addressed the biological implications of these conditions in benthic sediments. It is known that the decomposition of sedimentary organic matter can result in pore-water carbonate undersaturation and the subsequent dissolution of calcite and aragonite within bottom sediments (Aller 1982, Green & Aller 1998, 2001), with undersaturation being most pronounced at the sediment-water interface (SWI) (Green et al. 2013). As a result, this

could have major implications for newly settled benthic invertebrates that are restricted to the SWI as juveniles (Zwarts & Wanink 1989). For example, Green et al. (1993) suggested that surface-sediment undersaturation could account for ~100% loss of benthic foraminifera in Long Island Sound sediments on an annual basis. Also, Green et al. (2009) demonstrated that undersaturated sediments cause substantially heightened mortality rates of juvenile quahogs, *Mercenaria mercenaria* in the lab, while buffering sediments with crushed shell hash (i.e., adding calcium carbonate) led to increased quahog survival in the lab and a 3-fold increase in *Mya arenaria* recruitment in the field over 3 weeks. Although undersaturation was suggested as the cue for settling *M. mercenaria*, it is important to recognize that the effects of correlated chemical parameters (e.g. pH, pCO_2, Ω, dissolved inorganic carbon, total alkalinity, etc.) are difficult to distinguish from one another. Given the multitude of commercially and ecologically important species that reside within coastal sediments, understanding the biological implications of sediment acidification is critical to understanding the long-term effects of coastal acidification.

Another potential consequence of sedimentary carbonate acidification is reduced colonization by marine bivalves. It is well established that newly settled bivalves are able to use chemical cues to distinguish between favourable and unfavourable habitats (Snelgrove et al. 1999, Marinelli & Woodin, 2004). If the surrounding sediment is deemed unfavorable, bivalves may reject it and fail to burrow and/or increase post-settlement movement (i.e., entrainment and transport). Sediment acceptance and dispersal of juvenile bivalves can be affected by sediment type (Hunt 2004, Lundquist et al. 2004, St-Onge & Miron 2007), contamination (Roper et al. 1995), organic matter,

hydrodynamics (Butman 1987), organism size (St-Onge & Miron 2007), and the presence of hydrogen sulfide (H_2S) (M-J. Abgrall pers. comm.). Juveniles are also thought to select sediments based on Ω. Green et al. (2013) found that recently settled *Mercenaria mercenaria* actively rejected low Ω sediments under laboratory conditions, and that buffering sediments enhanced burrowing behaviour. However, since pH, pCO_2, dissolved inorganic carbon (DIC), and other chemical parameters such as Ω and proton concentration ($[H^+]$) are correlated, it is difficult to elucidate Ω as *the* cue influencing burrowing behaviour in these organisms. Given that changes in burrowing behaviour due to other factors are known to affect dispersal of juvenile bivalves (e.g. Hunt & Scheibling 1997, St-Onge & Miron 2007), increasing sediment acidity could potentially lead to increased dispersal and lower recruitment success, although this has yet to be established.

This study aimed to assess the effects of sediment acidification on burrowing behaviour and dispersal rates of juvenile soft-shell clams, *Mya arenaria*, in a laboratory flume experiment. The soft-shell clam is a common bivalve in eastern North America and can be considered an ecologically and economically important species. It has been reported that *M. arenaria* recruitment increased with sediment buffering with $CaCO_3$ (Green et al. 2009) in the field, but it is not known if this response was due to increased settlement, a reduction in mortality or dispersal, or some combination of the two. In the present study, juvenile *M. arenaria* were exposed to sediments subjected to varying levels of CO_2 to manipulate $[H^+]$ and Ω (reported with respect to aragonite ($\Omega_{aragonite}$), since *M. arenaria* shells are primarily comprised of this carbonate mineral form) in the laboratory. Burrowing behaviour in relation to $\Omega_{aragonite}$ and $[H^+]$ was assessed under still

water, while a factorial design was employed to determine whether or not the effects of $\Omega_{aragonite}$ and $[H^+]$ on clam dispersal were consistent across clam size classes and flow speeds in a flume experiment. I hypothesized that lower $\Omega_{aragonite}$ and higher $[H^+]$ sediments would impair burrowing and increase dispersal, particularly in higher flow conditions.

2.2 Methods

2.2.1 Flume

Laboratory experiments were conducted in a recirculating flume at the University of New Brunswick in Saint John, New Brunswick from 16-24 July 2012 (Figure 2.1). The working channel of the flume consists of an open acrylic channel 732 cm long, 50 cm high, and 50 cm wide (total volume of 1830 L). A 746-W motor powers a propeller placed at the opening of a 32 cm diameter PVC pipe, which runs below the working channel and feeds back into the flume at the opposite end of the channel via a circular opening of the same diameter.

The flume was filled to a depth of 20 cm with filtered seawater (to remove large particles that could interfere with the experiment) at an average temperature and salinity of 17.6ºC and 32.4 ppt, respectively (to mimic upper thresholds observed in Bay of Fundy tidal flats during the time of clam collection). Flow speeds were measured in the center of the channel at 19 heights between 0.1 and 18.2 cm above the bottom with a SonTek 16 MHz MicroADV controlled by a Velmex Unislide System to obtain a

vertical profile of flow velocities. Shear velocities (u_*) for each flow profile were estimated using the profile's slope in the log layer,

$$u(z) = \frac{u_*}{\kappa} \ln\left(\frac{z}{z_0}\right)$$

where $u(z)$ is the current velocity at depth z, z_0 is the bottom roughness parameter, and κ is von Kármán's constant, which predicts u_* to be proportional to the slope of the velocity profile in the log-layer ('law of the wall', Vogel 1994). Given that only 8 of the 19 measurements (up to 2.2 cm above bottom) fell within the log layer of the profile, flow measurements above 2.2 cm depth were not used to calculate u_*.

2.2.2 Sediment manipulation

The sediment used was obtained from a tidal flat in Little Lepreau, NB, Canada (45°7'28.82"N, 66°28'17.97"W), which is primarily comprised of fine sand, with a mean grain size of 174.7±11.8 µm (φ=2.52±0.09) and an organic content of 3.51±0.56%. Once collected, the sediment was brought back to the lab and sieved (1 mm) to get rid of any large rocks or animals. The initial mean pH of the sediment was relatively high (~7.8). Conditions of $\Omega_{aragonite}$ and [H+] were manipulated as continuous variables (n = 41). For each sediment sample (n = 41), an air hose and stone connected to a 50 lb. CO_2 cylinder was placed in the bottom of a jar of sediment to slowly diffuse CO_2 into the sediment. For each sample, the sediment was then gently mixed for 45 seconds for homogenization. CO_2 was turned on and diffused for random time intervals (between 0 and 40 sec) for each sediment sample to obtain a range of pH levels between 6.8 and 7.8 ($[H^+]$ 1.58×10^{-8}–1.58×10^{-7}) and $\Omega_{aragonite}$ between 0 and 2, comparable with field observations at several tidal flats in the Bay of Fundy (Clements unpublished data).

Although CO_2 addition could potentially alter other covariates contributing to organismal responses (i.e., hydrogen sulfide and oxygen), similar manipulation techniques are not known to influence these covariates (Green et al., 2009). Hence, I assumed bivalve responses were principally due to changes in sedimentary carbonate dynamics. Triplicate pH measurements for each sediment sample were taken in the top 1 mm of the surface sediment using an Accumet AB15 meter equipped with a MI-419 pH microelectrode (Microelectrodes Inc.), calibrated daily in pH 4, 7, and 10 buffers (Fisher Scientific). Measurements of pH were taken to the nearest 0.01 unit and were measured on the NBS scale. The majority of each jar of manipulated sediment was then transferred into a plastic cup (8 cm depth, 7.5 cm diameter), which was later placed in still water in the flume to be used in a given trial, while the remaining sediment from that sample was transferred into a 15 ml centrifuge tube for alkalinity titration analysis (see section 2.5); the procedure was repeated for each of the 41 experimental trials. Once in the flume, the top of the sediment was levelled off to ensure that the surface was flush with the bottom surface of the flume, to prevent altering water flow above the sediment.

2.2.3 Alkalinity titration analysis, $\Omega_{aragonite}$, and $[H^+]$

Alkalinity titration analysis was conducted for each experimental trial to determine $\Omega_{aragonite}$ values. Two 15 ml centrifuge tubes of sediment from each replicate were centrifuged at 3000 rpm for 5 min to extract pore water. Once centrifuged, pore water was transferred into a 20 ml scintillation vial. Alkalinity was then determined using the titration method described by Edmond (1970). Since centrifuged samples typically yielded less than 2 ml of pore water, each analysis was conducted on a 0.5 ml aliquot diluted with 1.5 ml of distilled water, and titrated with 0.01 N hydrochloric acid (HCl).

Initial pH (after addition of CO_2), temperature, salinity, and total alkalinity for each experimental sample were entered into the CO_2SYS program (Pierrot et al. 2006), along with the first and second dissociation constants of seawater carbonic acid described by Mehrbach et al. (1973) and refit by Dickson & Millero (1987), to obtain $\Omega_{aragonite}$ estimates. Although dissolved inorganic carbon (DIC) and the partial pressure of CO_2 (pCO_2) can also be used to estimate $\Omega_{aragonite}$, as well as being independent indicators of sediment carbonate chemistry, these parameters were not employed in the current study due to financial constraints. Proton (hydrogen ion) concentration ($[H^+]$) was also calculated from mean pH measurements, and was defined as the inverse -log of pH (10^{-pH}).

2.2.4 Porewater pH stability

To ensure that clams would be exposed to consistent $\Omega_{aragonite}$ over the duration of each experimental trial, an initial analysis of pH consistency was conducted in the lab. Control sediment (no CO_2) and sediment manipulated with respect to pH (addition of CO_2 for 25 s) were transferred into three individual cups per treatment. One cup from each pH treatment was then exposed to each of three water exposure treatments; no overlying water (NW), still water (SW) and flowing water (FW; 11 cm s^{-1}). At a later date, an additional experiment employing the same methods described above was conducted to ensure that porewater pH and $\Omega_{aragonite}$ remained stable under the high flow speed (23 cm s^{-1}) as well; this experiment only tested porewater pH and $\Omega_{aragonite}$ stability at the high flow speed (23 cm s^{-1}). In each treatment, triplicate measurements of porewater pH were taken once every 30 min for two hours to assess pH stability over time. Although logistics did not allow for $\Omega_{aragonite}$ measurements over time, $\Omega_{aragonite}$ was

assumed to be tightly coupled with pH, as it was in the remainder of the experiment (see section 3.3).

2.2.5 Sediment acceptance/rejection and dispersal

I assessed soft-shell clam burrowing behaviour and dispersal in still and flowing water, respectively, under various sedimentary pH and $\Omega_{aragonite}$ conditions. Water column pH and $\Omega_{aragonite}$ remained at ambient values (see section 3.1). Two size classes of juvenile *M. arenaria* (0.5-1.5 mm and 1.51-2.5 mm) obtained from Downeast Institute (Beals, Maine) were used in the experiment. Bivalves were kept in a recirculating seawater system at the University of New Brunswick, Saint John for approximately three months at a temperature of ~18°C and salinity ~31 ppt, and were fed daily with Instant Algae® Marine Microalgae Concentrates Shellfish Diet 1800 (ReedMariculture Inc., USA).

Dispersal of juvenile *M. arenaria* was examined for the two size classes of clams and two flow speeds (2 levels, 11 and 23 cm s^{-1}) in a factorial design. Flow speeds were selected based on preliminary observations to be slightly higher than the critical erosion thresholds of the clams (u_{9cm}=11 cm s^{-1}; u_*=1.24±0.08) and the sediment (u_{9cm}=23 cm s^{-1}; u_*=2.23±0.23), whereby the slower flow speed allowed clams to passively disperse without any sediment movement, while the higher flow speed inflicted minimal surface sediment movement. $\Omega_{aragonite}$ /$[H^+]$ level was a continuous variable ($\Omega_{aragonite}$: 0.2- 1.9, pH: 6.8–7.8, $[H^+]$: 1.58×10^{-8}–1.51×10^{-7}). There were 10-11 replicate runs, each with a randomly chosen $\Omega_{aragonite}$ /$[H^+]$ level, for each combination of clam size class and flow speed, yielding 41 trials in total (10 runs with varying $\Omega_{aragonite}$ and $[H^+]$ levels x 4

categorical treatment combinations (with one extra replicate for one treatment combination). For each run, a cup of manipulated sediment was placed into a hole of the same diameter in the bottom of the Plexiglas insert in the working section of the flume. The top of the cup was level with the bottom of the flume and the sediment surface was flattened using a straight-edge. A sediment trap (38 cm long, 2.5 cm wide, 14.5 cm deep) was located 25.5 cm downstream of the core. Thirty clams of a particular size class were then placed on the top of the cup in still water and given 20 minutes to burrow, based on preliminary burrowing observations. The experimental clam density was based on abundances of clams in field studies conducted in the Bay of Fundy (Bowen & Hunt 2009, Morse & Hunt, 2013). Sediment acceptance or rejection was quantified prior to flow exposure by visually counting the number of clams that burrowed into the sediment after 20 min for 21 of the 41 experimental trials (0.5-1.5 mm: n=11; 1.51-2 mm: n=10). We chose a time of 20 mins based on previous experiments showing that virtually all clams (>90%) will burrow in this timeframe under ambient conditions. Clams were considered to have accepted the sediment if they were anchored upright within the sediment or completely burrowed. The proportion of clams that accepted the sediment was calculated by dividing the number of upright or burrowed clams by the total number of clams used in the experimental trial (30). The flow in the flume was then turned on for 40 min. At the end of the 40 min, the contents of the bedload trap and sediment cup (top 2 cm) were then separately suctioned onto a 180 μm sieve. Sieved contents were preserved in 80% ethanol and examined under a dissecting scope to quantify the number of clams that no longer remained in (or dispersed from) the sediment cup. The proportion of clams that dispersed from the sediment was calculated by dividing the number of clams by the total number that had

left the sediment core (total clams initially – clams remaining) by the number of clams used in the experimental trial (30).

2.2.6 Statistical analyses

All statistical analyses were run with R version 2.14.1 (R Core Team, 2013). Linear regression was used to assess the relationship between pH and $\Omega_{aragonite}$. Repeated measures ANOVA was used to assess the stability of the two pH treatments across time (repeated measure) and varying overlying water treatments for CO_2 treated and untreated sediments, all of which were fixed factors. ANCOVA was used to test the null hypothesis that the relationships between initial burrowing versus $\Omega_{aragonite}$ and initial burrowing versus $[H^+]$ did not differ between the two clam size classes, with clam size as a fixed categorical factor (2 size classes), and $\Omega_{aragonite}$ and $[H^+]$ as covariates. ANCOVA was also used to test the null hypothesis that the relationships between dispersal versus $\Omega_{aragonite}$ and dispersal versus $[H^+]$ did not differ between flow speeds or size classes, with clam size and flow speed as a fixed categorical factors (2 size classes and 2 flow speeds), and $\Omega_{aragonite}$ and $[H^+]$ as covariates. α=0.05 was used for all statistical tests. For ANOVA and ANCOVA tests, assumptions of normality and homoscedasticity were tested using Q-Q plots and Cochran's tests, respectively, while assumptions of linearity, normality and homoscedasticity for regressions were tested using component-residual plots, Q-Q plots, and non-constant error variance tests, respectively. Initial $\Omega_{aragonite}$ data violated the assumption of homoscedasticity, so it was log transformed. All other data met the assumptions of their respective statistical tests.

2.3 Results

2.3.1 Water column conditions

Conditions within the water column remained stable throughout the experiment. Temperature and salinity were maintained at 17.5±0.12°C and 32.3±0.10 psu, respectively. Total alkalinity, pH, and $\Omega_{aragonite}$ were also consistent at ~2905.76±168.86 µmol kg^{-1} seawater, 7.80±0.03, and 1.81±0.11, respectively.

2.3.2 Alkalinity titration analysis

Inputs of CO_2 resulted in sediment pH values from 6.82–7.80 ($[H^+]$ 1.58×10^{-8}–1.51×10^{7}). These values corresponded to a range in $\Omega_{aragonite}$ from 0.21–1.87, yielding both saturated ($\Omega_{aragonite}\geq1$) and undersaturated ($\Omega_{aragonite}<1$) sediments. There was a strong linear relationship between $\log(\Omega_{aragonite})$ and pH ($F_{1,39}=1989.51$, $P<0.0001$, $R^2=0.9808$) of the sediment, indicating that pH is a good predictor of $\Omega_{aragonite}$ (Figure 2.2).

2.3.3 pH stability

Sedimentary pH remained stable over a 2 hour exposure, regardless of overlying water treatment, for both CO_2-treated and untreated sediment (Figure 2.3a-d). The 3-factor repeated measures ANOVA revealed that pH did not differ significantly over the two hour period or between overlying water treatments (no overlying water, still overlying water, and the low flow speed) for sediment with (pH 7.23±0.03) or without (7.85±0.03) CO_2 input (Figure 2.3a-c; Table 2.1). An additional subsequent confirmed

that the high flow speed used in the experiments did not alter sedimentary pH over the time frame of the experiment also showed no difference in pH across time (Table 2.2; Figure 2.3d). While there were not replicate cups of the different OLW and CO_2 treatments (i.e., there was pseudoreplication), stable pH was observed in all treatments for this experiment.

2.3.4 Sediment acceptance/rejection and dispersal

2.3.4.1 Acceptance/rejection

ANCOVA yielded similar slopes for the relationship between the percentage of clams that initiated burrowing and $\Omega_{aragonite}$ across the two size classes of clams ($\Omega_{aragonite}$×Size Class; $F_{1,17}$=0.220, P=0.650). Consequently, the analysis was rerun without the $\Omega_{aragonite}$×Size Class interaction term. $\Omega_{aragonite}$ had a significant effect on burrowing, with a smaller percentage of clams burrowing into low $\Omega_{aragonite}$ sediments (Table 2.3; Figure 2.4). Similarly, for the relationship between the percentage of clams that initiated burrowing and $[H^+]$, similar slopes were evident for the two size classes of clams ($[H^+]$×Size Class; $F_{1,17}$=0.129, P=0.7238), so the analysis was rerun without the $[H^+]$×Size Class interaction. Results indicated that $[H^+]$ had a significant effect on the number of clams that initiated burrowing ($F_{1,18}$=6.651, P=0.0189), with no effect of size class ($F_{1,18}$=0.871, P=0.3629).

2.3.4.2 Dispersal

ANCOVA yielded similar slopes for the relationship between % dispersal and $\Omega_{aragonite}$ for the two flow speeds ($\Omega_{aragonite}$×Flow; $F_{1,33}$=0.779, P=0.384) and size classes

($\Omega_{aragonite}$×Size Class; $F_{1,33}$=0.288, P=0.595); other interactive effects were not statistically significant (Size Class×Flow: $F_{1,33}$=0.162, P=0.690; Size Class×Flow×$\Omega_{aragonite}$: $F_{1,33}$=0.277, P=0.602). As such, the analysis was rerun without the interaction terms. In this subsequent analysis, flow speed and $\Omega_{aragonite}$ both had significant effects on clam dispersal, with % dispersal decreasing with increasing $\Omega_{aragonite}$ (Table 2.4; Figure 2.5). Size class did not have any independent effects on clam dispersal (Table 2.4). Similar analyses were conducted for the relationship between % dispersal and $[H^+]$ across flow speeds and clam size classes. Similar slopes were evident for the relationship between % dispersal and $[H^+]$ at the two flow speeds ($[H^+]$×Flow; $F_{1,33}$=0.036, P=0.851) and size classes ($[H^+]$×Size Class; $F_{1,33}$=0.630, P=0.433); other interactions were also found to be insignificant (Size Class×Flow: $F_{1,33}$=0.044, P=0.836; Size Class×Flow×$[H^+]$: $F_{1,33}$=0.924, P=0.344). The analysis was then rerun without the interaction terms. There was a significant effect of flow on % dispersal ($F_{1,36}$=59.157, P=<0.0001), but no significant effect of $[H^+]$ ($F_{1,36}$=2.320, P=0.136). Again, size class did not show a significant effect on dispersal ($F_{1,36}$=<0.0001, P=0.993).

2.4 Discussion

Our results suggest that sediment acidification can negatively impact the burrowing success and dispersal of juvenile soft-shell clams. In the laboratory experiment, fewer clams initiated burrowing, and dispersal rate at both flow speeds was greater, in acidified sediment.

Although water column conditions were kept constant with high pH and $\Omega_{aragonite}$ throughout the experiment, clams still rejected more acidic sediments, suggesting that

pore water acidification may have a greater effect on juvenile soft-shell clam behaviour than water column acidification. Overlying water acidification (i.e., in the water column) can have effects on calcification of various bivalve species, with depressed calcification and shell dissolution occurring at low pH and saturation levels (e.g. Gazeau et al. 2007; Waldbusser et al. 2011). However, given that various other physiological processes (e.g. metabolism and respiration (e.g. Benaish et al. 2010, Lanning et al. 2011), general health (Beesley et al. 2008), tissue structure and growth (Beesley et al. 2008, Dickinson et al. 2012), and metamorphosis (Colen et al. 2012)) can be influenced by increasing acidification, and that the effects on these processes are highly species-specific (Fabry et al. 2008, Kurihara 2008, Doney et al. 2009, Gazeau et al. 2013), they should be assessed independently and interactively across a wide array of bivalve species to better understand the physiological effects driving the behavioural responses observed in this study. To date, the long term of effects of acidifying sediments have not been tested for *M. arenaria*, or any other infaunal bivalves.

Even though sediment pH and $\Omega_{aragonite}$ are known to be much lower than that of the overlying water (Ponnamperuma 1976, Green et al. 2009; predominantly driven by microbial activity) sediment acidification is grossly understudied. However, Green et al. (2013) recently suggested that $\Omega_{aragonite}$ may act as a recruitment cue for plantigrade-stage *Mercenaria mercenaria.* In laboratory experiments, a significant positive relationship was found between percent of *M. mercenaria* burrowed and $\Omega_{aragonite}$. They had also previously demonstrated that low surface-sediment $\Omega_{aragonite}$ led to substantial degrees of mortality via shell dissolution of recently-settled *M. mercenaria* in the lab (Green et al. 2009). In the field, *Mya arenaria* recruitment was increased three-fold over 30 days by

buffering sediment with crushed shell hash (increasing and stabilizing surface-sediment pH and saturation state), which they suggested was a consequence of reduced mortality, increased *M. arenaria* settlement, or a combination of both (Green et al. 2013). Given the findings of Green et al. (2009, 2013) and the results of this study, it is plausible that juvenile *M. arenaria* and other infaunal bivalves reject low $\Omega_{aragonite}$ sediments to avoid being subjected to potentially lethal conditions. However, given the data available to date and the fact that a multitude of chemical parameters (DIC, pCO_2, pH, Ω, etc.) are highly correlated, $\Omega_{aragonite}$ cannot be isolated as *the* recruitment cue for settling marine bivalves.

Although juvenile clams actively responded to acidified sediments in this study by not burrowing, the question remains as to what cue they were actually using to distinguish between favorable and unfavorable sediments; pH or $[H^+]$, $\Omega_{aragonite}$, pCO_2, or other chemical parameters associated with acidification. Since pH and $\Omega_{aragonite}$ were manipulated using CO_2 injection, pCO_2 may also play a role in the burrowing and dispersal patterns observed here. Future studies should focus on ways of isolating the effects of these factors separately to determine whether or not increased levels of CO_2 independently contribute to the burrowing behaviour and dispersal patterns reported here. Furthermore, although ANCOVA results for burrowing were similar for $[H^+]$ and $\Omega_{aragonite}$, for dispersal there was a significant relationship to $\Omega_{aragonite}$ but not $[H^+]$, suggesting that $\Omega_{aragonite}$ may better predict the movement of clams away from more acidic sediments. Dissolved inorganic carbon and other chemical parameters may also play a role in this process, and so future studies should, if possible, assess a variety of parameters when assessing the impacts of acidification on juvenile clam recruitment.

Other factors shown to act as recruitment cues for juvenile bivalves include the presence of bromophenols (secreted by capitellid polychaetes; Woodin et al. 1997) and sediment disturbance (Woodin & Marinelli 1991, Marinelli & Woodin 2002, 2004). The former is unlikely in my experiments, particularly given that sediment was sieved and polychaetes capable of releasing bromophenols would not have been active within the sediment for 2-3 days leading up to experiments. However, prior to experimental runs, the sediment was gently stirred in an attempt to homogenize pH and $\Omega_{aragonite}$. Sediment disturbance has been shown to depress bivalve recruitment by altering pore water chemistry with respect to oxygen and ammonium (Marinelli & Woodin 2002, 2004). Although sediment disturbance may have influenced the results, this effect would have been uniform across treatments and therefore could not be responsible for the significant effects detected in the present study. It is also unlikely that addition of CO_2 significantly altered geochemical covariates, such as oxygen, related to organic decomposition or diagenetic redox reactions as previous work with similar methods did not detect any significant alteration in dissolved oxygen (Green et al. 2009). Ultimately, the results suggest that the acidification of sediments may hinder burrowing and increase post-settlement dispersal in juvenile *Mya arenaria*.

Our results suggest that juvenile soft-shell clams reject more acidic sediments, but the reason why remains unclear. In comparison to the findings of Green et al. (2009, 2013), my results indicate that *Mya arenaria* also respond to more acidic sediments and may avoid burrowing and move away from them to reduce the risk of dissolution mortality, although larger individuals are expected to be less susceptible to dissolution mortality than recent settlers (Waldbusser et al. 2009). However, other factors such as

growth and development, metabolism, and health may be influenced by more acidified sediments and could potentially contribute to the sediment acceptance and dispersal patterns observed in this study. This is particularly true given that some species have the potential to increase calcification in more acidified conditions at a cost to other factors such as metabolism (Wood et al. 2008). Further studies should focus on isolating the precise adaptive advantage of rejecting acidified sediments by juvenile *Mya arenaria*. Furthermore, given that the effects of acidification appear to be highly species specific, future studies assessing the influence of sediment acidification on habitat selection and dispersal in other bivalve species are certainly warranted.

Our observations suggest that the main mode of dispersal for juvenile *Mya arenaria* under more acidic sediments is passive entrainment and bedload transport (opposed to resuspension, byssal drifting, or crawling), as the vast majority clams that dispersed from the sediment were found in the bedload trap downstream. These results support other studies suggesting that *M. arenaria* predominantly disperse by bedload transport (e.g. Emerson & Grant 1991, Hunt & Mullineaux 2002, Hunt et al. 2007, St-Onge et al. 2007). Depending on hydrodynamic conditions, juvenile soft-shell clams may be transported 1 – 10 m distances over a tidal cycle or storm, orders of magnitude higher than crawling (Roegner et al. 1995). If chemical conditions within a given area are relatively similar, juvenile clams would have to disperse longer distances before reaching suitable substrate or, if overlying water currents are insufficient for bedload transport, may be restricted to a more acidified area, increasing the chances of dissolution mortality (Green et al. 2009). Thus, it would be beneficial under suboptimal conditions to use bedload transport in an attempt to locate suitable habitat. Further

studies are needed to assess dispersal rates and distances across a wider range of flow speeds to determine minimum speeds for dispersal and how natural flow conditions interact with clam response to sediment acidification.

The pH and $\Omega_{aragonite}$ values used in the experiment were chosen based on field observations of natural pore water pH and $\Omega_{aragonite}$ levels on intertidal mudflats in the Bay of Fundy in southwestern New Brunswick, and were much lower than predicted future oceanic water column values, which are expected to have drastic impacts on calcifying organisms by the end of the century (IPCC 2007). The negative impacts of sediment acidification on *Mya arenaria* burrowing and dispersal observed in the laboratory in this study are likely to affect recruitment in the field. Such effects may be increased in the future if ocean acidification has similar effects on pore-water carbonate chemistry in coastal areas. Unfortunately, projections of future coastal acidification scenarios are limited and challenging (Duarte et al. 2013). Furthermore, sediment pH and $\Omega_{aragonite}$ in the intertidal zone in the Bay of Fundy are highly variable temporally, with observed pH changes upwards of 1.5 units within 24 hours (Clements unpublished data). The effect of this temporal variability on clam behaviour and physiology is currently unknown and makes it more difficult to detect effects of sediment acidification on patterns of clam abundances in the field. Further work is needed to assess how open-ocean and coastal acidification might influence sedimentary pH and Ω in future CO_2 scenarios, and to elucidate how varying pH and $\Omega_{aragonite}$ conditions influence the burrowing behaviour and recruitment of marine bivalves.

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Table 2.1. Summary for 3-factor repeated measures ANOVA for surface-sediment pH stability. Effects of overlying water treatment (OLW; no water, still water, and flowing water at 12 cm s^{-1}), CO_2 treatment (CO_2 and no CO_2), and time (0, 30, 60, 90, and 120 mins) were tested, with time as a repeated measure.

	df	SS	MS	F	*P*
Error: Replicate					
CO_2	1	8.458	8.458	5105.353	<0.0001***
OLW	2	0.001	0.001	0.425	0.664
CO_2×OLW	2	<0.001	<0.001	0.005	0.995
Residuals	12	0.020	0.002		
Error: Within					
Time	4	0.002	<0.001	0.514	0.726
CO_2×Time	4	0.002	<0.001	0.470	0.757
OLW×Time	8	0.008	0.001	1.119	0.368
CO_2×OLW×Time	1	0.005	0.001	0.718	0.674
Residuals	48	0.042	0.001		

*P<0.05; **P<0.01; ***P<0.001

Table 2.2. Summary for 2-factor repeated measures ANOVA for surface-sediment pH stability at the high flow speed (23 cm s^{-1}) only. Effects of CO_2 treatment (CO_2 and no CO_2), and time (0, 30, 60, 90, and 120 mins; repeated measure) were tested.

	df	SS	MS	F	*P*
CO_2	1	0.04	0.04	274.51	<0.0001***
CO_2×Time	1	3.0×10^{-4}	3.0×10^{-4}	2.18	0.20
Residuals	5	6.8×10^{-4}	1.4×10^{-4}		

*P<0.05; **P<0.01; ***P<0.001

Table 2.3. ANCOVA summary for sediment acceptance/rejection experiment. The % of juvenile *Mya arenaria* burrowed into sediment of different $\Omega_{aragonite}$ after 20 mins was tested for effects of $\Omega_{aragonite}$ and clam size class (0.5-1.5 mm and 1.51-2.5 mm).

	df	SS	MS	F	*P*
$\Omega_{aragonite}$	1	0.14507	0.14507	16.425	0.0007***
Size Class	1	0.00025	0.00025	0.028	0.869
Residuals	18	0.15898	0.00883		

*P<0.05; **P<0.01; ***P<0.001

Table 2.4. ANCOVA summary for clam dispersal experiment. The % of juvenile *Mya arenaria* dispersed from sediment of different $\Omega_{aragonite}$ after 1 hour was tested for effects of $\Omega_{aragonite}$, clam size class (0.5-1.5 mm and 1.51-2.5 mm), and flow velocity (u=11 cm s^{-1} (u_*=1.81±0.08) and 23 cm s^{-1} (u_*=2.23±0.23)).

	df	SS	MS	F	*P*
$\Omega_{aragonite}$	1	0.1525	0.1525	5.690	0.0224*
Size Class	1	0.0031	0.0031	0.117	0.735
Flow	1	1.7363	1.7363	64.776	<0.0001***
Size Class×Flow	1	0.0032	0.0032	0.118	0.7336
Residuals	36	868.8	24.10000		

*P<0.05; **P<0.01; ***P<0.001

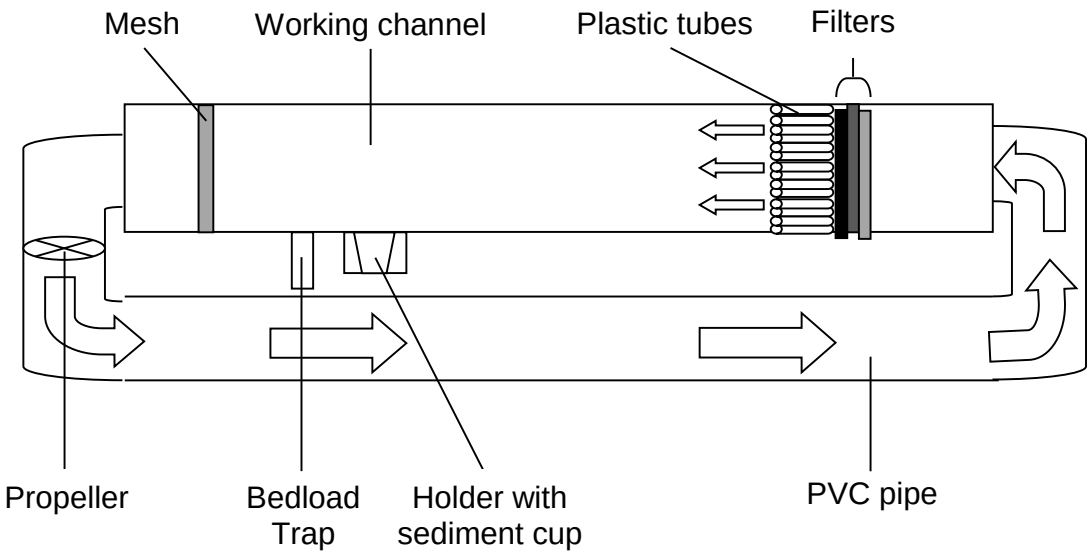

Figure 2.1. A detailed schematic of a side view of the laboratory flume used during the experiments. The propeller drives water movement, while filters clean the water as it comes out of the PVC pipe and plastic tubes produce laminar flow in the working channel. Arrows indicate the directionality of water flow.

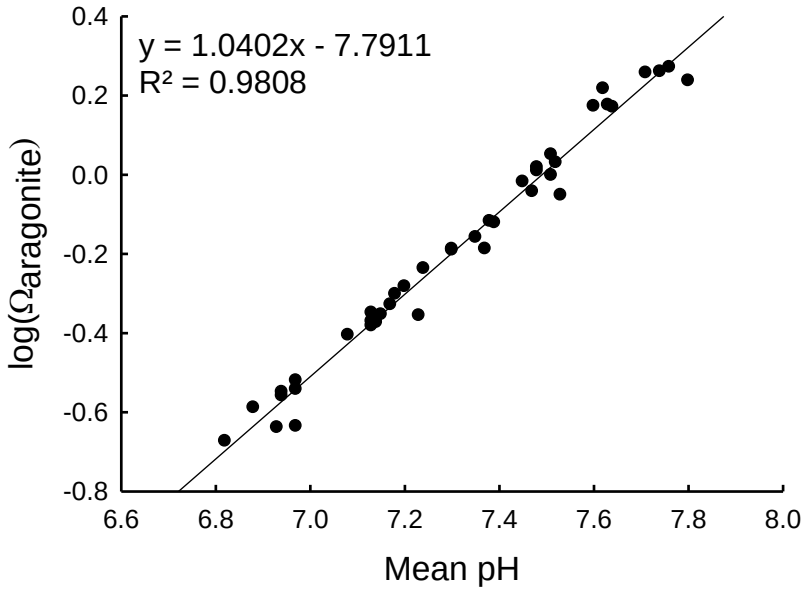

Figure 2.2. The relationship between mean pH (triplicate measurements) and $\log(\Omega_{aragonite})$ of CO_2-treated and untreated sediment across all 41 experimental trials.

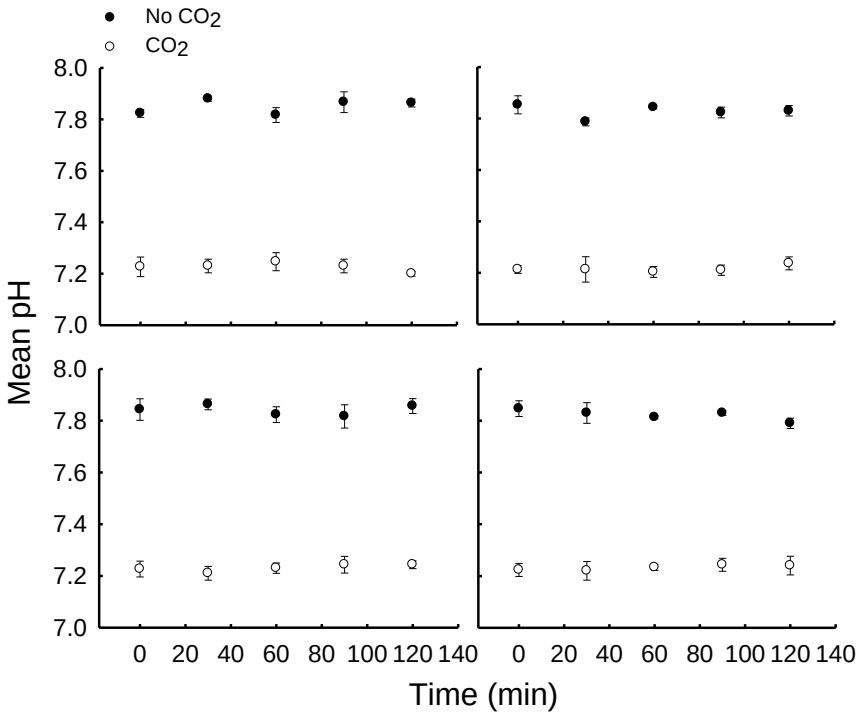

Figure 2.3. Stability of surface-sediment pH (top 1 mm) for CO_2 treated and untreated sediment in a) no overlying water (NW; top left); b) still water (SW; top right); c) flowing water (u_{9cm}=12 cm s^{-1}; u_*=1.81±0.08; bottom left); and d) flowing water (u_{9cm}=23 cm s^{-1}; u_*=2.23±0.23; bottom right).

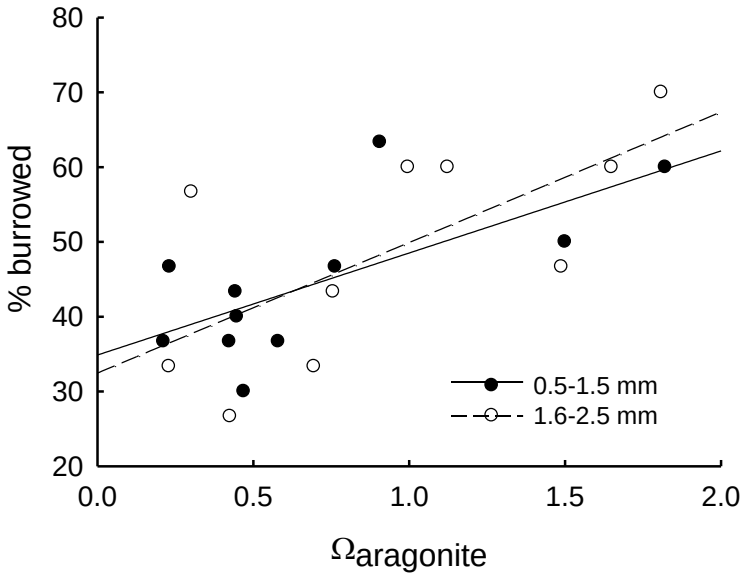

Figure 2.4. The relationship between % of *M. arenaria* burrowed (upright and anchored in sediment) within 20 min and surface-sediment $\Omega_{aragonite}$ (top 1 mm) for two size classes of clams (0.5-1.5 mm and 1.51-2.5 mm).

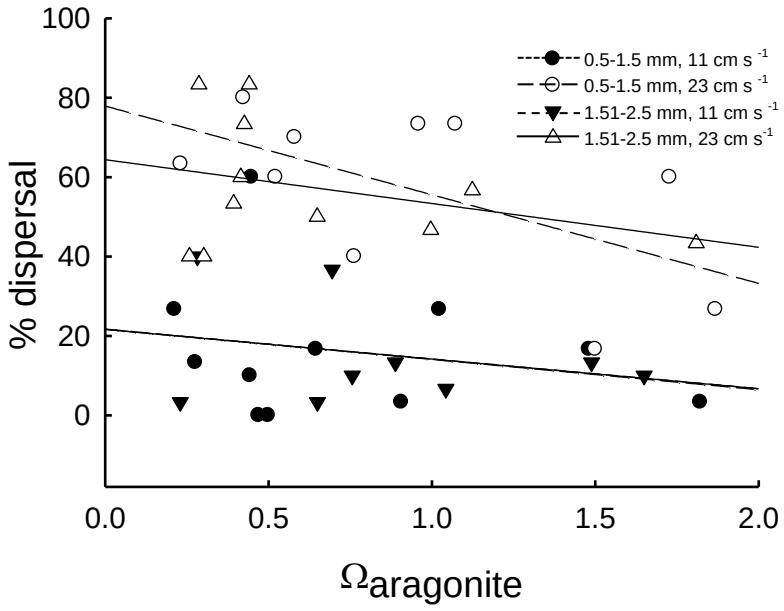

Figure 2.5. The relationship between % dispersal of *M. arenaria* and surface-sediment $\Omega_{aragonite}$ (top 1 mm) in the flume experiment at two flow speeds (u_{9cm} = 11 cm s^{-1} (u_* = 1.81±0.08) and u_{9cm} = 23 cm s^{-1} (u_* = 2.23±0.23)) for two size classes of clams (0.5-1.5 mm and 1.51-2.5 mm). Slopes for each size class at the low flow treatment overlap.

Chapter 3

Porewater acidification alters the burrowing behaviour and post-settlement dispersal of juvenile soft-shell clams (*Mya arenaria*)

This chapter is published the Journal of Experimental Marine Biology and Ecology:

Clements JC, Woodard KD, Hunt HL (2016) Porewater acidification alters the burrowing behaviour and post-settlement dispersal of juvenile soft-shell clams. J Exp Mar Biol Ecol 477:103-111

Abstract

Although ocean acidification will negatively impact marine organisms in the future, few studies have addressed the effects of sedimentary porewater acidification on benthic invertebrates. This study suggests that burrowing behaviour and dispersal of juvenile bivalves are altered by porewater acidification under present day conditions. I tested the efficacy of a novel method of stabilizing porewater pH using sediment underlain with food grade gelatin in both the lab and field, and then employed this method to test if porewater acidification could alter post-settlement clam dispersal under natural conditions. In the field, clams were exposed to a gradient of porewater acidification in manipulated (CO_2 added) sediments for 24 h to determine if acidification could alter dispersal patterns of juvenile clams under natural flow conditions; juvenile clam dispersal in the presence of different buffer types was also tested. In addition, juvenile clams were placed on unmanipulated, field-collected sediment cores in the lab which varied naturally with respect to acidification to test burrowing behaviour in response to

natural porewater pH. Gelatin stabilized porewater pH for 24-48 h and its presence did not influence clam burrowing behaviour. In the field, a significant negative relationship between the percent of clams dispersed and pH was observed, while stabilizing porewater pH significantly decreased clam dispersal. In the lab, there was a significant positive relationship between the percent of clams burrowed and porewater pH. This study suggests that porewater pH has the capacity to alter the burrowing behaviour and dispersal patterns of juvenile bivalves under natural conditions.

3.1 Introduction

Anthropogenic ocean acidification (OA) has lowered open-ocean pH by 0.1 units since the Industrial Revolution and is projected to lower the pH of the oceans another 0.3-0.4 units by the end of the century (Orr et al. 2005, IPCC 2014). While direct negative consequences of anthropogenic activity are documented for the open ocean, coastal ecosystems are exposed to an array of additional acid sources that the open ocean is not, such as terrestrial and freshwater runoff, coastal upwelling, ecosystem metabolism, changes to watershed dynamics (Duarte et al. 2013), and eutrophication (Wallace et al. 2014) among others. Consequently, coastal organisms already experience pH conditions at or below future OA projections (Duarte et al. 2013). However, the biological and ecosystem impacts of coastal OA are only beginning to be understood.

One consequence of OA in marine habitats is a reduction of carbonate saturation state (Ω) (Orr et al. 2005, Cao et al. 2007, Doney et al. 2009). The abiotic dissolution and precipitation of carbonate minerals depends primarily on acid availability and the titration of carbonate (CO_3^{2-}) according to the equation:

$CaCO_3 \leftrightarrow Ca^{2+} + CO_3^{2-}$

A decrease in the concentration of CO_3^{2-} ultimately results in a lowering of Ω, expressed as:

$$\Omega = [CO_3^{2-}][Ca^{2+}]/K'_{sp}$$

where K'_{sp} is the stoichiometric solubility product, dependent on the carbonate mineral phase (calcite, aragonite, low Mg calcite, high Mg calcite), temperature, salinity, and pressure. As waters become more acidic, excess hydrogen ions (H^+) bind to free CO_3^{2-} to create bicarbonate ions (HCO_3^-). Consequently, the concentration of carbonate ions is reduced and the carbonate saturation state, Ω, is decreased (since Ca^{2+} is most often highly available in seawater, Ω is almost entirely driven by $[CO_3^{2-}]$). Although carbonate thermodynamics are obviously altered as a result of OA, it appears that the kinetic constraints imposed on marine organisms in undersaturated conditions may drive species-specific calcification responses to OA (Waldbusser et al. 2013, 2015).

Increased acidity is likely to result in a variety of negative impacts for calcifying organisms in the near future. However, carbonate undersaturation is not responsible for all biological responses to OA (Guinotte & Fabry 2008, Green et al. 2009, Gazeau et al. 2013). For example, critical physiological functions such as ingestion (Fernández-Reiriz et al. 2011), respiration (Navarro et al. 2013) and metabolism (Beniash et al. 2010) have been reported to be impeded, and gene expression to be altered (e.g. for metabolism; Hüning et al. 2013), by increased levels of CO_2. Acidification has also been shown to alter the behaviour of some calcifying organisms (Clements & Hunt 2015). For example, Bibby et al. (2007) found that acidified conditions hindered shell production of the

intertidal gastropod *Littorina littorea* and subsequently increased avoidance behaviour in the presence of predators, resulting in the potential for altered biological interactions. Watson et al. (2014) reported that acidified conditions led to hindered antipredator-escape responses in the jumping conch, *Gibberulus gibberulus gibbosus* – a likely outcome of hindered $GABA_A$ receptor functioning ($GABA_A$ receptors are ligand gated ion channels found in the central nervous systems of vertebrates and some invertebrates which are involved in neurotransmission; they have a specific ionic conductance for chloride and bicarbonate ions). Acidic conditions can also cause substantial decreases in calcification, shell breaking resistance, and overall health in blue mussels (*Mytilus edulis*) and pacific oysters (*Crassostrea gigas*) (Gazeau et al. 2007, Beesley et al. 2008, Appelhans et al. 2012). Juvenile oysters (*Crassostrea virginica*) subjected to hypercapnic conditions also experience decreases in growth, tissue and shell mass, and increased mortality (Beniash et al. 2010), while lower pH conditions can increase mortality and shell growth of hard clams, *Mercenaria mercenaria*, although the effects may be size dependent (Waldbusser et al. 2009). Recently, saturation state has been proposed as the primary factor in the growth and calcification responses of calcifying organisms to acidified conditions (Waldbusser et al. 2015).

Many OA studies have assessed impacts of water column acidification on epifaunal organisms, but relatively few have documented the effects of sediment porewater acidification on infaunal organisms (Widdicombe et al. 2011). Although various factors can influence porewater pH (e.g. organic and inorganic content, oxygen concentration, nutrients, etc.), a reduction of overlying water pH has been suggested to result in reduced porewater pH as well (Widdicombe et al. 2009). As a result, organisms living

within sediments may be more vulnerable to increasingly acidified seawater than previously thought (Widdicombe et al. 2011). It is thus important to understand the impacts that porewater pH has on a variety of infaunal organisms to accurately address the potential impacts of near-future ocean acidification on these animals. One such group of organisms that appear to be vulnerable to effects of porewater acidification are juvenile bivalves. Aragonite (a calcium carbonate mineral) undersaturation in porewater has been reported to result in high levels of mortality (Green et al. 2009) and impede the burrowing behaviour (Green et al. 2013) of recently-settled hard clams (*Mercenaria mercenaria*). It has also been demonstrated that porewater acidification can reduce burrowing and increase dispersal rates of juvenile soft-shell clams (*Mya arenaria*) in the laboratory, whereby clams actively move away from low-pH sediments (Clements & Hunt 2014). However, the few studies assessing the impacts of porewater acidification on bivalve burrowing and dispersal have been primarily conducted in the laboratory and field studies are needed to fully understand the biological and ecological effects of porewater acidification.

Porewater geochemistry can vary on hourly scales in the lab and field (Wenzhöfer 1999, Yates & Halley 2006, J. Clements personal observations), making it difficult to maintain stable carbonate conditions in sediments for appropriate lengths of time to run field experiments. Consequently, there is a need to develop new methods to allow the transfer of porewater acidification studies from the laboratory to the field. Spiked polyacrylamide gel has been used to stabilize ammonium concentrations in sediment via ammonium diffusion in a study assessing the impacts of ammonium on the burrowing behaviour benthic organisms (Engstrom & Marinelli 2005), offering a potential method

to maintain carbonate chemistry. Though beneficial in that the diffusional properties of polyacrylamide gel are well understood, acrylamide is highly toxic, known to act as a neurotoxin and a potential human carcinogen (McAuley 2004), making it desirable to explore different methods of geochemical stabilization for studies assessing biological responses of infaunal organisms to porewater geochemistry.

This study employed four experiments assessing: 1. the efficacy of gelatin for stabilizing porewater pH in the lab and field; 2. the burrowing behaviour of juvenile soft-shell clams (*Mya arenaria*) in ambient pH sediments with a gelatin layer underneath; 3. the post-settlement dispersal of juvenile soft-shell clams from sediments of different pH in the field; and 4. the burrowing behaviour of juvenile soft-shell clams in sediments (from the Bay of Fundy) varying naturally with respect to pH. I first tested whether or not the presence of a gelatin layer underneath a thin (2.5 cm) layer of sediment could maintain porewater pH stability in marine sediments in both the field and the laboratory (Experiment 1), and tested whether or not the presence of gelatin altered the burrowing behaviour of juvenile clams under ambient sediment pH conditions (Experiment 2). I then used this method in a 24 h field experiment assessing the effect of porewater acidification and buffering by shell hash on dispersal of juvenile clams under natural flow conditions in a Bay of Fundy mudflat (Experiment 3). The burrowing frequency of juvenile clams was also quantified in cores collected from the Bay of Fundy, New Brunswick, Canada, which naturally varied with respect to porewater pH, to verify that the relationship previously observed between burrowing and laboratory-manipulated pH (Green et al. 2013, Clements & Hunt 2014) also exists under natural variation in pH (Experiment 4).

3.2 Methods

3.2.1 Sediment collection and manipulation

Sediment for all experiments (see Table 3.1) was obtained from mudflats along the northern shore of the Bay of Fundy in southwest New Brunswick, Canada (Table 3.2). For Experiments 1 and 2, the sediment was obtained from the Cassidy Lane mudflat in Little Lepreau; sediment for Experiment 3 came from the Red Head Road mudflat (Table 3.2). For each of these three experiments, sediment was collected from the surface (top 5 cm) of the mudflat with a shovel, transferred into buckets, and brought back to the lab (approx. 30 min transport time); anoxic sediment was avoided during collection. Upon return to the lab, sediment was immediately sieved (1 mm mesh) to remove any large rocks or animals and was allowed to settle in laboratory seawater for 24-48 h prior to experimentation.

For Experiment 4 (Table 3.1), sediment cores (6.5 cm diameter, 4-6 cm depth) were collected from four Bay of Fundy mudflats (Table 3.2) with acrylic cores. Cores were transferred from the field to the lab in a cooler with ice packs to ensure pH stability (M. Green, pers. comm.; Clements, unpublished data). Upon arrival to the lab, cores were placed in a recirculating seawater system (Aquabiotech Inc.) and were left to settle for 10 mins prior to experimentation.

To obtain lower sediment pH levels in experiments in which pH was manipulated (Table 3.1), CO_2 was added to a sample of sediment by sparging (flushing with CO_2 gas via an air stone and tube connected to a CO_2 cylinder) before starting the experiment. CO_2 was added for a given time period to obtain desired porewater pH levels. Sediment

was gently stirred immediately after sparging to homogenize each sample and the sediment was allowed to settle for approximately 10-15 min prior to initiating experiments (a detailed description of this methodology can be found in Clements & Hunt (2014)). Control pH treatments consisted of unsparged mud (i.e., no CO_2 added) which was stirred in a similar manner as the CO_2-treated mud prior to experimentation. Porewater pH was measured approximately 5-10 min after sparging/stirring. In each experiment, pH was measured using an Accumet AB15 meter equipped with a MI-4146 microelectrode (Microelectrodes Inc.). Sediment depths in Experiments 1-3 were 2.4 cm (underlain with a 2.6 cm deep layer of gelatin), while natural cores for Experiment 4 were 4-6 cm deep. For each sediment container in Experiments 1-3, pH was measured by hand at random depths within the top 2 cm of the sediment in three different spots (i.e., triplicate measures within a container) located approximately halfway between the centre of the container and the edges; pH was measured in the top 1 mm in Experiment 4 (see Methods section *2.6*) using a Velmex Unislide System to obtain precise depth measurements. All lab experiments were carried out in controlled overlying water temperature (21.7 ± 1.0), salinity (31.5 ± 1.1), and pH (7.87 ± 0.05) (n = 31) conditions; field experiments were exposed to natural variation in overlying temperature (21.1 ± 0.9), salinity (31.8 ± 0.9), and pH (7.42 ± 0.04) (n = 2) within the intertidal mudflats in which they were conducted.

3.2.2 Gelatin preparation

Gelatin used in Experiments 1-3 was Knox® Unflavored Gelatin purchased from a grocery store. For each gelatin sample, 9 pouches of gelatin powder (each pouch containing 15 ml of powder pre-measured to set 500 ml of liquid) were mixed with 450

ml boiling filtered seawater (salinity ~32 ‰, pH ~7.8) in square Ziploc® plastic containers (14.8 x 14.8 cm, 5 cm depth). This yielded a gelatin depth of 2.6 cm in each container; sediment samples without gelatin had an additional layer (2.4 cm) of sediment, rather than gelatin, underneath to ensure the sediment surface was at the same height in the container. Once the gelatin liquid was mixed, the containers were placed in a refrigerator for approximately 18 h to allow the gelatin to set. After 18 h, containers were removed and 2.4 cm of either control or CO_2-manipulated sediment (see next paragraph) was added to each container. The pH of the gelatin was measured in each sample (10 samples, triplicate measurements per sample) before setting, after setting, and upon the completion of the experiments.

3.2.3 Experiment 1: pH stabilization using gelatin in the lab and field

In the lab, 24 h and 7 d experiments were conducted to test the efficacy of gelatin in stabilizing porewater pH, while a 24 h field experiment tested the stabilizing capacity of gelatin in the field (Table 3.1). For the 24 h experiments in both the lab and field, a 2 × 2 factorial design was employed to test if gelatin placed underneath a layer of sediment stabilized pH in sediments. Two factors were assessed: gelatin (2 levels: sediment with or sediment without gelatin underneath) and sediment CO_2 (2 levels: spiked or unspiked sediment), yielding a total of 4 experimental sediment treatments (no gelatin, no CO_2; no gelatin, with CO_2; gelatin, no CO_2; and gelatin, with CO_2). Each treatment combination was replicated five times. For the 7 d lab experiment, the pH stabilization capability of gelatin in three separate CO_2 treatments (no CO_2, mid CO_2, and high CO_2; see below), along with a control treatment (no CO_2, no gelatin) was tested; each treatment contained five replicates. In the lab, sediment samples in the 24 h experiment were placed in still

seawater for the duration of the experiment, while the samples in the 7 d experiment were held in a recirculating seawater table. In the field experiment, sediment samples were deployed in the Red Head Road mudflat in southwest New Brunswick, Canada (Table 3.2) at low tide on 19 September 2013 and retrieved 24 h later on 20 September 2013. For each replicate, a hole was dug in the mud to the approximate depth of the container (~5 cm) the container was placed inside, and the mud surrounding it was flattened and smoothed so the surface of the mud in each container was level with surface of the mudflat. I chose a different mudflat for this experiment to avoid potential tampering by clam harvesters, whom were present at other mudflats in the area during this time.

For the CO_2 treatment in the 24 h experiments, porewater pH was manipulated by adding CO_2 for a 30 s period to each sample (by sparging; see Methods section *2.1*). For the 7 d experiment, the mid and high (equivalent to the CO_2 treatment in the 24 h experiment) CO_2 treatments were obtained by adding CO_2 for 15 and 30 s, respectively. The CO_2 treatment levels were chosen to obtain distinct levels of porewater pH (pH ~7.0 and 6.8) falling within the range of conditions observed on mudflats in the Bay of Fundy.

For the 24 h lab and field experiments, as well as the 7 d lab experiment, pH was measured as outlined above. In the lab, temperature, salinity, and pH were measured in the overlying water every hour for the 24 h experiment and every day for the 7 d experiment; the same measures were taken for the field experiment in seawater at the water line during low tide at the start and end of the field experiment with a Fisher Brand thermometer and a VEEGEE STX-3 Salinity 0-100 ‰ refractometer,

respectively. In the lab, triplicate sediment pH measurements were taken in each sample every 30 min for the first 4 h, every 60 min for the subsequent 8 h, and every 120 min for the remaining 10 h. In the field, triplicate pH measurements of sediment in the containers were taken prior to deployment and after sample collection. In both the lab and field, the pH meter was calibrated (3-point calibration; pH 4, 7, and 10 buffers) after every 10 samples (30 measurements) to avoid inaccurate geochemical measurements due to drift.

3.2.4 Experiment 2: Juvenile clam burrowing behaviour in response to gelatin

To ensure that the presence of gelatin did not act as a negative cue and impede the burrowing of juvenile soft-shell clams, a lab trial was conducted. Sediment with no CO_2 added was placed on top of one of two gelatin treatments (with or without gelatin), with four replicates per gelatin treatment. Gelatin and sediment were prepared in the same manner as described previously. Sediment samples were placed in still seawater and sixteen *Mya arenaria* (≤5 mm shell length) were placed onto each sediment sample and given 20 min to burrow, after which the number of clams burrowed into the sediment was recorded. The time period allowed for clam burrowing (20 min) was based on prior observations in the lab of the time after which virtually all (>90%) clams of this size will have burrowed below the surface. Clams were obtained from the same mudflat in Little Lepreau, NB as in Experiment 1, were kept in the lab under ambient seawater conditions (temperature ~ 21 °C, salinity ~32 ‰, pH ~7.83), and were fed daily with live algae (approximately 500 mL per 8 L holding container of each of three species: *Isochrysis galbana* (Tiso), *Monochrysis lutheri* (Mono) and *Chaetoceros mulleri* (Chagra) obtained

from the Coastal Zones Research Institute in Shippigan, NB). Temperature, salinity, and pH in the overlying water were not measured during Experiment 2 (assumed to be comparable to the other laboratory experiments).

3.2.5 Experiment 3: Dispersal of juvenile soft-shell clams in a Bay of Fundy mudflat

Using the gelatin method for stabilizing porewater pH, a field experiment was conducted to assess the effect of porewater acidification on recruitment and dispersal of juvenile soft-shell clams in a Bay of Fundy mudflat. In addition to manipulating porewater pH with CO_2 and using gelatin to stabilize the treatments, additional treatments using shell hash were included to determine whether juvenile soft-shell clam dispersal could be enhanced by stabilizing and/or raising porewater pH. Prior to the experiments, gelatin and sediment were prepared as described for the previous experiments.

To test the hypothesis that there is a negative linear relationship between clam dispersal and porewater pH, 15 containers lined with gelatin (on bottom) and sediment (on top of gelatin) were deployed in the mudflat. For comparability to a previous lab experiment examining the effect of sediment acidification on clam dispersal (Clements and Hunt 2014), a regression-style design was employed. The sediment in each container was sparged with a different, random amount of CO_2 (random time intervals between 0 and 40 s) prior to transport and deployment in the field to obtain a range of pH conditions comparable with field observations at several mudflats in the Bay of Fundy (pH: 6.26-7.54). To further explore the effects of porewater acidification and the

potential for buffering to reduce clam dispersal, additional treatments were run in parallel with the CO_2 treatment variable described above. Five replicates of sediment unmanipulated with respect to CO_2 were assigned to each of three treatments (hereafter called "buffering types"): 1. gelatin – gelatin placed below a layer of unmanipulated sediment (stabilize but not increase porewater carbonate parameters); 2. shell hash – sediment mixed with crushed shell hash (stabilize and increase carbonate geochemical parameters); and 3. control – unmanipulated sediment (neither stabilize nor increase carbonate geochemical parameters). Sediment in the experimental containers was then transferred to the mudflat and deployed as described for experiment 1. Twenty *M. arenaria* (≤2 mm) were then placed on top of each container. Clams were obtained and held prior to the experiment as described in Experiment 2, and were fed 15 drops of Instant Algae® Marine Microalgae Concentrates Shellfish Diet 1800 (Reed Mariculture Inc., USA) daily prior to deployment. Containers were deployed at the Red Head Road, NB mudflat (Table 3.2) at low tide on 19 September 2013 and retrieved 24 h later on 20 September 2014; the tidal range (semidiurnal tide) on these dates was 7.1 m. Upon retrieval, sediment from each sample was sieved (180 µm) and preserved in 80% ethanol and examined under a dissecting microscope to quantify the number of clams that dispersed from each sample. Prior to the deployment of each sample, porewater pH was measured as outlined above; pH was not measured at the end of the experiment due to a pH probe malfunction.

3.2.6 Experiment 4: Burrowing behaviour of juvenile soft-shell clams in sediments with naturally varying pH

To verify whether burrowing behaviour of juvenile *Mya arenaria* was affected by naturally induced acidification (i.e., field sediment unmanipulated with respect to porewater pH, but with naturally varying pH), the percentage of juvenile soft-shell clams burrowing into the sediment was assessed as a function of naturally-varying sediment porewater pH. Sediment cores (6.5 cm diameter, 4-6 cm depth) were collected from four mudflats from the Bay of Fundy, New Brunswick, Canada (Table 3.2) on 12 August, 2013 with acrylic cores. The experiment used 5-7 cores from each mudflat which varied naturally in pH. Sediment grain size and organic content were similar across three of the four mudflats, with Cassidy Lane having a higher grain size and %OC than the other three sites (Table 3.2). In each sediment core, 10 juvenile clams (2-6 mm shell length) collected from Cassidy Lane were placed on top of the sediment and were left to burrow for 5 h under continuous observation. Although the observation time employed in this experiment was far longer than previous experiments (i.e., 5 h versus 20 min), prior laboratory observations showed that, if they are going to burrow, virtually all clams (>90%) will burrow within 20 min and remain burrowed for up to 5 h afterwards (Woodard, unpublished data). After 5 hours, the number of clams remaining on the sediment surface was enumerated and the proportion of clams that had burrowed into the sediment was calculated. Temperature, salinity, and pH were measured as previously described.

3.2.7 Statistical analyses

All statistical analyses were run using R version 2.14.1 (R Core Team 2013). Changes in porewater pH over time in both the lab and field were analysed using linear mixed models to test for effects of treatment (all possible CO_2-gelatin combinations), container (individual replicates), and time (each measurement period in the lab; before and after in the field) on porewater pH stability; treatment and time were fixed factors, while container was random. For this repeated measures design, AIC model selection was used to determine the correlation structure which best fit the data for each experiment. That is, models with various correlation structures were constructed and AIC was used to select the best model for each experiment. The model for the 24 h lab experiment included a first order autoregressive correlation structure with homogenous variances while that for the 24 h field experiment included no correlation structure; heterogeneous variances did not improve the fit of the models.

Student's t-test (assuming equal variances) was used to test if the presence of gelatin had an effect on juvenile clam burrowing behaviour in the lab and dispersal in the field. To test the effect of intensity of porewater acidification on juvenile clam dispersal in the field, linear regression was employed. One-way ANOVA was used to test the effect of buffering type (gelatin, shell hash, and control (i.e., no buffer)) on clam dispersal and a Tukey HSD post-hoc test was employed to determine pairwise differences between each of the three treatments. For the burrowing experiment, one-way ANOVA was used to test for an effect of site on pH to ensure that site was not a confounding factor. The

relationship between porewater pH and % burrowing in natural sediment was assessed using linear regression. Assumptions of equal variance and normality were tested using Cochran's tests and Q-Q plots, respectively for ANOVAs and t-tests. For regression analysis, assumptions of normality, equal variances, and linearity were tested using Q-Q plots, non-constant error variance tests, and component residual plots, respectively. All data met the assumptions of their respective statistical tests.

3.3 Results

3.3.1 Experiment 1: pH stabilization using gelatin

The addition of CO_2 for 30 s resulted in pH conditions in the 24 h lab and field trials of 6.94 ± 0.04 and 6.82 ± 0.03, respectively (mean ± SE, n = 5 replicates; Figures 3.1, 3.2); the pH of the overlying seawater was stable throughout the laboratory experiment (7.86 ± 0.05). In comparison to laboratory conditions, seawater pH in the field was lower, but did not differ drastically between the start and end of the experiment (7.42 ± 0.04). In the 7 d lab experiment, the addition of CO_2 for 15 s (mid CO_2) resulted in an initial (day 0) pH of 7.05 ± 0.02, and a mean pH (over the 7 d) of 6.84 ± 0.18, while 30 s of CO_2 (high CO_2) yielded an initial pH of 6.85 ± 0.05 and a mean pH of 6.74 ± 0.10 (data are means ± SE, n = 5 replicates; Figure 3.3). Overlying seawater pH was comparable to those of the 24 h lab experiment (7.87 ± 0.06).

Gelatin pH was found to be acidic and was much lower than that of the sediment and overlying water. Gelatin pH values before setting and after setting were quite similar at 4.85 ± 0.02 and 4.95 ± 0.04, respectively (mean ± SE, n = 10 replicates). After 24 h,

gelatin pH had risen approximately 0.5 units to 5.37 ± 0.02 in both the lab and field (mean ± SE, n = 10 replicates).

In the lab, distinct differences in pH were initially evident between sediment with and without added CO_2, but not between treatments with and without gelatin (Figure 3.1). After approximately 7 h, pH in the "no gelatin, with CO_2" treatment had returned to levels prior to manipulation (i.e., levels without CO_2), while pH in all of the other treatment combinations remained relatively stable for the full 24 h (Figure 3.1). Porewater pH in the "no gelatin, no CO_2", "with gelatin, no CO_2", and "with gelatin, with CO_2" treatments showed very little change, decreasing by approximately 0.5%, 0.2%, and 0.6%, respectively. In contrast, porewater pH in the "no gelatin, with CO_2" treatment increased by approximately 5%. The linear mixed model indicated a significant interaction between treatment and time on porewater pH (Table 3.3). Although separation of treatments was evident between the CO_2 treated sediments with and without gelatin, within-treatment pH variability was quite high, likely due to the method of CO_2 manipulation (sparging) and the establishment of depth profiles in the presence of gelatin over time (depth profiles measured in a follow-up experiment and not during Experiments 1-3; data not shown).

Results for the 24 h field experiment were similar to those of the 24 h lab experiment, with only the no-gelatin CO_2-added treatment showing a large change in sediment pH over the course of 24 h (Figure 3.2). While pH in the "no gelatin, no CO_2" treatment increased slightly, pH in the "with gelatin, no CO_2" did not change and the "with gelatin, with CO_2" treatment decreased slightly. In contrast, porewater pH in the "no gelatin, with CO_2" treatment increased fairly dramatically. Over the course of 24 h

in the field, treatment and time had a significant interactive effect on porewater pH stability (Table 3.4).

In the 7 d experiment, gelatin was observed to stabilize porewater pH for a period of 24-72 h. pH in the high, mid, and low pH treatments with gelatin initially remained relatively stable, decreasing very slightly over 3 d (Figure 3.3). In contrast, sharp decreases in pH were observed after 3 d (Figure 3.3). Although separation of the treatments with and without CO_2 was maintained across the 7 d, the pH in gelatin treatments decreased after 24-72 h and the pH in the mid and high CO_2 treatments converged (Figure 3.3).

3.3.2 Experiment 2: Juvenile clam burrowing behaviour in response to gelatin

Porewater pH of the sediment for the trial testing the effect of gelatin on juvenile clam burrowing (i.e., digging into sediment) was similar in the two gelatin treatments, neither of which had added CO_2 (gelatin: 7.34 ± 0.05; no gelatin: 7.37 ± 0.04; mean ± SE, n = 4 replicates each). Juvenile clam burrowing behaviour was not influenced by the presence or absence of gelatin (t-test: $t_6 = -0.33$, $P = 0.750$). After 20 min, 93.8 ± 9.4 % and 92.2 ± 0.0 % of clams had burrowed into samples with and without gelatin, respectively.

3.3.3 Experiment 3: Dispersal of juvenile soft-shell clams in a Bay of Fundy mudflat

For the dispersal experiment, manipulation of porewater pH using CO_2 yielded conditions ranging from 6.26-7.54 prior to deployment (Figure 3.4), mimicking natural conditions in the Bay of Fundy (Clements unpublished data). Linear regression revealed that, under natural flow conditions, clams dispersed (i.e., moved away from sediment patch) at higher rates when exposed to more acidic sediments than less acidic sediments after 24 h (Figure 3.4).

For the buffering treatments, porewater pH prior to the start of the experiment was different between the three buffer treatments. ANOVA revealed a significant effect of buffer on clam dispersal, regardless of buffer type ($F_{2,13} = 9.44$, $P = 0.0041$). Juvenile soft-shell clams displayed lower dispersal (higher retention) in the gelatin and shell hash treatments in comparison to sediments without gelatin (Tukey HSD, $P < 0.05$); however, there was no significant difference observed between the gelatin and shell hash treatments (Figure 3.5). Only 19 % and 24 % of clams dispersed after 24 h in sediments with gelatin and shell hash, respectively, while 50 % dispersed from sediment treatments without gelatin or shell hash.

3.3.4 Experiment 4: Burrowing behaviour of juvenile soft-shell clams in sediments with naturally varying pH

Sediment cores collected from Bay of Fundy mudflats yielded pH conditions ranging from 6.84-7.37 (Figure 3.6). One-way ANOVA revealed no significant effect of

site on clam burrowing behaviour ($F_{3, 21} = 0.159$, $P = 0.922$). The burrowing behaviour of juvenile soft-shell clams varied significantly with intensity of natural porewater acidification. A significant positive relationship was observed between the percent of clams burrowed and pH. 100% of juvenile clams burrowed into sediments with the highest pH (7.37), even though the sediment was relatively low with respect to pH, while only 10% burrowed into the lowest pH (6.84) sediments (Figure 3.6).

3.4 Discussion

3.4.1 Ecological implications of porewater acidification with respect to juvenile bivalves

The results of this study suggest that gelatin can act to stabilize porewater pH over a 24-48 h period (Experiment 1) without altering the behaviour of burrowing bivalves (Experiment 2). This provides a novel tool to test the acute biological effects of porewater acidification in both the lab and field (but see below for limitations), as demonstrated by the successful test of the impacts of porewater acidification on the post-settlement dispersal of juvenile soft-shell clams in a Bay of Fundy mudflat (Experiment 3). Coupled with the results of Experiment 4 (i.e., a positive linear relationship between juvenile clam burrowing and sediment pH), it appears that sediments with low pH porewater can alter the burrowing behaviour of juvenile soft-shell clams such that fewer clams burrow into low pH sediment. Consequently, the post-settlement dispersal of these clams is also changed such that more clams are entrained and move away from low pH sediments, likely by bedload transport.

Although previous laboratory studies have examined the effects of porewater acidification on the burrowing behaviour and dispersal of infaunal bivalves (Green et al. 2013, Clements & Hunt 2014), the present study is the first to my knowledge to document these impacts using unmanipulated (natural, field) levels of water flow (Experiment 3) and sediment acidification (Experiment 4). While these results highlight the impacts of sediment acidification, other factors varying across cores such as grain size and organic content could have also influenced clam burrowing behaviour in Experiment 4. However, while the cores were collected from 4 sites which varied to some degree in sediment characteristics (Table 3.2), there was no significant effect of site on clam burrowing, suggesting that acidification, which varied considerably among cores within a site, was the primary factor impacting burrowing in Experiment 4. Thus, the results of Experiments 3 and 4 confirm those of Green et al. (2013) and Clements & Hunt (2014) obtained under laboratory conditions with sediment manipulated by the addition of CO_2. Together, the results of these 3 studies suggest that porewater acidification can negatively affect the burrowing behaviour of early life stage bivalves, can subsequently impact post-settlement dispersal, and may ultimately influence the recruitment patterns of these animals.

Although documented to be much more acidic than overlying seawater (Ponnamperuma 1976, Green et al. 2009), the biological impacts of porewater acidification are substantially understudied. Green et al. (2013) recently suggested that $\Omega_{aragonite}$ may be the recruitment cue for settling hard clams (*Mercenaria mercenaria*) and have previously demonstrated that undersaturated levels of $\Omega_{aragonite}$ led to substantial degrees of mortality of recently-settled hard clams in the lab, attributing such mortality

to shell dissolution from aragonite undersaturation (Green et al. 2009). In the field experiment (Experiment 3) exploring the efficacy of different buffers in reducing juvenile clam dispersal (increasing recruitment), buffering sediments (with respect to pH) significantly decreased clam dispersal relative to the control treatment (no buffer), with no significant difference observed between the two types of buffers. Buffering sediments with crushed shell hash has previously been reported to enhance the settlement of soft-shell clams (Green et al. 2009), with increased settlement attributed to resultant increase in $\Omega_{aragonite}$ from the addition of shell hash; however, it was unclear whether geochemical stability, geochemical enhancement (i.e., raised pH, saturation state, and lowered pCO_2), or the presence of conspecific shell acted as the settlement cue in this experiment. Given that shell hash was not present in the gelatin buffer treatment, that the gelatin acted to stabilize porewater pH without raising it, and there were no significant differences between the gelatin and shell hash treatments, it is likely that geochemical stability (i.e., reduced geochemical variability) is important in reducing dispersal and increasing recruitment.

In addition to porewater acidification, the presence of bromophenols secreted by capitellid polychaetes (Woodin et al. 1997), sediment disturbance (Woodin & Marinelli 1991, Marinelli & Woodin 2002, 2004), and sediment contamination (Phelps et al. 1983, Roper et al. 1995, Shin et al. 2002) have been shown to serve as recruitment cues for juvenile bivalves. The presence of bromophenols is unlikely in the experiments since all sediment was sieved 2-3 days prior to experimentation and any polychaetes capable of releasing bromophenols would not have been active within the sediment after sieving. The sediment was gently stirred to homogenize pH prior to experimentation, introducing

a potential confound of sediment disturbance, which can depress bivalve burrowing behaviour by altering porewater oxygen and ammonium (Marinelli & Woodin 2002, 2004). However, if disturbance was influencing the burrowing and dispersal results in this study, the impacts of disturbance would have been uniform across all treatments. It is also unlikely that other geochemical covariates, such as oxygen, which are related to organic decomposition or diagenetic redox reactions (i.e., the sum of reduction and oxidation of minerals within the sediment leading to geochemical changes), influenced the results reported here, as sediments never appeared black and were free of sulfurous smells. Furthermore, previous work with similar methods of altering porewater pH did not detect any significant alteration in dissolved oxygen (Green et al. 2009).

Although the results suggest that juvenile clams reject sediments with naturally acidic porewater and are transported away from them, the reason(s) for this are not clear. Carbonate geochemical parameters are highly correlated, making it difficult to determine the precise geochemical parameters responsible for observed biological impacts. Since pH was manipulated using CO_2 injection in this study, pCO_2 may also play a role in the observed dispersal patterns. Juvenile *Mya arenaria* may avoid burrowing into low $\Omega_{aragonite}$ to avoid dissolution mortality (Green et al. 2009, 2013), although larger individuals are expected to be less susceptible to dissolution mortality than recent settlers (Waldbusser et al. 2009). However, growth, metabolism, health, and other energetic attributes may also be depressed under more acidic conditions. Indeed some species have the potential to increase calcification in more acidified conditions, albeit at a cost to other physiological processes (Wood et al. 2008). It is also important to recognize that rejecting more acidic sediments is not always a beneficial behaviour for

juvenile and settling clams, as clams that do not burrow quickly are more susceptible to other post-settlement mortality factors such as transport and predation (Hunt & Scheibling 1997). Although high flow speeds do not appear to trigger the burrowing of juvenile clams in more acidic conditions (Clements & Hunt 2014), their burrowing response to porewater acidification in the presence of other mortality factors such as predators has yet to be determined.

3.4.2 Gelatin as a tool for pH stabilization in marine sediments

Because porewater geochemical parameters can vary over time scales of hours to days (Meyers et al. 1988, Marinelli 1994, Clements unpublished data), the transfer to field experiments beyond a few hours can be hindered by the absence of an effective technique for keeping porewater geochemistry stable for longer periods of time. For example, porewater pH on an intertidal mudflat can vary naturally over a time period of 24 h in the field by ±1.5 pH units (Clements unpublished data). Furthermore, although Green et al. (2009, 2013) conducted field experiments manipulating porewater carbonate geochemistry using crushed shell hash, potential complications arise when trying to discern biological effects, as organic compounds within shell may act as a cue rather than porewater geochemistry (Vasquez et al. 2013). Engstrom & Marinelli (2005) reported the use of spiked polyacrylamide gel for manipulating porewater geochemistry, specifically ammonia, in the laboratory. However, polyacrylamide gel can be toxic to living organisms and the efficacy of polyacrylamide gel in stabilizing porewater pH has never been tested. This study provides a simple method for stabilizing porewater pH for 24-48 h, while reducing the potential for toxicity (a concern when using polyacrylamide), providing a method to move from laboratory to field experiments.

Although Experiment 1 suggested that gelatin is effective in stabilizing porewater pH in the laboratory and the field, I did not test the mechanism for this. Amide peptide bond hydrolysis is a possible mechanism of gelatin's effect on pH, as bond hydrolysis can be catalyzed by an acid or a base (Steinhardt & Fugitt 1942); however, this process would not be expected to change the overall $[H^+]$. Given that gelatin is a protein based substance, the release of CO_2 from amino acids is biologically possible, but requires enzyme mediation (Chapelle & Luck 1957). Since enzyme mediation was not employed in this study, this mechanism seems doubtful. However, the presence of amino acids makes gelatin amphoteric (i.e., have properties of and react as either an acid or a base; Iqbal & Mido 2005). This property of gelatin may allow it to act as an external buffer/stabilizer, fixing pH within the sediment layer above for a brief period of time. Gelatin has been reported to act as a pH buffer in aqueous solutions via the common ion effect in studies of paper permanence (Baker 1997, Baty & Barrett 2007). Surprisingly, however, sediment without CO_2 underlain by gelatin maintained a much higher pH than the sediment with CO_2 underlain by gelatin, which seems to suggest that the gelatin alone is not generating enough protons to change porewater pH. In the 7 d lab experiment (Experiment 1), sediment pH remained stable for 24-48 h before beginning to decrease, although separation in pH was observed between treatments over the whole 7 d experiment (Figure 3.3). Furthermore, after 24 h, a gradient of decreasing pH was observed when gelatin was present below a sediment layer (same depth and grain size characteristics) in a follow-up experiment. As such, the slow diffusion of some acidic substance may be acting to stabilize porewater pH for a brief period of time, before lowering it gradually. Conversely, gelatin may be acting as a pH buffer as reported in previous studies (Baker 1997, Baty & Barrett 2007), by slowly bringing the porewater

pH down to an equilibrium point. It is important to note, however, that anoxic conditions were observed in the bottom 1-1.5 cm of sediment underlain with gelatin after 2-3 d in the 7 d experiment. Because gelatin is nitrogen rich, it likely results in bacterial degradation, producing CO_2 and potentially resulting in the gradual decrease in pH observed in the 7 d lab experiment (Experiment 1). Thus, microbial activity may be driving the longer-term influence of gelatin on porewater pH. Ultimately, however, a detailed understanding of how this method works is not possible without further studies testing the mechanism of its effect. Studies wishing to utilize this method in future experiments should test to see how long the geochemical parameters will remain stable under the particular experimental conditions being tested, as this may vary with sediment characteristics and flow conditions. However, although more work needs to be done with respect to understanding the mechanism by which gelatin is able to stabilize porewater pH and the specific experimental constraints that exist with using this method, the easy-to-use nature of commercial gelatin and its lack of toxicity (in contrast to polyacrylamide) makes it beneficial to the field of benthic ecology and allows studies manipulating porewater pH to move to the field. Given the growing awareness of ocean acidification and its potential to lower porewater pH (Widdicombe et al. 2009), coupled with the recent understanding that porewater acidification can also impact the survival, calcification, and behaviour of infaunal organisms (Green et al. 2009, 2013, Clements & Hunt 2014), the gelatin method described here seems promising for field studies assessing the biological impacts of porewater acidification.

3.4.3 Potential impacts of future ocean acidification

Experiments 3 and 4, coupled with the results of Clements & Hunt (2014), collectively suggest that juvenile soft-shell clam (*Mya arenaria*) burrowing behaviour can be hindered by porewater acidification and, upon sediment rejection, these clams may be dispersed from areas of low porewater pH. While these results are relevant to current sediment pH conditions experienced by these bivalves on intertidal mudflats, the results could also provide insight into how burrowing and dispersal patterns of juvenile *M. arenaria* may change under future ocean acidification scenarios. Given that porewater pH generally decreases as overlying seawater pH decreases (Widdicombe et al. 2009), it can be expected that, as the range of porewater pH conditions decreases in the future, juvenile *M. arenaria* (as well as settling hard clams, *Mercenaria mercenaria*; Green et al. 2013) may more frequently end up on sediment into which they will not burrow than they do under current conditions. However, it is important to note that current observations regarding the effects of porewater acidification on bivalve burrowing and dispersal are of acute impacts, and the chronic effects of such conditions are unknown. It is thus possible that these clams may be able to acclimate to the deteriorating pH conditions associated with future ocean acidification (Form & Riebesell 2011, Aase 2014, Munday 2014; but see Welch et al. 2014); however, more work is certainly necessary.

Sediment porewater may not always be negatively impacted by declining water column pH. For example, Gazeau et al. (2014) reported that near-future ocean acidification had no negative impacts on sediment processes in a shallow Arctic system. Such lack of coupling of sediment processes (particularly porewater pH) and ocean

acidification would prevent future impacts on the burrowing and dispersal patterns of juvenile clams such as *M. arenaria* in areas where this is the case. It is equally as important to note that the impact of water column acidification (and not necessarily the porewater) on bivalve burrowing are unknown and may in itself alter burrowing and dispersal patterns. However, since *M. arenaria* typically resides in coastal areas where pH conditions are highly variable and unpredictable due to the myriad of contributing acidic and basic sources (Duarte et al. 2013, Waldbusser & Salisbury 2014), the effects of future ocean acidification on bivalve burrowing behaviour are, at present, unpredictable.

Ultimately, these results suggest that naturally low porewater pH and water flow conditions observed in the Bay of Fundy are sufficient to reduce juvenile bivalve burrowing and increase post-settlement dispersal (i.e., neglecting to burrow soon after settlement or re-emerging from sediment after settlement and moving elsewhere). To adequately understand how future ocean acidification may impact bivalve burrowing, studies need to address how porewater pH and other sediment processes may change in a more acidified ocean for sediments with various compositions. Such knowledge would aid in predicting future porewater pH conditions and lead to a more comprehensive understanding of how juvenile bivalve burrowing and dispersal will be impacted in a high-CO_2 ocean.

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Table 3.1. Basic description of the four experiments conducted in this study.

	Type	Duration	Purpose	Sediment manipulation	Sediment depth	Porewater pH
Exp 1	Lab	24 h	Effect of gelatin on porewater pH stability	Sieved	2.4 cm	Manipulated
	Field	24 h	Effect of gelatin on porewater pH stability	Sieved	2.4 cm	Manipulated
	Lab	7 d	Effect of gelatin on porewater pH stability	Sieved	2.4 cm	Manipulated
Exp 2	Lab	20 min	Effect of gelatin presence on clam burrowing	Sieved	2.4 cm	Not manipulated
Exp 3	Field	24 h	Effect of porewater pH on clam dispersal	Sieved	2.4 cm	Manipulated
Exp 4	Lab	5 h	Effect of porewater pH on clam burrowing	Not sieved	4-6 cm	Not manipulated

Table 3.2. Coordinates, along with particle size (in micrometers, μm) and organic content (mean ± SD, n = 3) of sediment at the 4 mudflats where sediment was obtained for experiments conducted in this study. Experiments 1 and 2 used sediment from Cassidy Lane, while Experiment 3 used sediment from Red Head Road and Experiment 4 used sediment cores from all four sites.

Site	Latitude, longitude	Mean particle size (μm)	% OC
Cassidy Lane	45°7'28.82"N, 66°28'17.97"W	105.37 ± 5.88	3.51 ± 0.56
Little Lepreau	45°7'55.12"N, 66°28'22.02"W	77.69 ± 4.65	2.57 ± 0.33
Pocologan	45°7'11.98"N, 66°35'27.81"W	79.44 ± 38.81	2.74 ± 0.50
Red Head Road	45°6'50.00"N, 66°36'37.63"W	52.08 ± 2.25	2.67 ± 0.72

Table 3.3. Linear mixed effects model analysis of porewater pH after 24 h in the laboratory experiment.

	numDF	denDF	F-value	*P*-value
(Intercept)	1	381	36129.11	<.0001***
Time	1	381	0.87	0.3527
Sample	1	3	0.11	0.7655
Treatment	3	381	29.22	<.0001***
Time×Sample	1	381	0.04	0.8461
Time×Treatment	3	381	9.35	<.0001***
Sample×Treatment	3	381	0.35	0.7873
Time×Sample×Treatment	3	381	1.11	0.3466

*P<0.05; **P<0.01; ***P<0.001

Table 3.4. Linear mixed effects model analysis of porewater pH stability after 24 h in the field experiment.

	numDF	denDF	F-value	*P*-value
(Intercept)	1	21	37101.85	<.0001***
Time	1	21	0.03	0.8742
Sample	1	3	0.88	0.4166
Treatment	3	21	59.82	<.0001***
Time×Sample	1	21	1.13	0.2996
Time×Treatment	3	21	9.13	0.0005***
Sample×Treatment	3	21	1.71	0.1947
Time×Sample×Treatment	3	21	2.52	0.0860

*P<0.05; **P<0.01; ***P<0.001

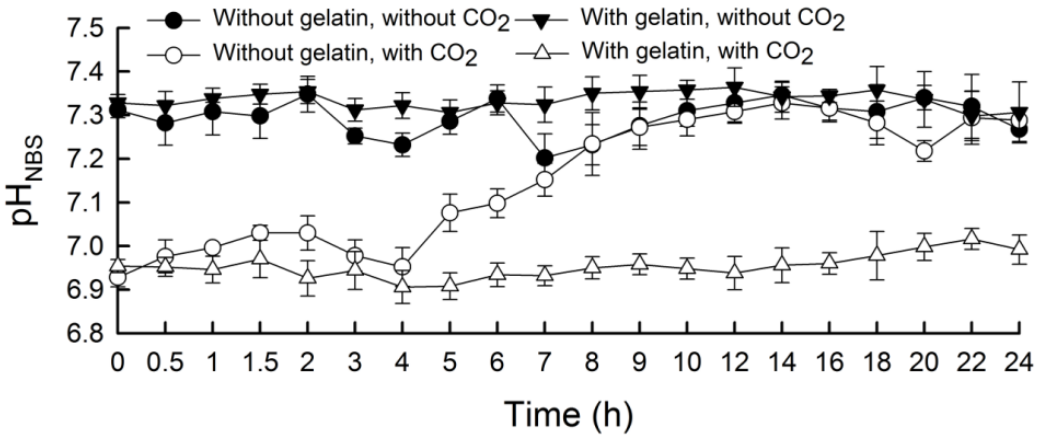

Figure 3.1. Stability of sediment pH under laboratory conditions in the four experimental treatments over time (24 h). Data are represented as means ± standard error.

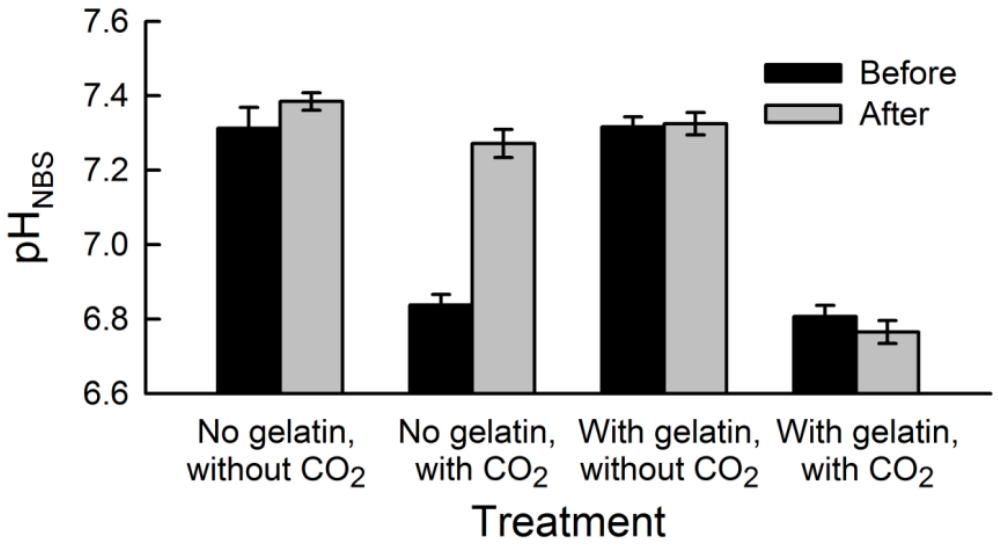

Figure 3.2. Sediment pH under field conditions in the four treatments immediately before and 24 h after the start of the experiment. Data are represented as means ± standard error.

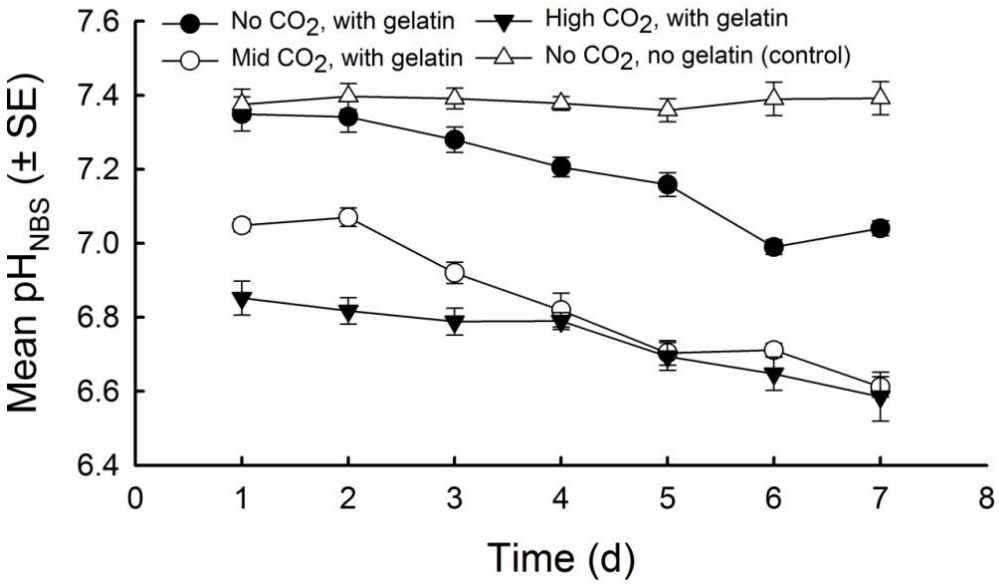

Figure 3.3. Sediment pH in four experimental treatments over time (7 d) under laboratory conditions. Data are represented as means ± standard error.

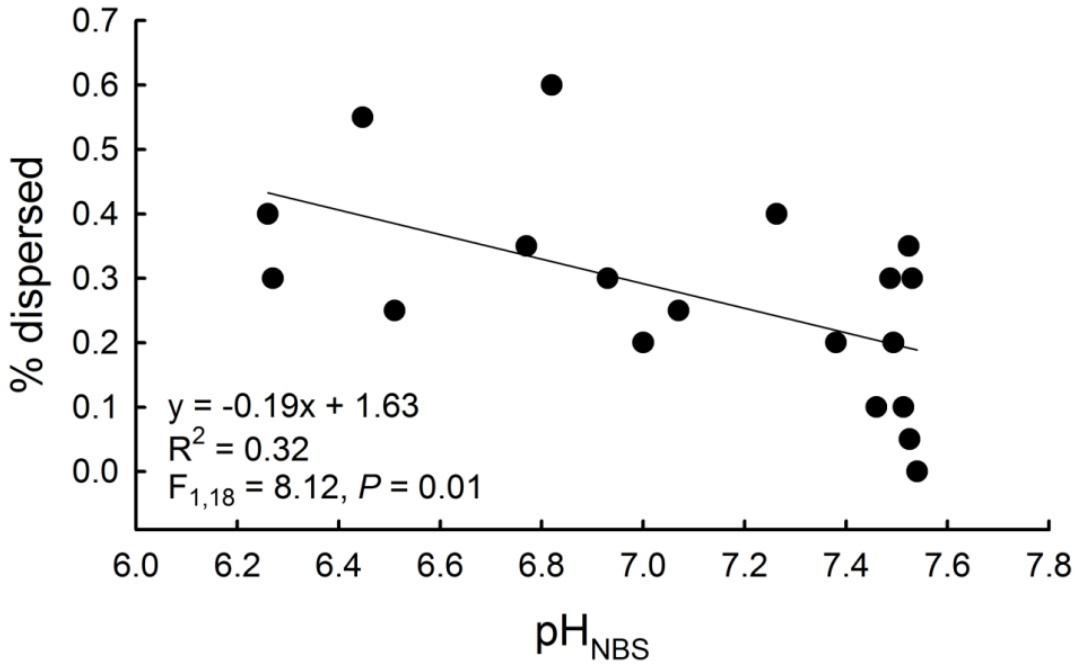

Figure 3.4. The relationship between the percentage of juvenile *M. arenaria* which had dispersed after 24 h (two tidal cycles) and initial pH in the field (n = 19 random CO_2 inputs, yielding 19 random pH).

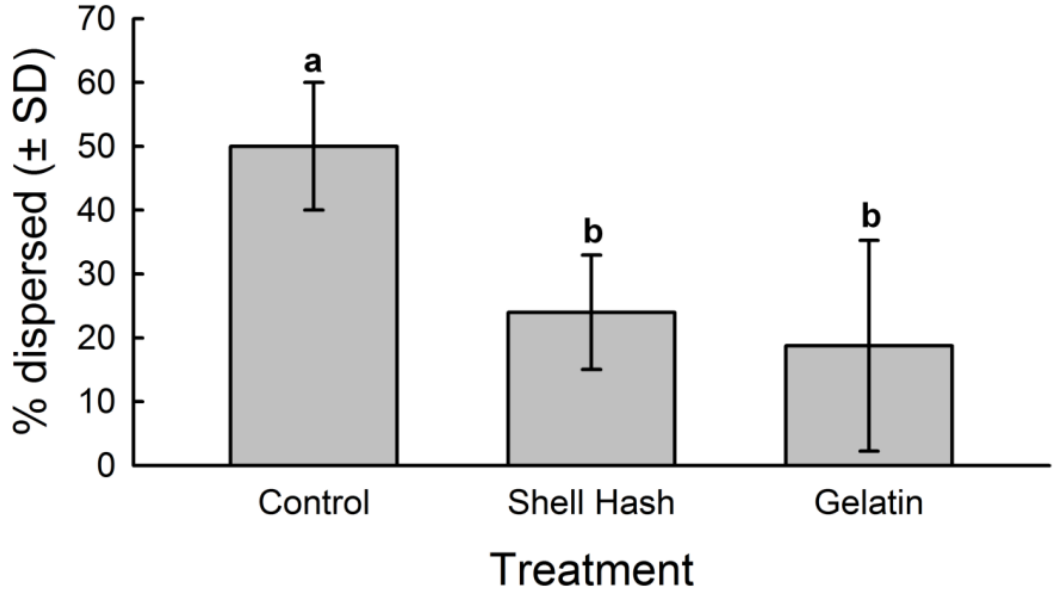

Figure 3.5. The percentage of juvenile *M. arenaria* that dispersed from each of the three buffer treatments after 24 h in the field (n = 5 samples per treatment, 20 clams per sample).

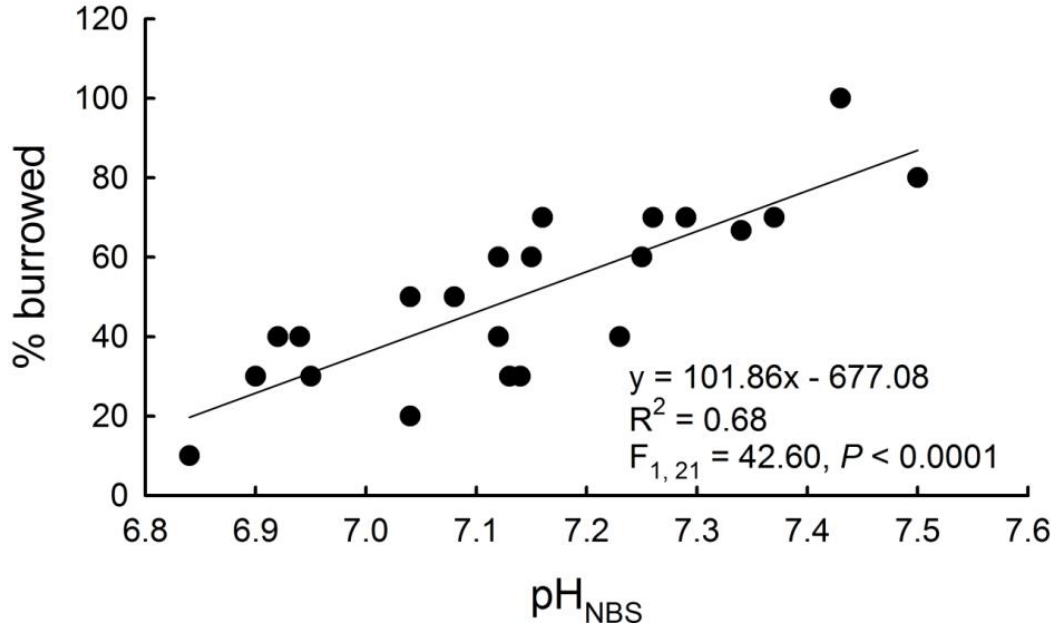

Figure 3.6. The relationship between the percentage of juvenile *M. arenaria* burrowed after 5 h and porewater pH in unmanipulated cores collected from the field (n = 22).

Chapter 4

Bivalve burrowing response to sediment acidification is a function of neurotransmitter interference and is also influenced by temperature

This chapter is to be submitted to Global Change Biology in April, 2016

Clements JC, Bishop MM, Hunt HH (2016) Bivalve burrowing response to sediment acidification is a function of neurotransmitter interference and is also influenced by temperature. Glob Change Biol.

Abstract

While ocean acidification (OA) is likely to affect marine vertebrate and invertebrate behaviour, how multiple stressors in conjunction with OA will elicit behavioural responses is not well known. Furthermore, although $GABA_A$ receptor interference appears widely responsible for OA effects on marine vertebrate behaviour, the mechanism(s) controlling invertebrate behavioural responses to acidification are poorly understood. Although OA will lead to changes in seawater carbonate chemistry, evidence suggests that it can also result in changes in sediment porewater (i.e., sediment acidification). I tested the interactive effects of seawater temperature and sediment pH on juvenile soft-shell clam burrowing. I also examined whether or not $GABA_A$-like receptor interference is the biological mechanism underpinning burrowing responses of these clams to sediment acidification. Elevated temperature alleviated the negative effects of low-pH sediment on burrowing, while clams treated with gabazine engaged in

normal burrowing behaviour under low pH conditions. This study suggests that sediment acidification impedes juvenile marine bivalve burrowing by interfering with $GABA_A$-like receptors in their pedal ganglia, and that elevated temperature can partially alleviate the negative impacts of acidification.

4.1 Introduction

Since the onset of the Industrial Revolution, approximately 30% of the excess atmospheric carbon dioxide (CO_2) emitted by anthropogenic activity has been absorbed by the ocean (Hoegh-Guldberg et al. 2014). As a result, surface ocean pH has experienced a 0.1 unit decline since the Industrial Revolution, with an additional 0.2-0.3 unit decline projected for the end of this century (RCP8.5; Hoegh-Guldberg et al. 2014). Consequently, this so called ocean acidification (OA) – the uptake of anthropogenic CO_2 by the ocean surface and the resultant drop in pH – is expected to elicit a myriad of effects on marine organisms (Fabry et al. 2008, Doney et al. 2009, Kroeker et al. 2010, Gazeau et al. 2013, Clements & Hunt 2015). A number of studies have paid particular attention to the physiological effects of OA on marine organisms (e.g. Pörtner *et al.*, 2004; Pörtner, 2008). However, it has become increasingly recognized that OA can have major implications for animal behaviour in vertebrates (fishes) and invertebrates (Munday et al. 2009, Briffa et al. 2012, Clements & Hunt, 2015).

Although OA is expected to elicit chemical and biological impacts in the open ocean, coastal systems are exposed to an array of acidic sources that the open ocean is not, such as acidic terrestrial and freshwater runoff, coastal upwelling, changes to watershed dynamics, and eutrophication (among others; Duarte et al. 2013). Given that

these sources of acid are currently abundant in many estuaries and coasts, carbonate system conditions in many coastal areas are already much more acidic than future open-ocean projections and often exhibit substantial fluctuations in seawater pH and carbonate chemistry (Reum et al. 2014, Wallace et al. 2014). Consequently, organisms residing in coastal zones may already be exposed to pH and carbonate system conditions beyond those projected for the open ocean by 2100 (Duarte et al. 2013; Waldbusser & Salisbury 2014). Since coastal zones already experience conditions beyond near-future OA projections, it has been argued that OA is strictly an open ocean problem (Duarte et al. 2013). However, the processes contributing to coastal acidification can act to alleviate or amplify CO_2-induced OA in nearshore waters (Waldbusser & Salisbury 2014). Furthermore, the more acidic and highly fluctuating conditions in coastal zones do not necessarily mean that coastal organisms will be able to better adapt to increasing CO_2 (Jansson et al. 2013). As such, understanding how organisms residing within coastal areas are currently affected under atypically low pH conditions can provide a window for understanding long-term biological impacts of near-future OA in both coastal and open-ocean systems.

While many studies have focused on the behavioural impacts of water column acidification on pelagic and epifaunal organisms, relatively few have examined the impacts of sediment acidification on infaunal organisms. A variety of processes influence sediment pH, including the cycling of organic matter and calcium carbonate, along with tidal pumping and groundwater discharge (Waldbusser & Salisbury 2014). However, a reduction of overlying water pH can result in reduced sediment pH and elicit impacts on infaunal organisms directly exposed to porewater conditions (Widdicombe et

al. 2009). Consequently, infaunal organisms are unlikely to be as immune to the effects of OA as previously assumed (Widdicombe et al. 2011). With respect to behaviour, settling and juvenile bivalves appear to be particularly vulnerable to sediment acidification. For example, Green et al. (2013) reported that sediment acidification acted as a negative recruitment cue for settling *M. mercenaria*, as clams failed to burrow into aragonite undersaturated sediments. Similar results have been reported by Clements & Hunt (2014), who found that juvenile soft-shell clams (*Mya arenaria*) failed to burrow into more acidic sediments, and subsequently were entrained when exposed to flow. Although carbonate saturation state has been suggested as the negative recruitment cue (Green et al. 2013), as undersaturated sediments can be a significant mortality factor for settling bivalves (Green et al. 2009), the mechanism(s) by which settling and juvenile bivalves reject more acidic sediments is currently unknown.

While a mechanism for bivalve rejection of more acidified sediments is unknown, $GABA_A$ receptor functioning has been suggested as a mechanism by which OA affects marine animal behaviour in vertebrates (Nilsson et al. 2012, Hamilton et al. 2014, Chivers et al. 2014, Lai et al. 2015) and cephalized invertebrates (Watson et al. 2014). GABA (gamma-aminobutyric acid) is a primary inhibitory neurotransmitter found widely in the nervous systems of vertebrates (central and peripheral) and some invertebrates (peripheral) (Jessen et al. 1979). This neurotransmitter opens ion channels and allows ions to flow in and out of cells (Nilsson et al. 2012), and is associated with two neuroreceptors – $GABA_A$ and $GABA_B$ – with the former having a specific conductance for Cl^- and HCO_3^-. Under ambient CO_2 conditions, Cl^- and HCO_3^- concentrations are slightly higher outside the cell than inside the cell. As a result,

equilibrium potential is near resting membrane potential, so when GABA binds to $GABA_A$ receptors and the receptors open, these ions flow into the cell to prevent depolarization, keep membrane potential negative, and reduce neural activity (Nilsson et al. 2012). In contrast, when CO_2 concentrations are elevated, Cl^- flows out of the cell and HCO_3^- is accumulated from the external environment (i.e., seawater) to avoid acidosis (e.g. Heuer & Grosell 2014), thus altering the ion gradient across the neural membrane and potentially resulting in depolarization, neural excitation, and, ultimately, changes in behaviour. While relatively well described in cephalized marine animals, the behavioural impacts of OA on non-cephalized invertebrates are poorly understood and a mechanistic understanding of how behaviour in such organisms can be affected by OA has yet to be explored.

To date, the potential biological effects of OA in isolation have been well documented. However, the relative influence of OA in the context of multiple environmental factors has only recently been tested, and the biological effects of other environmental stressors such as temperature in conjunction with sediment acidification are currently unknown. Of the few studies that have assessed the impacts of OA on animal behaviour in the context of multiple parameters, most suggest that OA in the presence of elevated temperature will yield different results than OA tested in isolation (Dissanayake & Ishimatsu 2011, Landes & Zimmer 2012, Nowicki et al. 2012, Zittier et al. 2012, Burnell et al. 2013, Domenici et al. 2014, Ferrari et al. 2015, Queirós et al. 2015), with negative effects of OA in isolation seemingly alleviated under elevated temperature (Clements & Hunt 2015).

The role of temperature in mediating the burrowing response of marine bivalves is not well defined. Optimal burrowing in hard clams (*Mercenaria mercenaria*) and surfclams (*Spisula solidissima*) has been reported to fall between 25°C-30°C and 15°C-20°C, respectively (Savage 1976). Monari et al. (2007) also reported negative effects on burrowing behaviour of *Chamelea gallina* at 30°C. While temperature appears to influence burrowing, the interactive effect of sediment acidification and temperature on the burrowing behaviour of infaunal bivalves has yet to be tested.

Given the lack of knowledge of how multiple environmental stressors, including sediment acidification, interact to elicit biological responses, I assessed the effects of temperature and sediment acidification on the burrowing behaviour of juvenile soft-shell clams. Furthermore, since $GABA_A$ receptor functioning appears to be impacted under OA conditions in vertebrates and cephalized invertebrates, coupled with the fact that GABA receptors exist in bivalve pedal ganglia (Vitellaro-Zuccarello & De Biasi 1988, Karhunen et al. 1995;, Welch et al. 2014), I aimed to determine whether or not $GABA_A$ receptor functioning is the mechanism by which bivalve burrowing is impeded in low-pH sediments (Green et al. 2013, Clements & Hunt 2014, Rodríguez-Romero et al. 2014).

4.2 Methods

4.2.1 Animal collection and husbandry

Juvenile *M. arenaria* (≤ 3 mm) were collected from a Bay of Fundy mudflat in southwest New Brunswick, Canada (Lepreau Basin: 45°7'28.82"N, 66°28'17.97"W) under Department of Fisheries and Oceans Section 52 Permit No. 333579. Clams for

Experiment 1 (see below for Experiment 1 and 2 descriptions) were collected in early August, 2015, while clams for Experiment 2 were collected in late September, 2015; the animals for each respective experiment were kept separate during rearing and husbandry. Clams were collected on different dates because Experiment 2 was conceived after Experiment 1, and there were not enough clams remaining from Experiment 1 to conduct Experiment 2. Clams were collected by scooping the surface sediment of the mudflat at low tide into 5 gal buckets. The buckets of sediment were covered and transported back to the laboratory where the sediment was then transferred into trays (40 cm length × 25 cm width × 7 cm depth) in approximately 5 cm thick layers and submerged in aerated seawater. Sediment was then sieved on a 0.5 mm sieve, transferred into Petri dishes with seawater, and each Petri dish was then thoroughly searched for any clams 0.5-3 mm in size (shell length) using a dissecting microscope and ocular micrometer (1X magnification; 10 µm = 1 mm). Previous work determined that burrowing responses to low-pH sediment were consistent for clams spanning this size range (Clements & Hunt, 2014; Clements *et al.*, under review). For Experiment 1, an equal number of clams (400) were placed in each of 2 buckets containing approximately 8 L of aerated seawater of a given temperature for acclimation prior to the experiment (18°C or 21°C) (see Experiment 1). While these temperatures exceed maximum summer sea-surface temperatures in the Bay of Fundy, these clams often experience these temperatures at low tide and can experience similar temperature at the southern limits of their geographic range. For Experiment 2, clams were similarly housed in a bucket containing ~8 L of aerated seawater; seawater temperature was 16°C during this experiment. Seawater pH during rearing was kept at ambient conditions (7.8), and salinity was kept constant at 31-32. Clams for both experiments were fed every 2 days

with a single species of live algae (*Monochrysis lutheri*; obtained from Coastal Zones Research Institute, Shippigan, NB, Canada) at an approximate concentration of 100 mL L seawater^{-1}. Clam buckets were thoroughly cleaned and rinsed once every 2 days.

4.2.2 Experimental protocol

I conducted two separate experiments: one to test the interactive effects of temperature and sediment pH on clam burrowing behaviour, and another to test the effects of gabazine (a $GABA_A$ antagonist) and sediment pH on clam burrowing behaviour (the latter was conducted at a single temperature; 16ºC). Each experiment employed a 2×2 factorial design with the same two levels of sediment pH: control and acidified (Table 4.1).

Sediment for both experiments was collected from the same mudflat that the clams were collected from and was primarily comprised of fine sand (mean grain size = 174.7 ± 11.8 µm; φ = 2.52 ± 0.09) with a % organic content of 3.51 ± 0.56 %. Once collected, the sediment was brought back to the lab and sieved (1 mm) to remove large rocks and live animals and was left to settle in aerated seawater until experimentation (approximately 24 h). Sediment pH was manipulated and measured according to the methods described in Chapter 1. In brief, prior to the experiment, CO_2 was sparged into a bucket of sediment through an air stone and tube connected to a 50 lb CO_2 cylinder for approximately 30 s to attain the desired pH for acidified treatments (~6.5 as compared to the 7,3 control; Table 4.2); sediment was subsequently stirred for 1 min for homogenization, transferred into individual cups, and left to sit for 5-10 mins prior to measuring pH. Although the pH of the acidified treatment far exceeds future projections

for OA scenarios, such conditions are already experienced in the surface sediment of mudflats of the Bay of Fundy (Clements unpublished data) and in other Northwest Atlantic surface sediments (e.g. Hales & Emerson 1996, Green & Aller 1998, Green et al. 2009). Further, these conditions may become even more acidic and more frequent under future OA scenarios (Widdicombe et al. 2009). Sediment pH (NBS) was measured in each replicate (i.e., sediment cup) with an Accumet AB115 pH meter (Fisher Scientific) and either a MI-4146 (Experiment 1; Microelectrodes Inc.) or MI-411 (Experiment 2; Microelectrodes Inc.) pH microelectrode standardized in pH 4 and 7 buffers (Ferris Chemicals). The pH was measured by inserting the probe into the top 2 cm of the sediment and recording the pH first measurement that was stable for $\geq$ 20 s. While depth profiles in pH can exist in the top 2 cm of sediments, it is unlikely that depth profiles would develop over the short duration of the experiments and influence the results (i.e., < 40 mins). Single pH measurements were taken in each replicate; previous measurements suggest that within-replicate (i.e. within a sediment cup) variability using these methods is negligible (Clements pers. obs.). Total alkalinity (TA) was also measured in 2 of the 4 samples for each pH-temperature treatment combination in Experiment 1. Briefly, 0.5 mL porewater samples were obtained by centrifuging a ~15 mL sediment sample from each replicate (cup) at 1000 rpm for 5 min. Porewater was then extracted using a syringe, transferred into a 20 mL scintillation vial, and titrated with 0.1 N HCl according to the protocol of Edmond (1970) (2-point alkalinity titration). Temperature, salinity and pH were also measured in the overlying seawater prior to and after each experiment using a FISHERbrand thermometer, VEEGEE STX-3 Salinity 0-100 ‰ refractometer, and Accumet pH meter/probe (as described above), respectively. The mean TA from each pH-temperature treatment combination in Experiment 1 (i.e.

TA was not measured in Experiment 2; Table 4.2), along with water temperature and salinity, and individual sediment pH measurements (i.e., pH in each replicate) from each experiment, were used to calculate pCO_2, $\Omega_{aragonite}$, and $[HCO_3^-]$ in the sediment porewater for each replicate from Experiments 1 and 2. Calculations were derived using CO_2SYS (Pierrot et al. 2006), with the first and second dissociation constants reported by Mehrbach et al. (1973) refit by Dickson & Millero (1987). The control pH treatment consisted of sediment stirred for 1 min so that differences in acidification treatments would not be confounded with effects of sediment disturbance on bivalve burrowing (Woodin & Marinelli 1991, Marinelli & Woodin 2002, 2004). Similar sediment manipulation techniques (i.e., CO_2 addition) have been reported to alter sediment carbonate chemistry without affecting other abiotic covariates such as oxygen and hydrogen sulfide concentrations (Green et al. 2009) and I therefore assumed that any burrowing responses observed between pH treatments were primarily due to changes in sediment carbonate chemistry.

I used burrowing proportion (# of clams burrowed ÷ # of clams deployed) as a metric for clam burrowing behaviour. Clams in each replicate were allowed to burrow for 20 min, with the number of clams burrowed in each replicate being enumerated afterward. Preliminary experiments have shown that virtually all clams that are going to burrow will do so within 20 min (Clements, pers. obs.). Upon completion of the experiment, clams were euthanized by freezing at -80ºC for 24 h.

4.2.3 Experimental design

4.2.3.1 Experiment 1

For the first experiment, temperature treatments consisted of a control treatment (18°C) and an elevated temperature treatment (21°C), which were maintained during clam rearing and husbandry, as well as during experimentation. The control temperature was chosen to mimic the upper boundary of summer temperatures in shallow tidal flats of the Bay of Fundy, while the elevated temperature treatment was chosen to mimic predicted temperature increases under ocean warming (+3°C). During rearing, the control temperature treatment was maintained by housing the clam bucket (see above) in a recirculating seawater system with temperature control and the elevated temperature treatment was maintained using a standard aquarium heater (Hagen Inc.). Clams were allowed to acclimate to their respective temperature treatment for 1 week prior to experimentation; seawater pH was kept at ambient conditions during this time (~7.8). During experimentation, the control temperature treatment was carried out in trays which were placed in the temperature-controlled recirculating seawater system; the seawater in the trays was acclimated to the temperature of the surrounding seawater in the recirculating system (18°C) prior to the experiment. The elevated temperature treatment was carried out in trays filled with seawater which was maintained at the experimental temperature (21°C) using an aquarium heater. Conditions other than temperature were similar across both temperature treatments (i.e., salinity ~ 32 and pH ~ 7.8).

There were 4 experimental replicates per temperature × sediment pH treatment combination. Each replicate consisted of sediment from one of the two pH treatments (i.e., 7.3 or 6.5) in a plastic cup, submerged under still (i.e., no flow) seawater maintained at one of the two temperature treatments. Once the sediment was submerged in seawater, it was allowed to settle (i.e., become stable) for 10 min in seawater, after which 20 clams from the corresponding temperature treatment were placed on the sediment surface and given 20 min to burrow (as described above).

4.2.3.2 Experiment 2

In the second experiment, the gabazine treatments consisted of administering (experimental treatment) or not administering (control treatment) gabazine to clams prior to the experiment and the same two pH treatments as in Experiment 1. Gabazine is a $GABA_A$ antagonist which blocks GABA from binding to the $GABA_A$ neurorecptor, keeping the $GABA_A$ neurorecptor closed. Thus, if $GABA_A$ is the physiological mechanism responsible for bivalve burrowing responses to sediment acidification, clams drugged with gabazine should burrow into sediment regardless of porewater pH, while clams not drugged would reject lower pH sediments. There were 10 replicates per gabazine × pH treatment combination. Gabazine was administered to a subset of clams by transferring them into separate beakers containing seawater (same temperature as during rearing; 16°C) with a gabazine (Sigma-Aldrich SR-95531) concentration of 5 mg L seawater $^{-1}$. Although a slightly higher dosage than previous studies (e.g. 4 mg L seawater $^{-1}$; Nilsson et al. 2012, Chivers et al. 2014, Hamilton et al. 2014, Watson et al. 2014, Lai et al. 2015), this concentration was used given the large number of animals being administered gabazine simultaneously; there was no mortality resulting from

gabazine administration. Heat (24-26°C) and gentle stirring were used to dissolve the gabazine; the temperature in each beaker was then acclimated to the same temperature at which the clams were held and the water was aerated before placing the clams from each temperature treatment into their respective gabazine treatments for 30 min prior to being placed onto the sediment. Each cup of sediment (as described in Experiment 1) from one of the two pH treatments was submerged under still seawater kept at the same rearing temperature of the clams (16°C) in a recirculating seawater system. As with Experiment 1, sediment was given 10 min to settle after it was placed in seawater, after which 8 clams from the corresponding gabazine treatment were transferred to the sediment surface and given 20 min to burrow.

4.2.4 Statistical analyses

All statistical analyses were conducted using R version 3.2.1 (R Core Team 2015) with a significance level of $\alpha = 0.05$. Separate two-way ANOVAs were used to test for the effects of seawater temperature and sediment pH, and gabazine and sediment pH, on clam burrowing in Experiment 1 and 2, respectively. Tukey HSD post hoc analysis was used to test for pairwise significance where significant interactions existed. Assumptions of normality and homoscedasticity were tested using Q-Q plots and Levene's tests, respectively. Data did not violate these assumptions.

4.3 Results

4.3.1 Sediment carbonate chemistry and overlying seawater conditions

In general, geochemical conditions were quite similar within their respective pH treatments for both experiments, regardless of temperature (Table 4.2). pCO_2 and $[HCO_3^-]$ were consistently higher in the low pH treatments. In Experiment 1, pCO_2 was ~1100 µatm in the control pH treatments and ~7600 µatm in the low pH treatments, while $[HCO_3^-]$ was ~ 2100 µmol kg^{-1} SW in the control pH treatments and ~2250 µmol kg^{-1} SW in the low pH treatments (Table 4.2). Similarly, $[HCO_3^-]$ was ~ 2100 µmol kg^{-1} SW in the control pH treatments and ~2250 µmol kg^{-1} SW in the low pH treatments in Experiment 2, while pCO_2 was higher in both pH than in Experiment 1 and was ~1500 µatm in the control pH treatment and ~9500 in the low pH treatment (Table 4.2). Sediments in all pH treatments were undersaturated with respect to aragonite, and $\Omega_{aragonite}$ was consistently lower in low pH treatments (Table 4.2). In both experiments, TA was ~2150 µmol kg^{-1} SW in control pH treatments and ~2250 µmol kg^{-1} SW in low pH treatments.

Over the course of each experiment, overlying water conditions remained stable. For Experiment 1, overlying water temperature, salinity, and pH were similar before and after the experiment in both sediment treatments. Temperature, salinity, and pH, respectively, were 18.5°C, 32.5, and 7.87 before the experiment and 18.5°C, 32, and 7.82 after the experiment for the high temperature treatment, and 21.5°C, 32, and 7.89 before and 21.5°C, 32, and 7.84 after the experiment and for the low temperature

treatment. Likewise, in Experiment 2, temperature, salinity, and pH, respectively, were 16°C, 32, and 7.80 before the experiment and 16°C, 32, and 7.85 after the experiment.

4.3.2 Effects of seawater temperature and sediment acidification on juvenile soft-shell clam burrowing

For Experiment 1, two-way ANOVA revealed significant independent effects of sediment pH and temperature on the proportion of juvenile soft-shell clams, with higher temperature and higher pH eliciting higher burrowing proportions. Under control and low pH sediments respectively, elevated temperature (21°C) increased the proportion of clams burrowed by 21.3 % and 51.2 % from proportions observed under the control temperature (18°C) (Figure 4.1). There was no significant temperature × pH interaction (Table 4.3). Under elevated temperatures, low-pH sediment resulted in a 9.2 % reduction in burrowing proportion compared to control pH sediment at the same temperature (Figure 4.1). In contrast, a 29.3 % reduction in burrowing proportion was observed under lower (control) temperatures (Figure 4.1).

4.3.3 Effects of gabazine and sediment acidification on juvenile soft-shell clam burrowing

There was a significant interaction between pH and gabazine on the proportion of juvenile soft-shell clams burrowed (Table 4.4). When gabazine was not administered, sediments with low pH (burrowing proportion = 0.20) had 67.2 % lower burrowing proportion than control pH sediments (burrowing proportion = 0.61) (Tukey HSD: $P < 0.001$; Figure 4.2). However, when gabazine was administered, similarly high burrowing

proportions of 0.61 ± 0.14 and 0.75 ± 0.11 (18.6% difference) were observed for low-pH and control-pH sediments, respectively (Tukey HSD: $P = 0.232$; Figure 4.2).

4.4 Discussion

The results of this study suggest that the burrowing responses of juvenile soft-shell clams can be influenced by sediment pH and overlying water temperature, with burrowing positively related to sediment pH and seawater temperature. Furthermore, these results suggest that neurotransmitter interference (at $GABA_A$-like receptors) serve as the biological mechanism governing juvenile bivalve burrowing responses to sediment pH. To my knowledge, this is the first study to report the effects of sediment acidification on juvenile bivalve burrowing in the context of ocean warming, and is also the first to elucidate the biological mechanism responsible for behavioural impacts of acidification in a non-cephalized invertebrate.

In this study, low-pH sediment reduced the proportion of clams burrowing, while elevated temperatures increased burrowing proportion, suggesting potentially offsetting effects of ocean warming and acidification on juvenile bivalve burrowing and recruitment (although this needs to be interpreted with caution given the lack of statistical significance for the pH×temperature interaction). More acidified sediment has been reported to depress the burrowing proportion of bivalves at various life history stages, including settling (pediveliger, <1 mm) *Mercenaria mercenaria* (Green et al. 2013), juvenile (2-5 mm) *Mya arenaria* (Clements & Hunt 2014), and adult (~40 mm) *Ruditapes philippinarum* (Rodríguez-Romero et al. 2014). Similarly, adult (24-88 mm shell length) bivalve burrowing has been linked to temperature, with burrowing being

positively related to temperature up until a certain threshold, after which the effects of temperature become negative (*M. mercenaria* and *Spisula solidissima*: Savage 1976; *Chamelea gallina*: Monari et al. 2007). While studies have reported the independent effects of sediment pH and seawater temperature on bivalve burrowing responses, the interactive effect of these two parameters has, to my knowledge, not been tested. In this study, alongside increasing overall burrowing, elevated temperature also reduced the % difference in burrowing proportion between low-pH sediments and control pH sediments (Figure 4.3), suggesting a potentially alleviatory effect of increasing temperature with respect to the negative impacts of sediment acidification on juvenile bivalve burrowing. However, given the lack of a significant temperature × pH interaction, this should be interpreted with caution. A number of previous studies have found interactive effects of temperature and water column acidification on marine invertebrate behaviour. For example, Dissanayake & Ishimatsu (2011) reported that elevated temperature (25°C) alleviated the negative impacts of elevated CO_2 conditions far beyond near-future projections on prawn (*Metapenaeus joyneri*) swimming behaviour. Likewise, Queirós et al. (2015) found that increasing temperatures by 2°C alleviated the negative effects of near-future acidification on snail foraging and activity observed under ambient temperatures. In contrast, elevated temperature and CO_2 were found to have a synergistic positive effect on sea urchin (*Amblypneustes pollidud*) grazing (Burnell et al. 2013), while synergistic negative impacts of elevated temperature and CO_2 were observed when assessing activity capacity in crabs (*Hyas araneus*) (Zittier et al. 2013). Furthermore, increasing both temperature and acidification appeared to have no influence on prey handling time in European green crabs (*Carcinus maenas*), nor their interaction with intertidal snails (*Littorina littorea*) (Landes & Zimmer 2012). Given the

observed species specificity in the behavioural responses to elevated CO_2 and temperature, the results observed herein may not be generalizable across all marine bivalves. However, since similar burrowing responses to acidified sediments have been observed in settling *Mercenaria mercenaria* (Green et al. 2013) and adult *Ruditapes philippinarum* (Rodríguez-Romero et al. 2014), it appears that sediment acidification can influence the burrowing behaviour of many infaunal marine bivalves across their ontogeny. The role of temperature in bivalve burrowing responses to sediment acidification deserves more attention across a wider range of taxa.

The results of the experiment suggest that $GABA_A$-like receptor functioning likely acts as the biological mechanism by which soft-shell clam burrowing behaviour is impeded in CO_2-acidified sediments. While clams not administered gabazine had significantly lower burrowing frequencies in low-pH sediments, this trend was completely absent in clams that were administered gabazine, with gabazine restoring normal burrowing under low pH conditions. The restoration of normal behaviour under elevated CO_2 by gabazine in vertebrates (fishes) has been reported in several studies. For example, Lai et al. (2015) reported that adult sticklebacks (*Gasterosteus aculeatus*) reared under elevated CO_2 and treated with gabazine elicited almost identical behavioural lateralization patterns to that of control fish, while fish exposed to elevated CO_2 and not administered gabazine had altered lateralization patterns. $GABA_A$'s role in driving behavioural responses to more acidified conditions has been highlighted in a number of other fish species as well (Nilsson et al. 2012, Chivers et al. 2014, Hamilton et al. 2014). While this mechanism is well established in fishes, its role in controlling invertebrate behaviour in more acidified conditions is less well known. Watson et al.

(2014) observed that gabazine restored predator escape behaviour in CO_2-reared conch snails to levels observed under control conditions (i.e., control CO_2 without gabazine). Given the close phylogenetic relationship between bivalves and gastropods (Kocot et al. 2011), it is intuitive that such a mechanism could also regulate bivalve behavioural responses to low pH conditions. Furthermore, while studies specific to *Mya arenaria* are lacking, GABA receptors are present in the pedal ganglia (i.e., nerves in bivalve feet) of many bivalves (Vitellaro-Zuccarello & De Biasi 1988, Karhunen et al. 1995, Welch et al. 2014). However, it is important to note that the pharmacology of gabazine in molluscs is poorly understood and other mechanisms may be working to drive these behavioural responses to both gabazine and low pH sediments (Watson et al. 2014). As such, further studies assessing the pharmacology of gabazine in bivalve and gastropod molluscs are needed to determine if $GABA_A$ neuroreceptors are responsible for observed behavioural responses, or if some other mechanism or physiological disruption is at play. Ultimately, bivalve burrowing responses to low pH sediments may be mediated, biologically, by $GABA_A$-like neurotransmitter interference, but future studies describing the pharmacology of gabazine in marine molluscs are necessary to confirm $GABA_A$-like neurotransmitter interference as the biological mechanism driving bivalve burrowing responses in sediments subjected to CO_2-driven acidification. In addition, the potential influence of this mechanism in mediating behavioural changes under OA conditions in other invertebrates should be explored. Finally, understanding the specific mechanisms that govern the association between acid-base regulation and $GABA_A$(-like) receptor functioning could help to explain observed species specificity in behavioural responses under OA conditions and will aid in elucidating an overarching theory of OA effects on marine ecosystems.

I tested bivalve burrowing responses in relation to sediment pH in this study. However, it is likely that pH is not the specific abiotic factor acting to reduce bivalve burrowing behaviour. If pH was the strict carbonate geochemical parameter acting to influence burrowing behaviour, one would expect to see similar % differences in burrowing proportion between sediments with low and control pH across the temperature treatments assessed in this study (Figure 4.1), given that the pH conditions of each sediment pH treatment were almost identical at all temperatures. However, the % difference in proportion of clams burrowing between low and control pH sediments decreased at the higher temperature (although temperature × pH interaction was non-significant). Furthermore, when looking at the % difference between control and low pH sediments in Experiment 2 (conducted at 16°C), the difference is even greater than that at 18°C in Experiment 1, even though the pH conditions in each sediment pH treatment were comparable to those in Experiment 1 (Table 4.2). As such, it may be that some other geochemical correlate influenced by temperature is responsible for reducing bivalve burrowing in low pH sediments, or that elevated CO_2 is impairing the bivalve's ability to cope with temperatures outside a natural range. Previous studies have suggested that carbonate saturation state (Ω) may act as the geochemical cue responsible for settling (pediveliger) bivalve settlement, burrowing, and recruitment (Green et al. 2013). However, given that $GABA_A$-like receptors are most likely controlling burrowing responses to sediment pH, it is unlikely that Ω is the carbonate geochemical cue responsible. More likely, the association between bicarbonate (HCO_3^-) and chloride (Cl^-) ion concentrations may drive the observed bivalve burrowing responses to sediment pH reported in this and previous studies. $GABA_A$ receptors have a specific conductance for chloride (Cl^-) and HCO_3^- ions. Under normal (ambient) pH conditions, [HCO_3^-] and [Cl^-]

are slightly higher extracellularly than intracellularly and the equilibrium potential of the cell is near resting membrane potential, causing HCO_3^- and/or Cl^- to flow into the cell when the $GABA_A$ receptor is opened (Nilsson et al. 2012). This ultimately prevents depolarization, maintains a negative membrane potential, and reduces neural activity. Under elevated CO_2, however, this ion gradient can be reversed (i.e., HCO_3^- and/or Cl^- flow out of the cell when the ion channel is opened) in order to avoid acidosis, which can result in membrane depolarization, excitation of the neural pathway, and ultimately altered behaviour. Given that gabazine restored normal burrowing behaviour in sediments with low pH, it seems likely that alterations to the ion gradient associated with a $GABA_A$-like neuroreceptor are responsible for behavioural changes in burrowing bivalves in response to CO_2-induced acidification in sediment porewater. However, the pharmacology of gabazine in marine molluscs needs to be determined in order to confirm or refute this hypothesis and the mechanisms governing the association between acid-base regulation and altered behaviour via $GABA_A$ interference need to be elucidated. Furthermore, studies isolating burrowing responses to specific, isolated geochemical parameters (e.g. Gazeau et al. 2011, Waldbusser et al. 2015) will help to support or reject the idea that sediment [HCO_3^-] has a causal effect on bivalve burrowing responses to sediment pH observed in this and other studies (Green et al. 2013, Clements & Hunt 2014, Rodríguez-Romero et al. 2014, Clements et al. 2016).

While the results of this study clearly show that seawater temperature and porewater pH can influence the burrowing proportion of juvenile bivalves, the broad-scale ecological consequences of such changes remain speculative. Indeed other factors are known to influence infaunal burrowing activity, including predators (e.g. Flynn & Smee

2010), sediment geochemistry not related to the carbonate system (Engstrom & Marinelli 2005, Cummings et al. 2009), contamination (Phelps et al. 1985, Shin et al. 2002), sediment disturbance (Marinelli & Woodin 2002), sediment type (Alexander et al. 1993, Tallqvist 2001, St. Onge et al. 2007, Sassa et al. 2011), and water flow (St. Onge et al. 2007, Clements & Hunt 2014). In particular, predation on juveniles is thought to play a major role in controlling clam recruitment (Beal 2006), although other factors may be particularly important in areas where predation is low or absent. While it can be speculated that under more acidic sediment conditions juvenile bivalves would be more vulnerable to predation due to decreased burrowing (i.e., "sitting ducks"), burrowing habits are known to change plastically in response to predators (e.g. Flynn & Smee 2010). As such, it is important for future studies to elucidate the impacts of sediment acidification (as well as other physicochemical parameters) on the predator-prey dynamics of juvenile bivalves and their predators. Bivalves are considered ecosystem engineers and clams can play important roles in the ecosystems in which they reside. Ultimately, reduced bivalve recruitment resulting from sediment acidification can potentially have a wide range of ecological consequences, although much more work is needed to elucidate such impacts.

Ultimately, this study suggests that sediment acidification can hinder juvenile marine bivalve burrowing, but that this effect may be alleviated under elevated temperature, suggesting potentially offsetting effects of ocean warming and acidification on juvenile bivalve burrowing and recruitment. Furthermore, it appears that $GABA_A$-like neurotransmitter interference is responsible for reduced burrowing in response to low pH sediment. Future studies should now focus on describing the pharmacology of gabazine

in marine molluscs, assessing the role of the $GABA_A$ mechanism in behavioural responses to OA for other marine invertebrates, and elucidating the interactive effects of sediment and pelagic acidification (in the context of other stressors) on infaunal bivalve burrowing behaviour. Additionally, elucidating the mechanisms governing the association between acid-base regulation and $GABA_A$ functioning could explain species specificity in behavioural responses to ocean acidification and lead to a more comprehensive understanding of how acidification influences animal behaviour. Finally, determining the broader-scale ecological consequences of reduced bivalve burrowing as a result of sediment acidification in the context of changes in various environmental drivers will lead to more accurate predictions regarding the ecological impacts of anthropogenically driven changes.

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Table 4.1. Sediment carbonate chemical conditions for each treatment in Experiments 1 and 2. Temperature (°C), salinity (psu), pH (NBS), and total alkalinity (A_T; µmol kg^{-1} SW) were measured directly; partial pressure of CO_2 (pCO_2; µatm), bicarbonate concentration ($[HCO_3^-]$; µmol kg^{-1} SW), and aragonite saturation ($\Omega_{aragonite}$) were calculated in CO_2SYS. CT = control temp, ET = elevated temp, CpH = control pH, LpH = low pH.

	Measured				Calculated		
Treatment	Temp	Salinity	pH_{NBS}	A_T	pCO_2	$[HCO_3^-]$	$\Omega_{aragonite}$
Exp. 1							
HT-CpH	21.5 ± 0.0	32 ± 0.0	7.35 ± 0.05	2195.5 ± 10.9	1090.3 ± 36.3	2113.0 ± 2.6	0.49 ± 0.02
HT-LpH	21.5 ± 0.0	32 ± 0.0	6.56 ± 0.04	2253.6 ± 40.9	7544.2 ± 615.3	2240.4 ± 1.1	0.08 ± 0.01
CT-CpH	18.5 ± 0.0	32.3 ± 0.4	7.36 ± 0.04	2113.2 ± 53.9	1118.6 ± 111.1	2036.8 ± 7.2	0.45 ± 0.04
CT-LpH	18.5 ± 0.0	32.3 ± 0.4	6.55 ± 0.06	2266.7 ± 64.1	7736.9 ± 733.9	2253.5 ± 1.4	0.08 ± 0.01
Exp. 2							
CpH	16 ± 0.0	32 ± 0.0	7.26 ± 0.03	2154.3 ± 57.2	1479.8 ± 119.8	2094.1 ± 4.5	0.35 ± 0.03
LpH	16 ± 0.0	32 ± 0.0	6.50 ± 0.06	2260.2 ± 44.5	9531.8 ± 1349.1	2249.6 ± 1.7	0.07 ± 0.01

Table 4.2. Summary of ANOVA results for the effects of temperature and sediment pH on bivalve burrowing (Experiment).

	df	SS	MS	F-value	*P*-value
pH	1	0.114	0.114	10.668	0.007
Temperature	1	0.191	0.191	17.927	0.001
pH × Temperature	1	0.013	0.013	1.185	0.298
Residuals	12	0.128	0.011		

Table 4.3. Summary of ANOVA results for the effects of gabazine and sediment pH on bivalve burrowing (Experiment 2).

	df	SS	MS	F-value	*P*-value
pH	1	0.756	0.756	29.938	<0.001
Gabazine	1	0.756	0.756	29.938	<0.001
pH × Gabazine	1	0.189	0.189	7.485	0.010
Residuals	36	0.909	0.025		

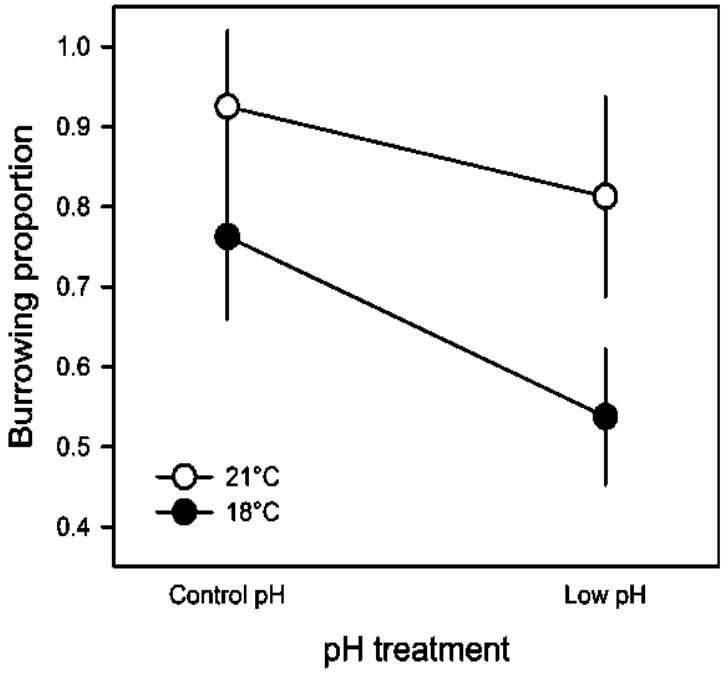

Figure 4.1. Mean burrowing proportion (± SD) in low pH (~6.5) and control pH sediment (~7.3) after 20 min of clams from the control (18°C, n = 4).

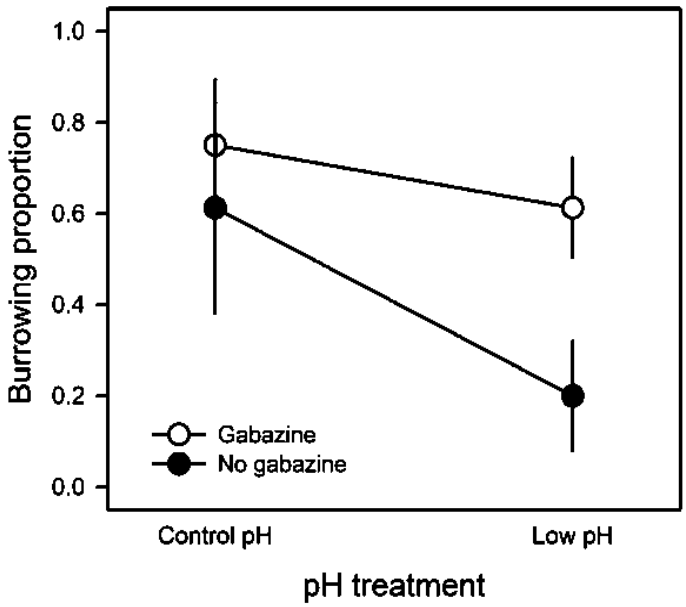

Figure 4.2. Mean burrowing proportion (± SD) after 20 min of gabazine treated (5 mg l^{-1}) and untreated juvenile soft-shell clams exposed to low pH (~6.5) and control pH sediment (~7.3).

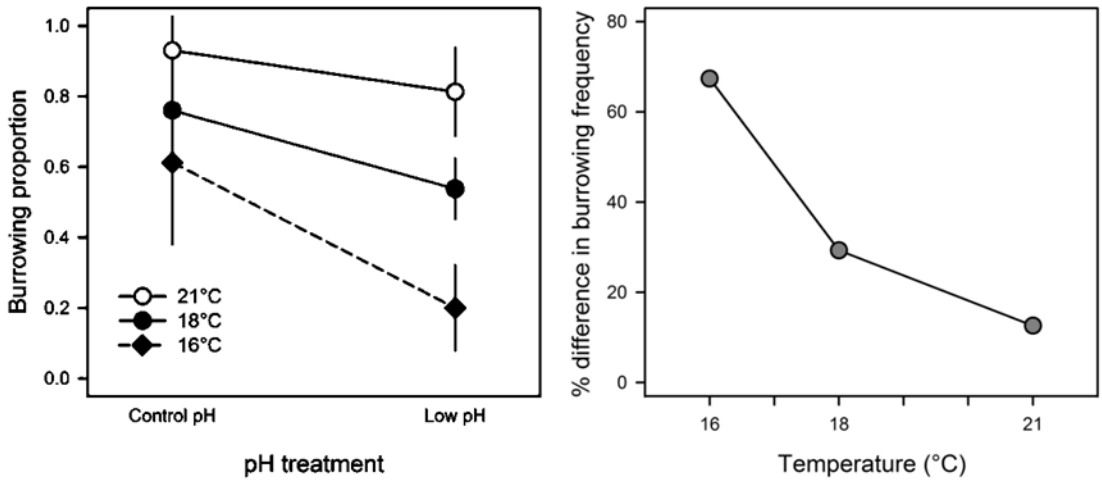

Figure 4.3. Left: Mean burrowing proportion (± SD) in low pH (~6.5) and control pH sediment (~7.3) after 20 min of clams from the control (18°C, n = 4) and elevated (21°C, n = 4) seawater temperature treatments in Experiment 1 (circles), as well as ambient temperature in Experiment 2 (16°C, n = 10; diamonds). Right: The percent difference in burrowing proportion of juvenile soft-shell clams between low pH (~6.5) and control pH sediment (~7.3) at three temperatures: 21°C and 18°C (Experiment 1, n = 4), and 16°C (Experiment 2, n = 10).

Chapter 5

Variability in juvenile soft-shell clam (*Mya arenaria*) abundance on the northern shore of the Bay of Fundy in relation to sediment pH, temperature, and precipitation

This chapter is to be submitted to Estuaries and Coasts in April, 2016

Abstract

While soft-shell clams (*Mya arenaria*) can serve important ecological roles on intertidal mudflats and are an important social and recreational resource in the northwestern Atlantic, predicting their abundance and recruitment can prove difficult in areas where source populations of larvae are unknown. I conducted a field study assessing relationships between juvenile soft-shell clam abundance and spatial, temporal, and environmental variables in coastal mudflats of the northern shore of the Bay of Fundy. Sediment pH and water temperature (low tide) were monitored *in situ* on a biweekly–monthly basis over the course of the *Mya arenaria* settlement season in 2012 and 2013, as well as on a daily basis over two 4 d outings in August, 2013, at four study sites to quantify diel variation in sediment pH and temperature. Core samples were also collected to quantify *M. arenaria* (<6 mm) abundance. Sea-surface and air temperature, rainfall, and *in situ* sediment pH and water temperature were then used to predict clam abundance in 2012. Sediment pH was spatially and temporally variable, while *in situ*

temperature was stable and declined over the sampling seasons. While clam recruitment was too low to detect environmental relationships in 2013, AIC model selection indicated that site and rainfall best predicted patterns of 2012 recruitment when site, date, and environmental variables were included in linear models. When only environmental variables were included, the best model of the variation in clam abundance included air temperature and sediment pH. This study suggests that complex spatial and environmental effects can influence soft-shell clam recruitment.

5.1 Introduction

The recruitment of infaunal marine bivalves can be modulated by pre-settlement, at-settlement, and post-settlement processes. Although early studies suggested that recruitment is predominantly controlled by pre- and at-settlement processes, such as larval supply and patterns of settlement, it has become increasingly recognized that post-settlement processes can influence benthic populations (e.g. Ólafsson et al. 1994). For example, post-settlement burrowing behaviour, organism transport, and predation can ultimately influence the recruitment patterns of infaunal bivalves and invoke substantial changes to the spatial patterns of bivalve populations at various spatial scales (Günther 1992, Armonies 1994, Hunt & Mullineaux 2002, St. Onge et al. 2007). Furthermore, additional environmental sources of mortality following settlement, such as sediment acidification, have the capacity to greatly reduce the number of individuals recruiting to a population (Green et al. 2009).

Infaunal recruitment can be influenced by many factors (Rodríguez et al. 1993). However, two highly important post-settlement factors influencing infaunal bivalve

recruitment are burrowing behaviour (i.e., the acceptance or rejection of habitat; Butman et al. 1988, Woodin et al. 1995, Hunt et al. 2007, Valanko et al. 2010) and mortality (Gosselin & Qian 1997, Hunt & Scheibling 1997, Hunt & Mullineaux 2002). There are numerous biotic and abiotic factors (sediment conditions, water flow, predation, etc.) that can influence post-settlement burrowing behaviour and mortality, which have the potential to interact with one another and alter patterns of recruitment (e.g. St. Onge et al. 2007). As such, understanding how these various biotic and abiotic conditions influence post-settlement processes is critical for garnering an accurate understanding of infaunal bivalve recruitment to natural populations.

Although the burrowing behaviour and recruitment cues of infaunal species are not well understood, studies have attempted to elucidate factors responsible for promoting and/or impeding burrowing. For example, sediment disturbance and chemical secretion by co-habiting species have been reported to influence the burrowing behaviour and recruitment of infaunal bivalves and polychaetes (Woodin et al. 1993, Woodin et al. 1997, Marinelli & Woodin 2002, da Rosa & Bemvenuti 2004). Various environmental factors can also influence patterns of bivalve recruitment. For example, water temperature can modulate burrowing in hard clams (*Mercenaria mercenaria*) and surfclams (*Spisula solidissima*) (optimal temperatures of 25°C-30°C and 15°C-20°C, respectively; Savage 1976). Monari et al. (2007) also reported increased burrowing of *Chamelea gallina* up to 30°C after which negative impacts were experienced. Sediment type and overlying water flow can also play a role in the burrowing behaviour of juvenile bivalves (St. Onge et al. 2007, Clements & Hunt 2014). Sediment (porewater) chemistry can also drive burrowing patterns, as ammonium concentrations (Engstrom &

Marinelli 2005) and sediment carbonate chemistry (Green et al. 2013, Clements & Hunt 2014, Rodríguez-Romero et al. 2014, Clements et al. 2016) have been reported to influence bivalve burrowing in a host of species and at different life stages (settlers, juveniles, and adults).

Post-settlement mortality – which can increase when infaunal bivalves neglect to burrow quickly – can also have a drastic influence on infaunal bivalve recruitment patterns. Mortality at the time of settlement can be influenced by various abiotic and biotic conditions. For example, laboratory and field experiments have suggested that sediment acidification can result in substantial shell dissolution and mortality of settling hard clams, *M. mercenaria* (Green et al. 2004, 2009). Predation is also thought to play a major role in post-settlement mortality and has been suggested to be the most important driver of infaunal bivalve population dynamics (Beal 2006). In a predator exclusion experiment, Hunt & Mullineaux (2002) reported a 76% reduction in juvenile soft-shell clam, *M. arenaria*, abundance in uncaged (predation allowed) plots in comparison to caged (predation excluded) plots. Although the mechanism of reduced abundance was not specifically tested in that study, predators can have direct and indirect impacts on the recruitment of their prey via consumption (direct; Micheli 1997, Richards et al. 1999), disturbance and erosion (indirect; Hunt 2004), or by modifying the behaviour of their prey, (indirect; Nakaoka 2000, Hunt 2004), suggesting that post-settlement mortality can be directly or indirectly driven by predation. Temperature has also been suggested to indirectly impact the recruitment of marine bivalves by modulating predator abundances, with harsher winters reducing predation and subsequently increasing bivalve recruitment in the Wadden Sea (Beukema & Dekker 2014). Hydrodynamics (i.e., current velocity)

and physical disturbance also can influence post-settlement mortality in marine bivalves, although the effects of current velocity appear species specific (Eckman 1987). Other factors potentially influencing to post-settlement mortality of benthic marine bivalves include delayed metamorphosis, physiological stress, and competition (Hunt & Scheibling 1997).

Given the large body of experimental evidence highlighting potential biotic and environmental factors that could potentially influence bivalve recruitment, I conducted a field study to elucidate important predictors of bivalve recruitment on mudflats of the outer Bay of Fundy using the soft-shell clam (*Mya arenaria*) as a model species. Spatial and temporal variability in juvenile *Mya arenaria* abundance, as well as sediment pH and *in situ* temperature, were measured. Model selection was used to determine which factors (including environmental variables: sediment pH, water temperature, air temperature, and precipitation) best predicted the observed variability in juvenile clam abundance at four mudflats in southwest New Brunswick, Canada.

5.2 Materials and methods

5.2.1 Study sites

This study was conducted across four mudflats along the northern shore of the Bay of Fundy, west of Saint John, New Brunswick, Canada. The sites were selected based on previous observations of recently settled *M. arenaria* (Bowen and Hunt 2009; Hunt, unpublished data) (Figure 5.1): Cassidy Lane (CL: 45°7'28.82"N, 66°28'17.97"W), Little Lepreau Road (LLR: 45°7'55.12"N, 66°28'22.02"W), Pocologan (POCO: 45°7'11.98"N, 66°35'27.81"W), and Redhead Road (RHR: 45°6'50.00"N, 66°36'37.63"W). The study

sites formed two pairs, with sites within each pair (CL and LLR; POCO and RHR) located 0.5-1.5 km apart, while the two pairs of sites were separated by ~10 km (Figure 5.1). The four study sites were comparable with respect to sediment grain size (except for RHR which was muddier) and organic matter (% OM), and were predominantly comprised of sediment grain sizes of 50-105 μm with low % OM (Table 5.1): Tides in this area are semi-diurnal with an average height of 7 m (Bowen and Hunt 2009). While the precise time of tidal inundation was slightly different across the four mudflats, sites were similar in steepness and the time it took for the tide to completely cover each mudflat with water was comparable (10-13 min.; Clements, pers. obs.); all four mudflats are exposed for approximately 4 h during low tide (2 h before and after low tide).

5.2.2 Field sampling design

Sediment pH (top 2 cm), water temperature (tidepools at low tide) and clam abundance were sampled at each of the four sites during low tide on a biweekly–monthly basis during the settlement period of *M. arenaria* in 2012 and 2013. Sampling dates were selected based on previous observations of temporal patterns of *M. arenaria* settlement at or near my study sites (Bowen and Hunt 2009; Hunt pers. obs.). There were a total of five sample outings in 2012, spanning from late August to early November, while the timing of sampling in 2013 was adjusted based on the 2012 data, with three sampling trips between early August and mid-September in 2013. In addition to the biweekly–monthly sampling, two four-day outings of daily sampling were conducted in 2013 to observe diel variation in environmental factors; clam abundance was sampled only on the 4^{th} day.

On each sampling date at each site, three 60 m transect ropes were laid out perpendicular to the shore approximately 20 m apart and approximately 7.6 m (25 feet) from the high water mark on the shore beginning at the same permanent markers each time. Core samples (acrylic cores; 6.5 cm diameter, 2 cm depth) were taken at 5 random distances (1 m increments) along the three transects at each site directly beside the transect ropes; core samples for clam abundance (defined as the total number of clams found in a core) were taken directly beside pH core samples. In total, 30 cores per site were collected on each sampling date, 15 for sediment pH and 15 for clam recruitment. Once collected, cores samples were transferred to a cooler (to preserve sediment pH; M. Green, pers. comm.) and transported back to the lab.

5.2.3 *In situ* sediment pH and overlying water temperature

Upon return to the laboratory, triplicate pH (NBS scale) measurements were taken in the top 2 cm of the sediment surface in each core sample using an Accumet AB15 pH meter (Fisher Scientific) and an MI-419 microelectrode (Microelectrodes Inc., New Hampshire, USA). Precise depths were achieved using a Velmex Unislide (Velmex Inc., New York, USA) system to control the position of the probe's tip. Triplicate measurements were averaged to provide a mean pH per core. The pH meter & probe were standardized (pH 4 and 7 buffers; Ferris Chemicals Ltd., Saint John, NB) after every 10 samples to avoid potential inaccuracies. Loss of sample contents and lack of interstitial water in some samples resulted in the processing of 3-5 cores per transect for pH (10-15 cores per site). Additionally, water samples from tide pools near each transect at each site on each date were taken to measure *in situ* water temperature at low tide (n =

3 samples site^{-1} date^{-1}). Temperature was measured immediately upon collection (i.e., on site) with a FisherBRAND thermometer.

5.2.4 Additional environmental variables

In order to understand how a number of additional environmental conditions might influence juvenile soft-shell clam abundance, I obtained sea surface temperature, air temperature, and precipitation data. Sea surface temperature data were gathered from the St. Lawrence Global Observatory (SLGO; www.slgo.ca), which provided 2-week mean SSTs (i.e., mean SST over 2 weeks prior to sampling date) for each site on each date. While the exact latitude and longitude for each site were not available through this database, I selected the temperatures from coordinates that minimized the overall difference between the available SLGO coordinates and the actual coordinates for each site; this provided coordinates within 0.01 and 0.04 decimal degrees (latitude and longitude, respectively) of the actual site coordinates. I also obtained measures of air temperature and precipitation from Environment Canada. Air temperature measures were maximum air temperature (AT_{max}), minimum air temperature (AT_{min}), and average air temperature (AT_{avg}). Due to the limited spatial resolution of Environment Canada data, air temperature and precipitation data were the same between CL and LLR, and the same between RHR and POCO, but CL-LLR values were different than RHR-POCO values. Given that SST data were 2-week means, I also used 2-week means for each AT measure. Because precipitation could impact clam abundance in different ways (e.g. metabolic shock via desalination and acidification, physical damage/harm), I calculated three different measures of precipitation: 2 day cumulative rainfall (prior to sampling

date), 2-week mean rainfall (prior to sampling date), and the total number of days with rainfall in the 2 weeks prior to a sampling date.

5.2.5 Soft-shell clam recruitment

Samples for clam recruitment (i.e., juvenile clam abundance) were brought back to the lab, sieved on a 180 µm sieve, and preserved in 85% ethanol. Prior to examining sample contents, each sample was sieved on stacked 500 and 180 µm sieves and the retained sediment from each sieve was examined separately. To elutriate the sediment retained on the 180 µm sieve and separate out the clams, the sediment for each sample was placed in a bucket, swirled for 5 s, and the supernatant (containing the suspended clams and other lighter material) was poured off; the complete contents of the 500 µm sieve was examined. Aliquots of sediment from the contents of 500 and 180 µm sieves were then transferred into Petri dishes and examined under a dissecting microscope to count soft-shell clam abundance; clams were measured using an ocular micrometer. Clams > 6 mm were not included in the analyses because most clams of this size are found at depths > 2 mm. Size-frequencies were plotted to validate that my sampling protocol adequately captured the soft-shell clam settlement season. Clam recruitment data is included for an additional sampling date in early August 2012 to determine the onset of the settlement period; environmental factors were not sampled on this date due to pH probe malfunctions. For the 2012 sampling season, I tested for differences across transects for the sites and dates with the highest abundance of clams (CL and LLR in Late Aug. and Early Sept.) to determine if there were significant differences between transects. That analysis revealed no differences across transects (see *Juvenile clam recruitment* in Results), and I therefore concentrated effort in processing samples on a

single transect (Transect 1) per site to assess the impacts of spatial (site), temporal (date), and environmental factors (*in situ* water temperature, SST, AT measures, precipitation measures, and sediment pH) on clam recruitment. As such, all subsequent analyses of clam recruitment data are based on one transect per site only. Due to low abundances (max. recruit abundance = 10 clams core^{-1}) of clam recruits in 2013 (likely a result of the re-opening of clam harvesting at 3 of the 4 sites: Cassidy Lane, Little Lepreau Road, and Pocologan), the relationship between juvenile abundances and environmental factors was examined only for 2012.

5.2.6 Statistical analyses

All statistical analyses were conducted in R version 3.2.1 (R Core Team 2015). Nested ANOVA was used to test for differences in clam abundance and pH within- (i.e., differences between transects) and between-sites across two dates (CL and LLR in Aug. and Early Sept.) in 2012, with site, and date as fixed factors and transect as a random factor nested within site. I built linear models without interactions to determine how well environmental variables predicted the observed variability in mean juvenile soft-shell clam abundance across sites and dates in 2012 (n = 20 site and date combinations). Linearity was assessed visually with scatterplots of clam abundance *versus* each predictor variable. Assumptions of homogenous variances and normality were tested using Levene's tests and Q–Q plots, respectively. Log (x+ 1) transformed clam abundance data was used as the dependent variable in the final models because there was a non-linear relationship between untransformed clam abundance and environmental variables and the data included 0 values. Correlations between all independent variables were assessed to determine which could be reasonably included

together in the same model. Models including two or more independent variables with a correlation of ≥ 0.70 were discarded.

AIC model selection (AICcmodavg package in R) was used to determine which variables best predicted site means of soft-shell clam recruitment across dates in 2012. Two sets of models were developed, the first which included spatial, temporal, and environmental variables and the second which included environmental variables only (Table 5.2). Once models including ≥ 2 highly correlated variables were removed, the first set included 165 models and the second set 55 models, with both sets including a null model. Models were selected based on AIC and log likelihood values. I considered all models with a delta AIC (Δ AIC) ≤ 2 as top models. Subsequently, I derived the overall R^2 and *P*-value for the best models in each selection round.

5.3 Results

5.3.1 Temporal and spatial variability in *in situ* sediment pH and temperature

Sediment pH sampled on the biweekly–monthly scale was variable across dates. The magnitude of temporal variability was higher in 2012 than 2013 and pH appeared to be much more variable across sites in 2012 than 2013. However, differences across sites were quite date-dependent and within-site variability was also quite substantial (Figure 5.2). In 2012, biweekly–monthly ranges in average pore-water pH were largest for LLR and RHR (1.07 and 1.13 pH units, respectively), and smaller for CL and POCO (0.66 and 0.96 pH units, respectively). In 2013, the average pore-water pH range was highest at RHR (0.96 pH units), while ranges across the other three sites were very similar (0.75,

0.74, and 0.73 pH units at CL, LLR, and POCO, respectively). The lowest average pH was recorded at RHR in both 2012 and 2013 (6.36 and 6.68, respectively), while the highest occurred at CL in 2012 (7.99) and at LLR in 2013 (7.66). Interestingly, pH was much lower at the beginning of the settlement season in 2013 than in 2012 (Figure 5.2). Because of the large variability in sediment pore-water pH across weeks, diel sampling was carried over two 4-day periods. During these two sampling periods, average pH appeared relatively consistent across sites, but showed substantial diel variability across the four sampling days (Figure 5.3). Nested ANOVA revealed significant date×site and date×transect interactions on sediment pH (Table 5.3). While no consistent temporal trends were evident in pH and salinity on the biweekly–monthly scale, temperature gradually decreased as the sampling season progressed in both years (Figures 5.2, 5.3). On shorter time-scales, temperature was relatively consistent within and across sites (Figures 5.2, 5.3).

5.3.2 Juvenile clam recruitment

Size-frequency distributions confirmed that I had adequately captured the soft-shell clam settlement period in 2012 and that settlement occurred at approximately the same time (i.e. Early August-Early September) at all four sampling sites (Figure 5.4). Initial analysis (nested ANOVA) of clam recruitment across transects at two sites on two dates in 2012 (CL and LLR in Late Aug. & Early Sept.; sites and dates chosen based on the highest number of clams and thus the best chance of detecting an effect) showed no significant effects of any factors, nor their interactions, on clam abundance (Table 5.4; Figure 5.5). Consequently, samples were processed for a single transect at each site on all other sampling dates.

A clear trend in juvenile clam abundance was evident across the 2012 sampling season, with abundance decreasing as the sampling season progressed at all 4 sites. Clam abundance was initially higher at CL and LLR than at POCO and RHR, with gradual declines over time at all four sites (Figure 5.4). While initial abundance (i.e., settlement) was highest at CL and LLR (Figure 5.4), overall clam recruitment was highest at CL, followed by LLR, POCO, and RHR, respectively (Figure 5.6).In 2013, clam recruitment was low (near 0) across the entire sampling season, with no apparent peaks in clam abundance (Figure 5.6).

5.3.3 Predicting variability in juvenile clam recruitment

Among the set of models that included both spatial (site) and temporal (date) factors as well as environmental variables, AICc indicated that the best model predicting variability in mean juvenile clam abundance at a site included site, 2 d cumulative rainfall, and 2 week average rainfall. This best model explained almost all of the variability in site means of juvenile clam abundance across the 2012 sampling dates (R^2 = 0.94; Table 5.5). All other candidate models (i.e., Δ AIC $\leq$ 2) also included site along with some measure of rainfall (2 week average rainfall and/or rainfall days; Table 5.5). When only environmental variables were included (i.e., no models including site and date), the best model included minimum air temperature and sediment pH, and explained almost 70% of the variation in juvenile clam abundance (R^2 = 0.68; Table 5.6). Other candidate models also included sediment pH and/or some measure of air temperature, except for the two worst candidate models, which included average rainfall (along with pH) and *in situ* temperature (Table 5.6). Minimum air temperature and sediment pH both displayed a positive relationship with clam abundance, with abundance being higher

under higher temperatures and pH levels (Figure 5.7). Conversely, clam abundance showed a negative relationship with 2 d cumulative rainfall (Figure 5.7).

5.4 Discussion

The results of this study suggest highly variable environmental conditions in Bay of Fundy mudflats on various spatial and temporal scales, along with variable patterns of juvenile soft-shell clam recruitment. While variable, clam recruitment was best predicted by rainfall and site, while temperature and pH were less useful in predicting clam variability when only environmental factors were considered. Further, I only included data for the sampling period, and measurements over longer temporal durations will likely better predict recruitment.

Coastal areas are highly dynamic systems with drastic fluctuations in environmental and biotic conditions (Duarte et al. 2013, Waldbusser & Salisbury 2014). For example, highly variable seawater pH and carbonate conditions have been recorded in various coastal systems across the globe (e.g. Hauri et al. 2009, Gagliano et al. 2010, Shaw et al. 2012, Johnson et al. 2013, Lienweber & Gruber 2013), although predictable temporal and spatial trends are not always evident (Gagliano et al. 2010**)**. These results confirm the high degree of variability in coastal environmental conditions, especially with respect to sediment pH, and are in agreement with the results of studies suggesting a lack of highly predictable patterns. Furthermore, juvenile clam abundance was also variable and a link between clam recruitment and environmental variables (rainfall, air temperature, and sediment pH) was evident.

Various sources of acid (other than atmospheric CO_2) contribute to coastal acidification in the water column and (potentially) in the sediment, including biological activity, atmospheric deposition of various materials, upwelling, and terrestrial and freshwater input (Duarte et al. 2013, Waldbusser & Salisbury 2014). For example, biological processes have been reported to drive seasonal variation in pelagic $CaCO_3$ saturation states in the Arctic Ocean (Bates et al. 2009), while annual patterns in sediment carbonate geochemistry correlate with cyclical trends in the abundance of benthic organisms (foraminiferans; Green et al. 1993). It is likely that biological activity contributed (at least partially) to the variability in sediment pH observed on Bay of Fundy mudflats, as sedimentary pH is largely driven by the bacterial consumption of organic material at the sediment surface (Green et al. 1993, Wenzhöfer 1999, Yates and Halley 2006). Acidic freshwater input can also contribute to conditions of low pH. Such freshwater often originates from rivers and contributes to localized areas of low-pH seawater around river mouths (Hinga 2002, Blackford & Gilbert 2005, Salisbury et al. 2008). However, rainfall can also impose drastic changes and substantial variability in coastal systems, with negative relationships observed between rainfall and pH (i.e., increased rainfall, lower pH) (Doney et al. 2007). Most of the extremely low pH conditions observed in this study occurred during or soon after the mudflats were inundated with amounts of rain >5 mm (low pH). For example, on 9 August 2013, southwestern New Brunswick received 48.4 mm of rain and pH fell sharply, remaining low for ~3 d (Figure 5.3). Given that these rain events occur relatively often over the *M. arenaria* settlement period (Table 5.7), it is likely that rainfall events can drive large environmental changes, particularly in sediment pH and salinity, and may cause stress-induced mortality. Furthermore, it is possible that the sheer force of rainfall pounding

the mudflat surface at low tide can result in physical damage to juvenile bivalves confined to the sediment surface and may result in physical mortality. Indeed, when site was included as a factor, juvenile *M. arenaria* abundance was best predicted when measures of rainfall were incorporated into models, with clam abundance being negatively correlated with increased rainfall. Ultimately, it appears that biological activity and rainfall can contribute to temporal variation in sediment pH on the northern shore of the Bay of Fundy, and that temperature, sediment pH, and rainfall contributed to the observed variability in juvenile *M. arenaria* observed here. However, it remains uncertain whether or not these variables are causing the trends in clam abundance observed in this study, as they may correlate with other causal factors involved (e.g. predation). Further research is thus necessary to determine the mechanistic processes underlying these predictions.

Although it is likely that numerous factors contributed to the observed variability in sediment pH, much of the variability in *in situ* temperature may simply be a function of tidal cycle stage and the fact that water samples for *in situ* temperature were taken from tidepools. While the physical conditions of a tidepools do not fluctuate as much as the exposed environment during low tide, their temperature can change drastically in relatively short periods of time and are regulated to a large degree by the tidal cycle (Metaxas and Scheibling 1993). Although water samples for temperature and salinity were taken quite soon (within 1 h) after the tide had receded at each site to minimize the influence of heating and evaporation on the measurements, it is likely that the observed variability in temperature and salinity were due to tidal cycle stage and, at least in part,

to small-scale changes across tidepools. Furthermore, like sediment pH, biweekly-monthly variation in temperature and salinity may actually reflect diel variation.

Given the high degree of variability in environmental factors at multiple temporal and spatial scales within the intertidal zone, clams and other intertidal organisms are exposed to extreme conditions regularly, particularly during low tide. As such, organismal responses to such variable environmental conditions can be variable as well. While many factors likely contributed to the observed variability in clam recruitment, AIC model selection suggested that rainfall, air temperature, and sediment pH explained a large degree of the variability in clam recruitment. However, given that models including site explained the most variation in clam recruitment, other factors likely contributed to variation in *M. arenaria* abundance in this study. Furthermore, the variables included in the models could have been correlated with other unmeasured factors (e.g. predation) that may have contributed to clam mortality and the observed variability in juvenile clam abundance. Other factors known to influence clam recruitment include (but are not limited to) harvesting and predation (Micheli 1997, Richards et al. 1999, Nakaoka 2000, Hunt & Mullineaux 2002, Hunt 2004), erosion and transport (Hunt & Mullineaux 2002), sediment geochemistry not related to the carbonate system (Engstrom & Marinelli 2005, Cummings et al. 2009, Tallqvist 2001), contamination (Phelps et al. 1985, Shin et al. 2002), sediment disturbance (Marinelli & Woodin 2002), sediment type (Alexander et al. 1993, Tallqvist 2001, St. Onge et al. 2007, Sassa et al. 2011), and water flow (St. Onge et al. 2007). Of these, predation has been suggested to play a major role in controlling clam recruitment (Beal 2006). It has been suggested that increasing temperature can increase predation and subsequently

depress clam recruitment (Glude 1955, Beal 2006), and that depressions in predator abundance after harsh winters can lead to successful recruitment events in bivalves (Beukema & Dekker 2014). On the other hand, increased temperatures (in comparison to Bay of Fundy averages) may allow for expedited growth in juvenile clams, reducing the amount of time that they spend exposed to predation (e.g. larger clams burrow deeper and are thus able to better avoid predation; Zaklan & Ydenberg 1997). Furthermore, adult clams have been reported to maximize burrowing under particular temperature ranges (although temperature responses in juveniles are unknown; Savage 1976). In the absence of predation (or in areas of low predation), clams would be free to burrow and recruit without predator interference, with recruitment being maximized at optimal temperatures and sediment pH (as well as other factors such as grain size, water flow, oxygen, etc.).

Given that I did not include predation as a factor in my analyses, it is possible that predation could contribute to the unexplained variability in juvenile *M. arenaria* abundance, or could actually be the cause of some of the variability that was explained by the predictor variables included in the models (i.e., environmental variables and site). For example, Bowen (2007) reported that while there appeared to no relationship between juvenile (<5 mm) soft-shell clam abundance and green crab (*Carcinus maenas*) density on Bay of Fundy mudflats, juvenile clam abundances decreased exponentially with increasing *Nereis virens* densities, with direct predation by *N. virens* observed in the lab (although follow-up lab experiments found considerably lower rates of *Nereis* predation compared to *C. maenas* ; H. Hunt, unpublished data). Given that the sites sampled in the aforementioned study were comparable to those explored in this study

(i.e., sandy-mud sediment with one overlapping site – POCO), it is plausible that *Nereis* predation could serve as the functional cause of mortality observed in this study, as it could correlate with my predictor variables. On the other hand, environmental variables employed here may very well have correlated with the *Nereis* densities reported in Bowen (2007) and acted as the causal mechanism for their observed clam density patterns. In addition, small-scale population dynamics of infaunal bivalves have been suggested to be primarily influenced by local, small-scale hydrodynamic and environmental factors (e.g. LeBlanc & Miron 2005), which could also contribute to the unexplained variation in clam abundance in this study. Ultimately, while the analyses suggests that rainfall, air temperature, and sediment pH can explain a large degree of the variability in juvenile *M. arenaria* recruitment in the outer Bay of Fundy, they may not be the actual causes of the mortality and observed abundance patterns. As such, further studies are necessary to strictly elucidate the causal factors of clam mortality and associated abundance patterns along the northern shore of the Bay of Fundy.

Although pH and air temperature were related to variation in clam recruitment, if these factors are the cause of decreases in abundance, it is unknown whether clams are dying in or moving away from locations with low sediment pH and air temperature. It has been reported that settling clams exposed to carbonate undersaturated sediments dissolve and die in such conditions in the laboratory (Green et al. 2004, 2009). However, when exposed to such conditions, settling and juvenile bivalves show reduced burrowing and increased post-settlement dispersal away from unsuitable habitat (Green et al. 2013, Clements & Hunt 2014, Clements et al. 2016). Similarly, adult soft-shell clams have been reported to reject burrowing under lower temperatures and likely move away from

such conditions as well (Savage 1976), although temperature responses in juveniles are unknown. Furthermore, most clams found here were >0.5 mm and may have been large enough to avoid dissolution in undersaturated sediments (Green et al. 2009). As such, clams may be engaging in post-settlement dispersal to move away from low pH conditions and avoid dissolution. However, while post-settlement dispersal within a site may help settling and juvenile clams avoid acidification- and temperature-related mortality, dispersal increases their vulnerability to other sources of mortality such as tissue erosion, burial, and predation (Gosselin & Qian 1997, Hunt & Scheibling 1997, Hunt & Mullineaux 2002). Furthermore, since my model was based on site averages, clams would have had to disperse away from an entire site in order for post-settlement dispersal to explain the observed variability in clam abundance. Given that post-settlement dispersal of juvenile soft-shell clams typically occurs on the scale of meters (e.g. Roegner et al. 1995, Jennings & Hunt 2009), it seems unlikely that dispersal would explain decreases in juvenile clam abundance over time and between sites. Consequently, it is likely that mortality drove the patterns in juvenile clam abundance observed in this study.

Ultimately, this study suggests that rainfall, air temperature, and sediment pH can be used to predict the natural recruitment of juvenile soft-shell clams on mudflats in the Bay of Fundy to a moderate degree. However, these factors may not necessarily be the cause of the observed abundance patterns and other factors known to affect clam recruitment may correlate with the variables included in this study. As such, future studies should focus on incorporating other variables, most notably predation and wind speed/direction, in models of juvenile soft-shell clam recruitment in the Bay of Fundy,

and should focus on elucidating the causal factors driving mortality and the associated abundance patterns observed here.

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Table 5.1. Average grain size and % organic matter (±SD) of surface sediment (n = 5) at each of the four study sites along the northern shore of the Bay of Fundy in Southwest NB, Canda.

Site	Sediment grain size (μm)	Organic matter (%)
Cassidy Lane	105.37 ± 5.88	3.51 ± 0.56
Little Lepreau Road	77.69 ± 4.65	2.57 ± 0.33
Pocologan	79.44 ± 38.81	2.74 ± 0.50
Redhead Road	52.08 ± 2.25	2.67 ± 0.72

Table 5.2. Temporal, spatial, and environmental variables used to build predictive models for average clam abundance site $^{-1}$ date^{-1}. Asterisks indicate variables that had a Pearson correlation ≥ 0.70.

Variable type	Variable	Abbr.
Spatial	Site	-
Temporal	Date	-
Environmental	In situ temperature*	-
	Sediment pH (in situ)	-
	Sea surface temperature*	SST
	Daily max. air temperature (2 week mean)*	AT_{max}
	Daily min. air temperature (2 week mean)*	AT_{min}
	Daily avg. air temperature (2 week mean)*	AT_{avg}
	2 d cumulative rainfall	-
	2 week average rainfall*	-
	Rainfall days (2 weeks prior to sample date)	-

Table 5.3. Nested ANOVA results for the effects of date (n = 2; Late Aug. and Early Sept.), site (n = 2; CL and LLR), and transect (nested within site; n = 3) on sediment pH.

	df	SS	MS	F-value	*P*-value
Date	1	1.432	1.432	46.236	<0.001***
Transect	1	0.225	0.225	7.264	0.009**
Site	1	0.814	0.814	26.289	<0.001***
Date×Transect	1	0.259	0.259	8.368	0.006**
Date×Site	1	0.495	0.495	15.981	<0.001***
Transect×Site	1	0.003	0.003	0.083	0.775
Date×Transect×Site	1	0.087	0.087	2.792	0.101
Residuals	52	1.611	0.031		

Table 5.4. Nested ANOVA results for the effects of date (n = 2; Late Aug. and Early Sept.), site (n = 2; CL and LLR), and transect (nested within site; n = 3) on juvenile soft-shell clam abundance.

	df	SS	MS	F-value	*P*-value
Date	1	676	675.9	2.369	0.130
Transect	1	25	25.0	0.088	0.768
Site	1	156	156.1	0.547	0.463
Date×Transect	1	618	618.3	2.168	0.147
Date×Site	1	116	116.2	0.407	0.526
Transect×Site	1	47	47.1	0.165	0.686
Date×Transect×Site	1	30	30.4	0.107	0.746
Residuals	49	13977	285.3		

Table 5.5. AIC model selection summary for best linear models including combined and independent effects of all 11 variables (with exception of models including 2 or more variables with a ≥ 0.70 correlation) on juvenile soft-shell clam abundance (mean abundance $site^{-1}$ $date^{-1}$; n = 20).

Model	K	AICc	Δ AICc	AICc Wt.	Cum. Wt.	LL	R^2	*P*-value
Site + cum. rain + avg. rain	7	-4.41	0	0.47	0.47	13.87	0.94	<0.0001
Site + avg. rain + # rain days	7	-3.09	1.32	0.25	0.72	13.21	0.93	<0.0001
Site + avg. rain	6	-2.6	1.81	0.19	0.91	10.53	0.91	<0.0002

Table 5.6. AIC model selection summary for linear models including independent effect of sediment pH (pH) on recruit abundance for samples collected from 1 transect. A model including no variables (Null model) was also tested. The best model (model explaining the most variability in recruit abundance; based on AIC and log likelihood) is bolded. Data were standardized residuals of replicates.

Model	K	AICc	Δ AICc	AICc Wt.	Cum. Wt.	LL	R^2	*P*-value
Min. air temp.	3	15.89	0	0.13	0.13	-4.19	0.63	<0.0001
pH + min. air temp.	4	15.89	0.01	0.13	0.27	-2.61	0.68	<0.0001
pH + max. air temp.	4	17.22	1.34	0.07	0.34	-3.28	0.66	0.0001
pH + avg. rain	4	17.27	1.38	0.07	0.41	-3.3	0.66	0.0001
In situ temp.	3	17.66	1.78	0.06	0.46	-5.08	0.59	<0.0001

Table 5.7. Number of days with rainfall (>5 mm) during the 2012 and 2013 *M. arenaria* settlement season. Data were obtained online from Environment Canada.

	# days with >5 mm rain	
Month	2012	2013
August	4	2
September	5	5
October	8	5

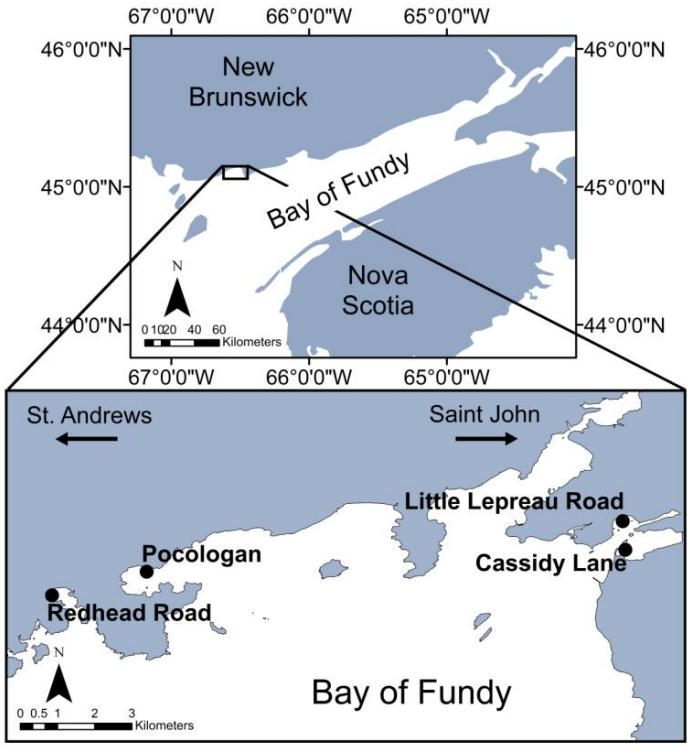

Figure 5.1. Map of the four study sites in the Bay of Fundy in southwest New Brunswick, Canada.

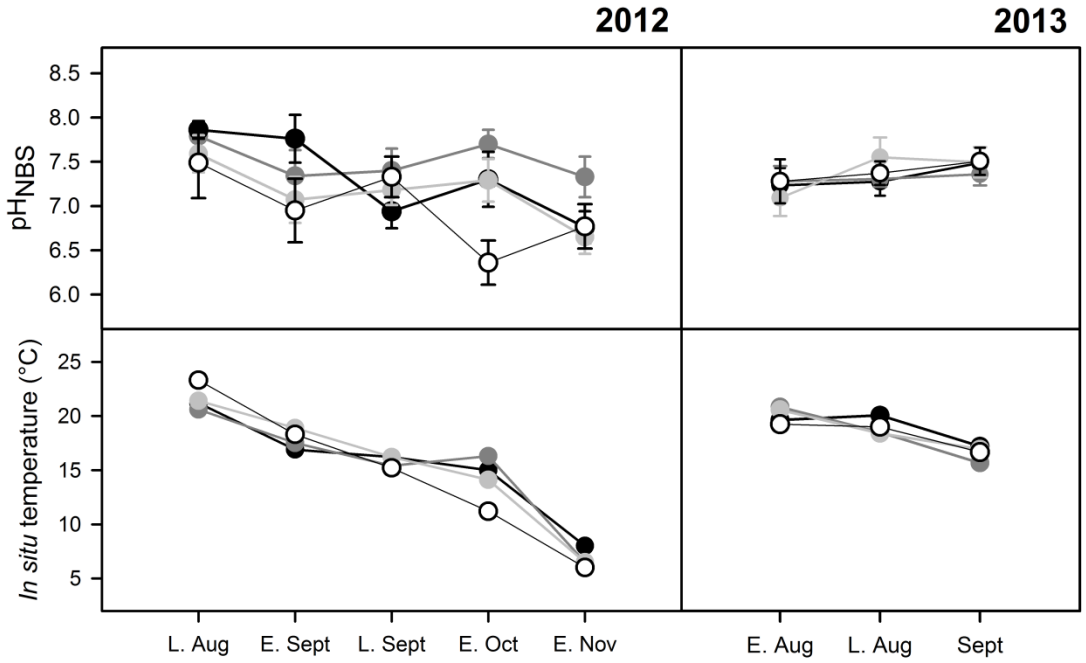

Figure 5.2. Bi-weekly variation in average sediment pH, and average *in situ* water temperature (tidepools) across each of the sample outings in 2012 and 2013. August, 2013 data reflect the conditions on the last of the 4 d sample outings (see Figure 3). Plot colours denote the four different sampling sites: Cassidy Lane (dark gray), Little Lepreau Road (black), Pocologan (light gray), and Redhead Road (white). For temperature, n = 3 site^{-1} date^{-1}; for pH, n = 10-15 site^{-1} date^{-1}.

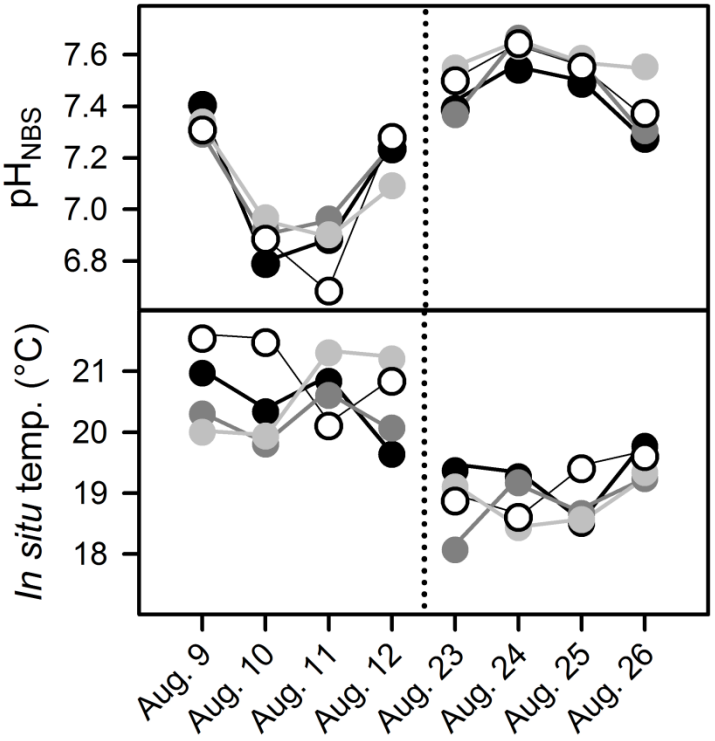

Figure 5.3. Diel variation in average sediment pH, and average *in situ* water temperature (tidepools) during the 2012 sampling season. Plot colours denote the four different sampling sites: Cassidy Lane (dark gray), Little Lepreau Road (black), Pocologan (light gray), and Redhead Road (white). For temperature, n = 3 site^{-1} date^{-1}; for pH, n = 10-15 site^{-1} date^{-1}. Dotted line separates two, 4 d outings.

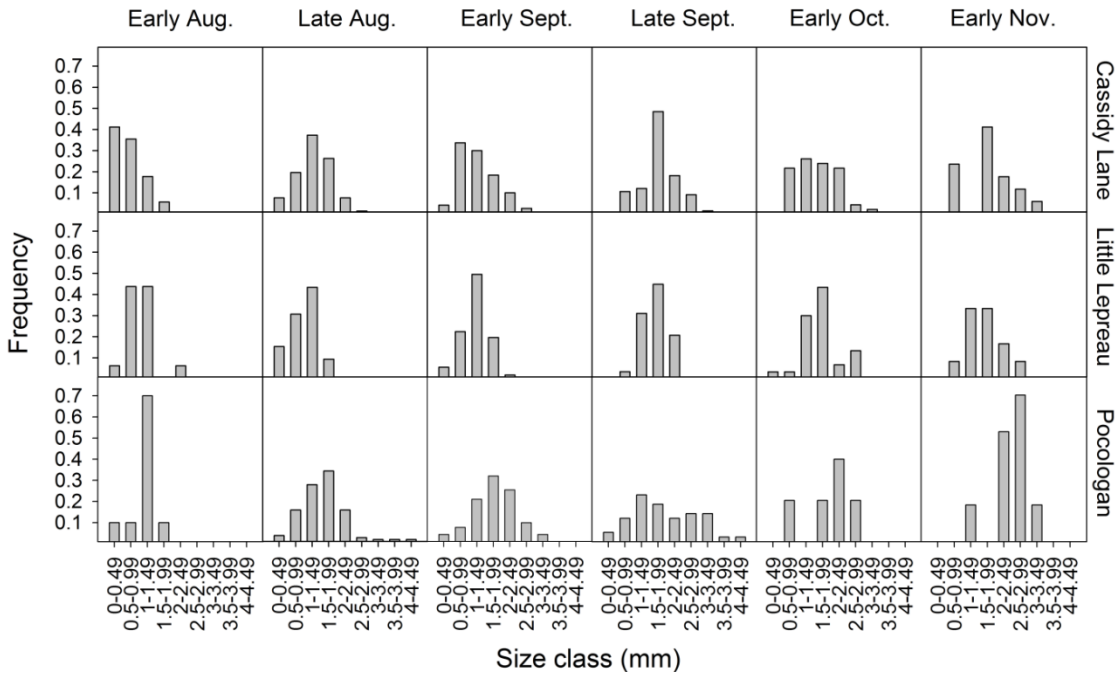

Figure 5.4. Size frequency distributions of juvenile *M. arenaria* at three of the four sites (rows; Redhead Road not displayed due to small sample size) on each sampling date (columns) in 2012.

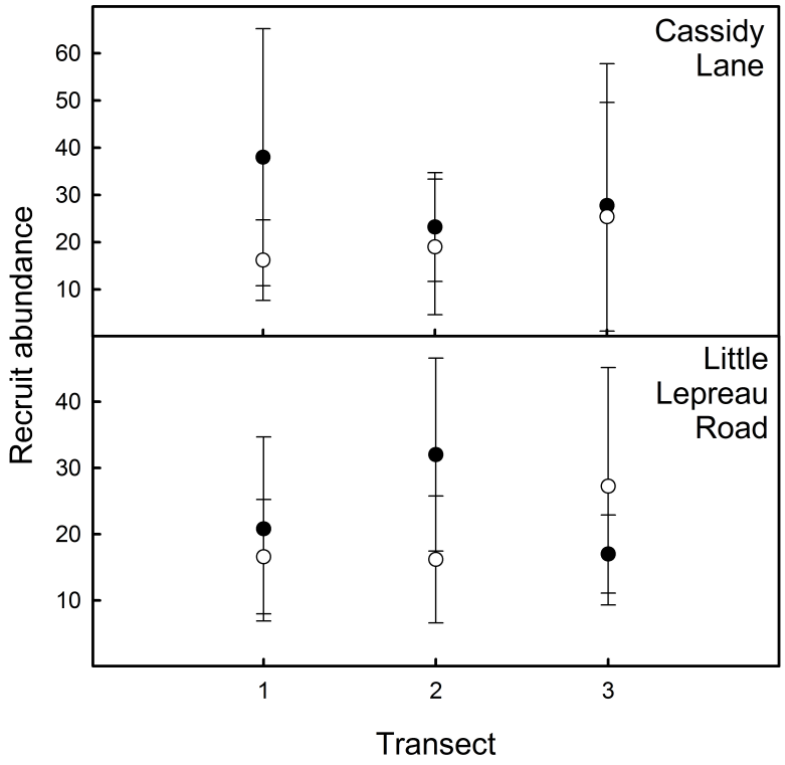

Figure 5.5**.** Mean recruit abundance (± SD) across the three transects at Cassidy Lane and Little Lepreau in late August (black plots) and early September (white plots), 2012.

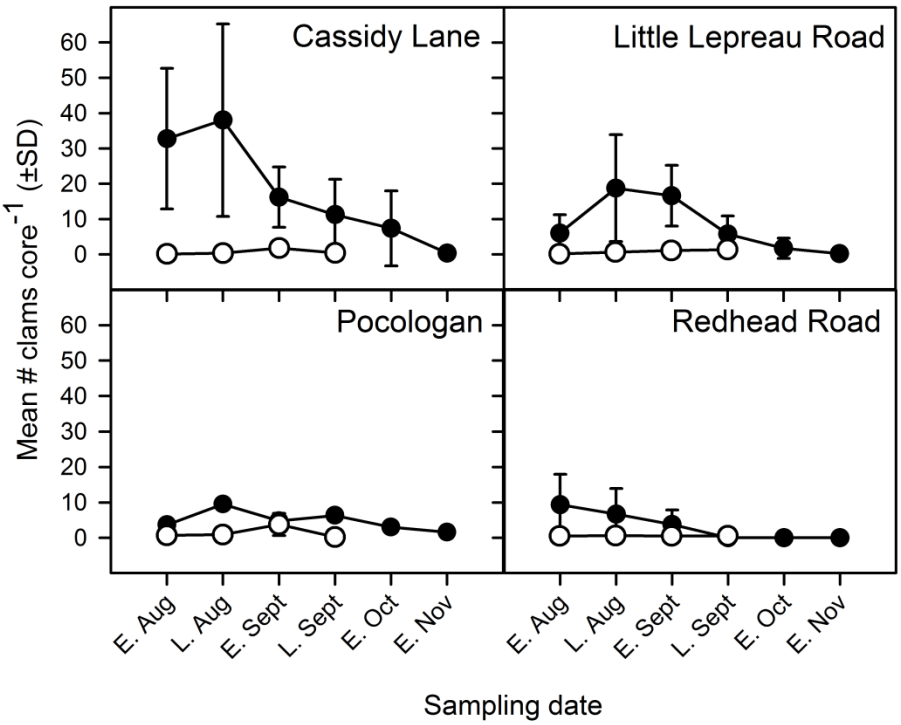

Figure 5.6. Temporal trends in mean recruit (< 6 mm) abundace (± SD) across the 5 sample outings in 2012 (black plots) and 2013 (white plots) at each of the four sites. n = 3-5 site^{-1} date^{-1}.

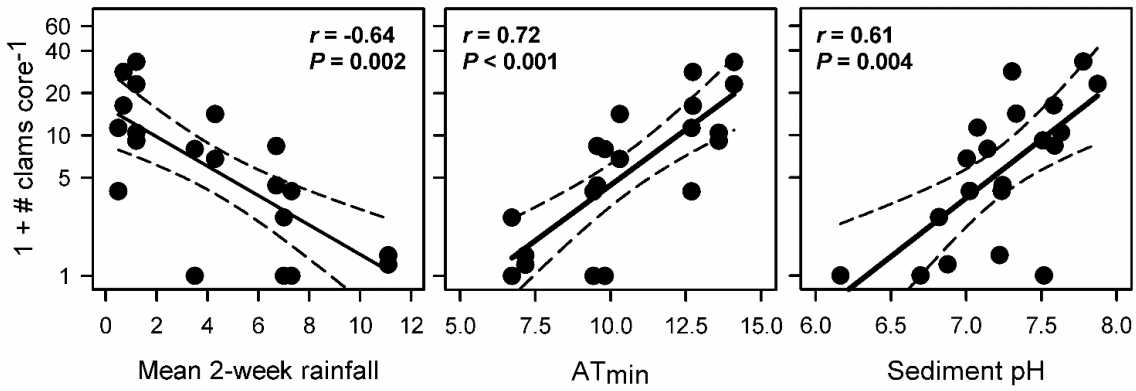

Figure 5.7. Linear relationships between mean clam abundance (site^{-1} date^{-1}) and mean 2-week rainfall, minimum air temperature (2-week mean), and mean sediment pH (site^{-1} date^{-1}) during the 2012 sampling season. Regression lines are lines of best fit, dashed lines ar 95% confidence intervals, and *r*- and *P*-values are Pearson correlation statistics. Y-axes are log scaled.

Chapter 6

General discussion: Influence of sediment acidification on the burrowing behaviour, post-settlement dispersal, and recruitment of marine bivalves

The results of this dissertation suggest that juvenile soft-shell clams (*Mya arenaria*) can be influenced by sediment pH conditions that they currently experience along the northern shore of the Bay of Fundy. More specifically, in Chapters 2 and 3, the burrowing behaviour and subsequent post-settlement dispersal of these clams was impacted by sediment acidification such that burrowing was depressed (also reiterated in Chapter 4) and dispersal was amplified. The results of previous studies have suggested similar effects on burrowing responses for other species of marine bivalves and at different life history stages. For example, Green et al. (2013) reported that low aragonite saturation states in marine sediments depressed the burrowing responses of settling (plantigrade stage) hard clams, *Mercenaria mercenaria*. Rodríguez-Romero et al. (2014) also found that adult manila clams (*Ruditapes philippinarum*) neglected to burrow into low pH sediments as well. Collectively, the results of these studies and this dissertation suggest that low-pH sediments can influence the burrowing responses in a wide array of marine bivalve species and can act to hinder bivalve burrowing across the ontogeny of these animals, which may contribute to declining populations in the Bay of Fundy.

While this dissertation, along with the results of Green et al. (2013) and Rodríguez-Romero et al. (2014), suggest that bivalve burrowing can be negatively affected by low

pH sediments, the results of Chapter 4 elucidated the biological mechanism responsible for these burrowing responses – $GABA_A$-like neurotransmitter interference. It is known that GABA neuroreceptors exist in the pedal ganglia of marine bivalves (Vitellaro-Zuccarello & De Biasi 1988, Karhunen et al. 1995, Welch et al. 2014). Given that these animals typically use their foot to test sediments for suitability, it appears that CO_2 and HCO_3^- conditions reverse ion gradients associated with $GABA_A$-like neuroreceptors in a fashion similar to that of fishes (Nilsson et al. 2012, Hamilton et al. 2014, Chivers et al. 2014, Lai et al. 2015) and cephalized molluscs (Watson et al. 2014). Furthermore, the partial alleviatory effect of temperature in Chapter 4 reinforces the hypothesis that CO_2 and HCO_3^- conditions – not pH or saturation state (e.g. Green et al. 2013) –drive marine bivalve burrowing responses in high CO_2, low pH sediments. Further studies are needed to confirm this hypothesis and isolate the specific carbonate system parameter(s) involved. More research is also needed to better understand the mechanisms governing the association between acid-base regulation and $GABA_A$(-like) receptor functioning in order to better predict the behavioural responses of marine fauna in a high CO_2 ocean.

Alongside impacts to bivalve burrowing, my dissertation contributes a comprehensive review of how ocean acidification impacts the behaviour of marine organisms, providing a necessary synthesis of research to date and setting the stage for future research in this field. Additionally, this dissertation provides a novel method for stabilizing sediment pH for 1-2 days by underlaying a thin (2.5 cm) layer of sediment with gelatin. This method allows studies assessing the effects of sediment acidification on infaunal organisms to move from the laboratory to field settings, providing more realistic assessments of these impacts under natural conditions. Finally, this dissertation

also suggests that a crucial attribute of marine bivalve population dynamics – post-settlement dispersal – can also be influenced by sediment acidification. Studies have reported that water velocity can also influence the burrowing behaviour of burrowing bivalves (e.g. St. Onge et al. 2007). However, in Chapters 2 and 3, clams neglected to burrow even when they were exposed to moving water, and, as a result, were entrained by the flow and moved away from areas of low-pH sediment, both in the laboratory and when transplanted to the field. This suggests that sediment acidification could play a role in structuring natural populations of these animals. Indeed, the results of Chapter 5 suggest that sediment acidification can contribute to observed variability in soft-shell clam recruitment in the Bay of Fundy. However, it is important to note that other predictors such as air temperature and rainfall appear to also be important in determining the population structure of soft-shell clams on mudflats in the Bay of Fundy, while other parameters not measured in Chapter 5 (e.g. wind speed and diretion) can also play a role.

In closing, the results of this dissertation suggest that the burrowing behaviour of infaunal marine bivalves can be influenced by sediment acidification. Soft shell clam burrowing was depressed under more acidic sediments and this response appears to be driven by alterations to $GABA_A$-like neurotransmitter functioning under high CO_2 conditions. Post-settlement dispersal increased under more acidified sediments as a result of the reduction in burrowing. While acidification depressed clam burrowing, increased temperature can, at least in part, alleviate the negative effects of sediment acidification. Given that ocean acidification and warming will happen in parallel, ocean warming will likely reduce the magnitude of effect that would be observed if acidification was occurring in isolation. Finally, given that sediment pH and air

temperature were able to account for 68% of the variability in clam recruitment in the field, it seems likely that soft-shell clam population dynamics will be impacted by global climate change, although more research focused on predicting these impacts is necessary.

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Curriculum vitae (abridged)

Candidate's name: Jeff C. Clements, B.Sc. (hons) biology, BA psychology

EDUCATION

Cape Breton University

Apr, 2011 – Completed four-year Bachelor of Science (Hons.) Biology program

- "Feeding Ecology of the Northern Moonsnail (*Euspira heros*) (Gastropoda: Naticidae) in Cape Breton, Nova Scotia" with Dr. Timothy Rawlings

Apr, 2011 – Completed four-year Bachelor of Arts program with a concentration in Psychology

SCHOLARSHIPS AND GRANTS

OCT, 2015 – UNB Marguerite & Murray Vaughan Scholarship ($3000)
OCT, 2014 – UNB Marguerite & Murray Vaughan Scholarship ($3000)
SEPT, 2014 – NBIF salary grant ($6000)
OCT, 2013 – UNB Marguerite & Murray Vaughan Scholarship ($2500)
OCT, 2012 – UNB Marguerite & Murray Vaughan Scholarship ($1200)
OCT, 2011 – UNB Marguerite & Murray Vaughan Scholarship ($1100)
JUN, 2007 – McDonald's scholarship ($500)
JUN, 2006 – Cape Breton University Entrance Scholarship ($2000)
JUN, 2006 – Royal Canadian Legion National Scholarship ($1000)
JUN, 2006 – Jason Simmons Memorial Scholarship ($500)
JUN, 2006 – Dr. Donald Ferguson Memorial Award ($500)

AWARDS

OCT, 2015 – Thurlow C. Nelson Award for best oral presentation – 107th Annual NSA Meeting in Monterey, CA
JUN, 2014 – Best oral presentation – ACCESS Meeting 2014 in Halifax, NS ($100)
JUN, 2014 – 2nd place poster presentation – ACCESS Meeting 2014 in Halifax, NS
MAY, 2012 – Best poster presentation – ACCESS Meeting 2012 in Halifax, NS ($100)
MAY, 2011 – CBU Book Award for outstanding contributions to the CBU Biology Department
MAR, 2011 – Best oral presentation – CBU SURF in Sydney, NS ($200)
MAR, 2011 – NSERC Representatives Student Undergraduate Award for best oral presentation – APICS AUBC in Halifax, NS ($150)
MAR, 2010 – Best oral presentation – CBU SURF in Sydney, NS ($100)

PUBLICATIONS AND PRESENTATIONS

Publications

<u>In prep, submitted, or under review</u>

1. **Clements JC**, Hunt HL. Effects of CO2-driven sediment acidification on infaunal marine bivalves: a synthesis. Mar Poll Bull. Under review.
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Presentations

12 APR, 2015 – Clements, J. & H. Hunt. Clams on acid: experimental effects of sediment acidification and elevated temperature on juvenile soft-shell clam burrowing

- CERF 2015: Portland, OR

12 APR, 2015 – Clements, J. & H. Hunt. Impacts of porewater acidification on recruitment and size of juvenile soft-shell clams in the Bay of Fundy.

- ACCESS Meeting 2015: Huntsman Marine Science Center, St. Andrews, NB

23 MAR, 2015 – Clements, J., K. Woodard, & H. Hunt. Clams on acid: experimental effects of sediment acidification on burrowing behaviour and dispersal of juvenile soft-shell clams

- 107th NSA meeting, Monterey Marriott, Monterey, CA

30 OCT, 2014 – Clements, J. & H. Hunt. Clams on acid: experimental effects of sediment acidification on burrowing behaviour and dispersal of juvenile soft-shell clams (*Mya arenaria*)

- SABS Benthic Workshop, SABS, St Andrews, NB

13 JUN, 2014 – Clements, J. & H. Hunt. Commercial gelatin as a tool for carbonate geochemistry stabilization in marine soft sediments and its application for benthic ecology

- ACCESS Meeting 2014: Saint Mary's University, Halifax, NS (2nd place poster)

13 JUN, 2014 – Clements, J. & H. Hunt. Clams on acid: experimental effects of sediment acidification on burrowing behaviour and dispersal of juvenile soft-shell clams (*Mya arenaria*)

- ACCESS Meeting 2014: Saint Mary's University, Halifax, NS (Best oral)

24 APR, 2014 – Clements, J. & H. Hunt. Clams on acid: experimental effects of sediment acidification on burrowing behaviour and dispersal of juvenile soft-shell clams (*Mya arenaria*)

- UNB Graduate Research Conference: UNB, Fredericton, NB

06 NOV, 2013 – Clements, J. & H. Hunt. Influence of sedimentary aragonite saturation state (Ωaragonite) on sediment acceptance and dispersal of juvenile soft-shell clams (*Mya arenaria* L.)

- CERF 2013: Town & Country Resort, San Diego, California

11 MAY, 2013 – Clements, J. & H. Hunt. Influence of surface-sediment pH and Ωaragonite on sediment acceptance and dispersal of juvenile soft-shell clams (*Mya arenaria*)

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- ACCESS Meeting 2012: Dalhousie University, Halifax, NS (Best poster)

25 MAR, 2011 – Clements, J. & T. Rawlings. 2011. Feeding ecology of the northern moonsnail (*Euspira heros*) (Gastropoda: Naticidae) in Cape Breton, Nova Scotia.

- CBU SURF: CBU, Sydney, NS (Best oral)

12 MAR, 2011 – Clements, J. & T. Rawlings. 2011. Feeding ecology of the northern moonsnail (*Euspira heros*) (Gastropoda: Naticidae) in Cape Breton, Nova Scotia.

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27 MAR, 2009 – Clements, J. & T. Bouman. 2010. Whole-plant plasticity of balsam fir and paper birch on extreme sites in eastern Cape Breton.

- CBU SURF: CBU, Sydney, NS (Best oral)