LABORATORY- AND FIELD-BASED APPROACHES FOR EVALUATING CONNECTIVITY IN A DYNAMIC COASTAL ENVIRONMENT: APPLICATIONS FOR MANAGEMENT AND CONSERVATION

by

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Abstract

Connectivity of marine populations, defined as the magnitude of discrete population units interconnected through dispersal, has important implications for the ecology and management of commercially harvested species. Sustainable management requires consideration of the spatial-temporal structure of exploited populations. Connectivity measurement requires accuracy in providing relevant spatial information. My thesis bridges laboratory and field based approaches to provide integrated and reliable estimates of connectivity. Using controlled laboratory experiments, I determined that the interaction of temperature and salinity influenced composition of juvenile Atlantic cod otoliths, thus questioning whether otoliths can reconstruct environmental history when environmental variables are studied in isolation. Utilizing a field survey, I demonstrated that otolith chemistry differences could discriminate among juvenile cod from adjacent bays and coasts of origin. Assignment of residuals derived from laboratory model predictions and field observations improved discrimination, illustrating underlying fine-scale biocomplexity in otolith chemistry, and potential influence of environment on assignment at small spatial scales. These results demonstrate the utility of otolith chemistry as a tool to evaluate contributions of sub-populations to Atlantic cod stocks, and, highlight limitations imposed by environmental variation at scales less than 100 km. In a second series of experiments that focused on larval American lobster, I demonstrated that swimming ability and vertical position in the water column varied significantly among ontogenetic stages and did not did not increase linearly with development. Through a series of common garden experiments, I demonstrated biogeographic variability in swimming ability and the influence of environment. Variability in

swimming apparently reflects ambient conditions of the pelagic habitat of origin. Utilizing a biophysical model incorporating observed swimming behaviours, I demonstrated that larval behaviour significantly influenced the magnitude, direction, and duration of dispersal, and that this influence varied both spatially and temporally. These results provide a biological-behavioural context to parameterize bio-physical models and an approach to improve accuracy of dispersal models and advance understanding of connectivity.

By improving aspects of design and testing assumptions, these analyses provide a template for future use of otolith chemistry and biophysical modelling, punctuating the need for calibration and validation of the assumptions of each strategy when applied to dynamic field conditions.

I dedicate this to my grandfather, Edward MacAulduff, a long time lobster fisherman from western Prince Edward Island, who instilled in me a respect of the ocean and a connection to the communities who rely on its perseverance.

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[1] 45.949°N , -61.828°W

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List of common nomenclature and abbreviations

Term		Description
ADCP	…….	Acoustic Doppler current profiler
CTD	…….	Conductivity temperature depth profiler
CHONe	…….	Canadian Healthy Oceans Network
DFO	…….	Department of Fisheries and Oceans Canada
D_{me}	…….	Partition coefficient (ratio of otolith to water chemistry)
DU	…….	Management designatable unit
ICP-MS	…….	Inductively coupled plasma mass spectrometry
ICP-OES	…….	Inductively coupled plasma optical emission spectrometry
JBARB	…….	Joe Brown Aquatic Research Building
LME/MM	…….	Linear mixed effects models
LOD	…….	Limits of detection
MPA	…….	Marine protected area
MUN	…….	Memorial University of Newfoundland
NSERC	…….	Natural Sciences and Research Council of Canada
PLD	…….	Pelagic larval duration (days)
PSU	…….	Practical salinity unit
NL	…….	Newfoundland and Labrador
NS	…….	Nova Scotia
RDC	…….	Research and Development Corporation of Newfoundland

SGB	…….	St. George's Bay, Nova Scotia
VPDB	…….	Vienna Pee Dee Belemnite
Ba	…….	Elemental barium
Ca	…….	Elemental calcium
Mg	…….	Elemental magnesium
Mn	…….	Elemental manganese
Sr	…….	Elemental strontium
$\delta^{13}C$	…….	Stable carbon isotope
$\delta^{18}O$	…….	Stable carbon isotope
Cod	…….	*Gadus morhua* (Linnaeus, 1758)
Lobster	…….	*Homarus americanus* (H. Milne-Edwards, 1837)
Common garden	…….	Organisms transplanted from different natal environments to a common set of conditions.

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Co-authorship statement

The research detailed in this thesis was designed and conceptualized by Ryan R.E. Stanley, with assistance from committee members Drs. Paul Snelgrove, Brad deYoung and Anna Metaxas. All data were collected and analyzed by R. Stanley. Chapters 2 and 3 (Otolith geochemistry) were conducted and written in close collaboration with Drs. Ian Bradbury and Claudio DiBacco. The biophysical model in Chapter 5 was jointly developed with Drs. Remi Daigle and Joël Chassé. All manuscripts resulting from this research were composed by R. Stanley, with editing assistance and creative direction from committee members and associated co-authors.

"If you want to build a boat, don't drum up the men and give them orders. Instead, teach them to yearn for the vast and endless sea." Antoine de Saint Exupéry

Chapter 1 – Tools for measuring connectivity in heterogeneous marine populations and the need for precision

1.1 Population structure and marine management

Sustainable management of marine species requires a comprehensive understanding of how populations are structured. Structuring of marine populations, to varying degrees, may occur over a broad spectrum of spatial and temporal scales, spanning ephemeral schools to established sub-components within a larger metapopulation (Hilborn et al. 2003, Ciannelli et al. 2013). The spatial structure of a metapopulation, regardless of scale, reflects a key aspect of its ecology, and thus should inform the sustainable management of the species. However, few examples in contemporary fisheries management utilize population spatial dynamics to inform design of management designatable units (DUs), bringing into question the capacity of current management practices to support sustainable exploitation (Begg et al. 1999). Often the lack of a mechanistic understanding of the relevant spatial-temporal scales at which populations operate forces management to rely on landscape and-or political boundaries, which rarely synchronize with the dynamics of the managed population(s). Resulting mismatched application of spatial management to marine populations limits the ability of management to achieve sustainability and objectively evaluate success (Ricker 1958, Ruzzante et al. 2006, Reiss et al. 2009, Kerr et al. 2014). Commercial fisheries run the risk of depleting local population components if the DUs do not reflect the population structure of the species (Neat et al. 2014).

Marine ecosystems encompass a heterogeneous landscape of environmental conditions that determine key population parameters such as survival and persistence

(Cowen et al. 2006). Indeed numerous studies document phenotypic (e.g. Morgan & Christy 1996, Miller & Morgan 2013), morphological (e.g. Harding et al. 1993, Marcil et al. 2006), and genetic (e.g. Ruzzante et al. 2000a, Reiss et al. 2009, Benestan et al. 2015) heterogeneity across marine species' ranges, all of which detail the underlying spatial structure of marine populations. These findings inspired the now widely accepted concept of 'metapopulations' for marine populations (Kritzer & Sale 2004), particularly for species with highly dispersive stages (Lipcius et al. 2008). A metapopulation may fall under a variety of definitions, many of which trace back to Levins (1969) seminal work on terrestrial insect populations, who described a metapopulation as a "population of populations", consisting of discrete population units interconnected through dispersal, the magnitude of which defines connectivity. Independence among metapopulation units, or subpopulations, assumes that varying environmental conditions impart variation in the probability of extinction. Here I consider a sub-population as a "*semi-independent, self-reproducing group of individuals within a larger population which undergo limited exchange of individuals with other areas within the population range*" (Smedbol & Stephenson 2001). Sub-populations are independent from each other in the complete absence of any connective processes (e.g dispersal; Grimm et al. 2003). The metapopulation approach focusses on defining those linkages between local- and regional-scale processes (Kritzer & Sale 2004), the degree of which defines 'connectivity', and thus provides a framework to assess a variety of fragmented marine populations, integral to the successful of spatially explicit marine management (Reiss et al. 2009, Kerr et al. 2014, Neat et al. 2014).

Mismatches between management and population structures can limit the effectiveness of sustainable management policies. Mismatches occur in two general

forms. First, management of multiple population units as a single identity can distort estimation of production (Sterner 2007, Kerr et al. 2014). In such situations, smaller, less productive populations may be more susceptible to local extinction and more likely to experience overexploitation first than more productive stocks (Ricker 1958, Reiss et al. 2009, Roy et al. 2012); both outcomes run counter to management objectives. Second, populations that overlap multiple contiguous DUs, but are nonetheless assessed independently, can hinder the evaluation of stock dynamics such as growth and harvest potential (e.g. Sterner 2007, Frisk et al. 2008). Both scenarios can lead to irreparable changes in the metapopulation, altering the functional role of populations units and, in extreme cases, the loss of spawning groups (Stephenson 1999, Hutchinson 2008).

Multiple published studies document the consequence of mismatched management; Atlantic cod, a relatively spatially structured species, offers numerous examples of mismatched application of spatial management (Ruzzante et al. 2000b), leading to failed objectives. For example, management and associated assessments of cod in the North Sea as a single large panmictic stock instead of a metapopulation consisting of spawning stock sub-populations, contributed to the depletion of several historical spawning stocks (Fox et al. 2008, Holmes et al. 2008). Similarly, simulations of *Gadus morhua* from the Northeast Atlantic, using dynamic population models, demonstrated that exploitation of a two-stock population under one management regime risked significant overexploitation of the less productive and thus more vulnerable stock (Fu & Fanning 2004, Sterner 2007, Kerr et al. 2014). In Newfoundland, both Trinity (Brattey & Healey 2008) and Placentia (Robichaud & Rose 2004, Mello & Rose 2005) Bays are thought to support both resident and migratory phenotypes of Atlantic cod, both of which are targeted by commercial inshore fishing. This combined fishery makes partitioning of

mortality to each stock difficult, especially if assessed as one population. Serial depletion of spawning components, resulting in part from the homogenous assessment, likely contributed significantly to the rapid decline and lack of subsequent clear recovery of cod from the Northeast Atlantic (Hutchinson 2008).

Managers must also consider the links between production from individual spawning components to areas of settlement, hereafter source-sink dynamics. Management objectives focussed on persistence, viability, and alignment between management efforts and ecological processes must incorporate connectivity among sources and sinks (Chen et al. 2014, Treml et al. 2015). Heterogeneous patterns in productivity common to many marine species arise from source-sink dynamics (e.g. Smedbol & Wroblewski 2002). Areas characterized by high productivity serve as '*sources*' supplying propagules to areas with lower productivity or '*sinks*'. Population dynamics derived from source-sink processes can produce several scenarios that span from closely linked source-sink populations (e.g. Murawski et al. 2005) to weakly connected metapopulations (e.g. Kenchington et al. 2009). Linkages among sources and sinks may be represented as either recruitment connectivity (productive area tied to less productive area) or ontogentic connectivity (origin and destination of different life history phases) (Cowen et al. 2000, Levin et al. 2009). Despite the intrinsic importance of understanding the spatial dynamics of production within a system, the lack of definitive estimates of connectivity often impedes successful marine management (e.g. marine reserves; Murawski et al. 2005, Sale et al. 2005).

Variation among sub-populations can also be referred to as the '*biocomplexity*' of a system or metapopulation. Biocomplexity in this sense represents the sum of genetic

and phenotypic variability within a larger metapopulation. Multiple authors link the ability of marine management plans to maintain underlying biocomplexity of an ecosystem or managed species to its potential sustainability (Sterner 2007, Kerr et al. 2014). For example, the stability of production from Sockeye salmon (*Oncorhynchus nerka*) in Bristol Bay, Alaska, was attributed to maintenance and conservation of the complexity of spawning stocks within the larger metapopulation, despite major climatic fluctuations (Hilborn et al. 2003). Even without clearly defined genetic differentiation among spawning groups, as some might require to constitute a true sub-population within a metapopulation context (e.g. Roy et al. 2012), the maintenance of phenotypic complexity in the system contributed to its sustainability. Indeed variation in phenotypic traits (e.g. size at maturation; Olsen et al. 2008) may occur at small scales, and the diversity of these traits contributes to the resilience of the managed stock to environmental and commercial pressures (Hilborn et al. 2003).

1.2 Tools for evaluating spatial structure in marine populations

Successful application of connectivity to management requires defining both population structure and the underlying biocomplexity of the system at some scale. Evaluation of these two topics is challenging, likely explaining more talk (avocation) than action (incorporation) in, fisheries management design. Marine ecologists struggle to measure directly the spatial dynamics throughout the ontogeny of a given species and population. While techniques such as direct tagging have successfully measured in-situ movement (e.g. Shapiera et al. 2014) and net displacement (e.g. Rowe 2002), these approaches often lack the spatial-temporal resolution to elucidate movement characteristics through the complete life history. Indeed, different life stages often require an entirely different toolbox of approaches driven by changes in size and physiology with

development. Pragmatic tools to measure the spatial dynamics of the early life history of marine species represent an even greater gap. The pelagic larval stages of meroplanktonic species, for example, generally disperse further, sometimes by orders of magnitude, than their benthic adult counterparts (Botsford et al. 1994); this high dispersal potential increases the difficulty in measurement. Despite the significant challenges in measuring dispersal directly, evaluating potential connectivity among sub-populations, or key population components within a well-managed system, nonetheless requires information regarding the spatial dynamics of the system.

These demands have led to development and application of several tools to evaluate connectivity in the marine environment and thus help to evaluate spatial dynamics of marine populations. Tools for evaluating connectivity fall into five broad groupings[2]: 1) tagging (e.g. acoustic telemetry; Morris et al. 2014, Shapiera et al. 2014); 2) genetic comparisons (e.g. genetic similarity; Planes et al. 2009, Benestan et al. 2015); 3) trait comparisons (e.g. phenotype; Harding et al. 1993, Westley et al. 2013); 4) trace elemental histories (e.g. otolith chemistry; Campana et al. 1999, Thorisson et al. 2011); 5) biophysical models (e.g. dispersal recruitment models; Cudney-Bueno et al. 2009, Incze et al. 2010). Though each broad category involves a specific measured attribute, methodology, and associated strengths and weaknesses, they collectively provide integral information on spatial structure and variability of the focal species.

The utility of a connectivity measurement tool to provide relevant spatial information depends on its precision and accuracy. My thesis focuses on testing how different biological and environmental drivers influence the information gained from

[2] These groupings represent simplified categorization of the diversity of approaches.

connectivity tools applied in the coastal, near-shore setting. Specifically I focus on the application of trace elemental histories and biophysical models, both of which have proven capacity to provide actionable information on stock structure to management (e.g. Campana et al. 1994, Kenchington et al. 2009). Both approaches include assumptions and sensitivities that remain untested, thus leaving the potential for improved estimates of connectivity. I viewed these untested aspects as opportunities to explore how application of information from controlled laboratory experiments could inform real world applications on the spatial dynamics of the species in question.

1.2.1 Otolith chemistry

Chapters 2 and 3 focus specifically on otolith geochemistry. Briefly, otolith geochemistry utilizes the earstones (otoliths) common to many fish species. Otoliths are a pair of calcified structures utilized by teleost fishes for hearing and/or balance. Otoliths provide several unique pieces of information that can be used to understand aspects of the life history of a fish. Fish otoliths are metabolically inert calcified structures that grow continuously throughout development, and integrate at least some elements from the surrounding water into the calcium carbonate matrix in proportion to ambient concentrations (Campana et al. 2000). Continuous growth provides a retrospective tool to not only look at environmental history but also potentially differentiate the spatial history of fishes (e.g. Campana et al. 1994, D'Avignon & Rose 2013). Indeed, in regions with sufficient spatial variation in water chemistry and environmental conditions, analysis of otolith geochemical composition offers a natural marker, or 'fingerprint', of both time and space to infer spatial dynamics of marine populations. Because otolith fingerprinting can provide effective measures of connectivity even in high gene-flow environments, it offers a particularly attractive approach to elucidating spatial dynamics of past developmental

stages (Campana 1999, Elsdon et al. 2008, Syedang et al. 2010). Otoliths offer a less conservative, and thus more sensitive, tool for elucidating spatial patterns than genetic data alone (Reiss et al. 2009).

Otolith chemistry has limitations. First, the species and element specific incorporation of trace elements limits inferences across taxa and otolith constituents. Therefore, whether otolith composition reflects ambient water chemistry varies with the magnitude of spatial variation in water chemistry and the physiology of the species of interest. Second, the influence of environment, namely temperature and salinity, also influences otolith deposition within different species (e.g. Reis-Santos et al. 2013). The interactive effects of temperature and salinity on otolith chemistry (Elsdon & Gillanders 2002) constrain extrapolation of otolith chemistry from one species to another in resolving environmental histories. Although the use of otolith composition to inform spatial variation does not necessarily require information on environment (Campana et al. 2000), the significant influence of environmental factors complicates interpretation without information on environmental conditions (Martin & Wuenschel 2006), particularly in dynamic coastal environments.

Controlled laboratory experiments provide an important vehicle to explore how environmental drivers, namely temperature and salinity, interact to control otolith composition (Elsdon & Gillanders 2002). Empirical observations detailing the influence of temperature and salinity on composition offer an important baseline that can inform interpretation of environmental histories and variation in otolith chemistry in the field.

I chose Atlantic cod (*Gadus mohura*), hereafter cod, as a model species to evaluate otolith chemistry as a tool to evaluate spatial dynamics in the coastal

environment. Historically, cod was heavily exploited commercially on the eastern coast of Canada and assessed and managed using spatially structured DUs (DFO 2013b). Significant past effort has applied otolith chemistry tools to cod, developing an empirical baseline and history of successful application. In Atlantic Canada, otolith chemistry has been utilized to discriminate broad-scale spawning locations (D'Avignon & Rose 2013), differentiate among adults in a mixed population (Campana et al. 1999), model environmental change (Gao 2002, Jones & Campana 2009), and evaluate juvenile distribution (Dalley & Anderson 1997). Analogous studies focussed on eastern Atlantic cod (e.g. Wright et al. 2006, Limburg et al. 2011, Thorisson et al. 2011, Pampoulie et al. 2012, Neat et al. 2014). Baseline information detailing the influence of environment on cod otolith composition has accumulated for a variety of constituents, evaluating the influence of temperature (Townsend et al. 1995, Hoie et al. 2004) and salinity (Weidman & Millner 2000) on both isotopes and elements. Retrospective analysis of otoliths could provide a tool to link adult spawning groups to nursery areas, and thus provide important information on underlying complexity of exploited stocks.

To date, no study has assessed the influence of temperature and salinity on otolith composition of cod in tandem, nor has any previous study assessed the influence of these variables on the incorporation of isotopic *and* elemental otolith constituents. Given the history of retrospective analysis of otolith composition to assign environmental histories (e.g. Jones & Campana 2009), attributing unresolved variation to an uncontrolled variable, as attempted in other species (e.g. Elsdon & Gillanders 2002, Miller 2011, Reis-Santos et al. 2013), leaves uncertainty in past and future application, particularly in coastal environments where salinity and temperature can both vary at relatively small temporal and spatial scales (e.g. coastal Newfoundland; Colbourne et al. 1998, Craig &

Colbourne 2004). Evaluation of the impact of environmental heterogeneity on otolith discrimination-assignment tests requires a comprehensive understanding of the singular and interactive effects of temperature and salinity on a broad suite of otolith constituents.

Most applications of otolith chemistry focus on large-scale (100s km) or geographically (land) separated areas (e.g. D'Avignon & Rose 2013). However, adult cod move in variable patterns (Rose et al. 2011), and although migrations may extend hundreds of kilometers, small, inshore groups in eastern Newfoundland represent an estimated 44 % of all known spawning groups (Robichaud & Rose 2004). The application of otolith discrimination analyses, as well as underlying variation in otolith chemistry at smaller scales (e.g. within bays; <100 km), remains unresolved. Documentation of significant small-scale variation in otolith chemistry could extend retrospective spatial analyses, particularly for delineating natal and spawning aggregations at finer scales than previously explored. At the same time, failing to account for small-scale variation could limit the precision of otolith discrimination analyses (Campana 1999). Significant variation in environmental conditions that can also occur at small (<100 km) scales exacerbates this concern. To date no study has examined the effect of unresolved small-scale variation in otolith chemistry on discrimination analyses.

The first two experimental chapters of my thesis apply otolith chemistry to juvenile cod. In Chapter 2 I develop the relationship between juvenile otolith chemistry and environment through an orthogonal experiment that manipulates temperature and salinity in the laboratory to provide critical baseline data on environmental effects on juvenile cod otolith chemistry. Chapter 3 builds on these experiments with a series of field surveys of juvenile cod from 5 major embayments in Newfoundland. I evaluate

otolith composition from captured fish to determine the scale at which otolith chemistry effectively differentiates natal habitats of juvenile cod. Using different subsets and combinations of capture locations, I evaluate how variation within the scale of an embayment can mediate successful discrimination. Finally, using a laboratory derived predictive model from Chapter 2 and environmental measurements from the field, I partition the underlying complexity of the system (water chemistry, diet, etc.) from the observed environmental heterogeneity. Partitioning environmental variability from the complexity of the system provides information critical for applying otolith methodologies at scales of hundreds of kilometers or less, and, how much 'noise' can be attributed to environmental variability. Collectively these two chapters provide important insight into the application of otolith chemistry to evaluate the spatial dynamics of juvenile cod, and the scales at which researchers or managers could use retrospective otolith analysis to delineate spatial patterns of juvenile cod. Such information could then feed application of otolith fingerprinting to establish connectivity between nursery areas and exploited adult stocks.

1.2.2 Biophysical models

Chapters 4 and 5 focus on the use of biophysical models to simulate and predict dispersal. The scale and extent of connectivity linking sub-populations or spatially delineated areas depends on the exchange of individuals, which many marine taxa achieve primarily through pelagic dispersal during their early life history (Cowen et al. 2007). Pelagic dispersal results from the combined effects of physical (circulation and temperature) and biological processes (mortality and behaviour) (e.g. Moksnes et al. 2003, Fiksen et al. 2007, Vikebo et al. 2007, Stanley et al. 2012). The logistics of

physically tagging early life history stages and the large distances over which they can disperse constrains direct measurements of dispersal. Although genetic (e.g. Planes et al. 2009) and otolith (see Section 1.2.1; Gillanders & Kingsford 2000) discriminations offer important insight into spatial complexity, they typically cannot provide information on which factors produce the spatial patterns they describe as well as the underlying source-sink dynamics. Because genetic and otolith methodologies do not detail the factors that influence dispersal, they offer limited utility in predicting the implications of environmental change for dispersal. Biophysical models can help fill this information gap, providing a means to model dispersal, predict connectivity, and evaluate factors that mediate larval transport (Metaxas & Saunders 2009). Combined with post-settlement indices, such as genetics or otolith fingerprinting, biophysical models can provide information that helps define populations, identifies source-sink dynamics, and highlights the magnitude and variability in connectivity (Treml et al. 2015).

Larval transport reflects a complex interaction between ocean circulation, the vertical position of dispersing larvae, and the duration of dispersal (Levin 2006, Moksnes et al. 2014). Biophysical models of larval transport ideally incorporate both biological and physical factors. The accuracy of biophysical models depends on how well the physical and biological variables within the model reflect natural processes (Metaxas & Saunders 2009). Particularly in the near-shore environment, complex vertical structure in both temperature and circulation can produce significantly different dispersal outcomes, depending on larval release time and location, as well as vertical resolution of the model (Daigle et al. 2015). Inclusion of realistic biological information, including swimming

speeds and vertical movement becomes increasingly important as physical models improve in spatial resolution (Treml et al. 2015).

Most early studies model dispersal as a primarily passive process (e.g. Hjort 1914, Petrie & Drinkwater 1978, Lough et al. 2006), however, increasing evidence suggests that behavioural effects over even small scales (mm-cm • s^{-1}) can significantly alter dispersal outcomes both during the planktonic phase of dispersal (Metaxas 2001, Corell et al. 2012, Lloyd et al. 2012a) and particularly at settlement (Butman et al. 1988, Snelgrove et al. 1995). For example, vertical migration of larvae over a tidal cycle can alter dispersal outcomes relative to larvae maintaining a fixed position (selective tidal stream transport; Dibacco et al. 2001). Simulations of larval Atlantic cod dispersal demonstrated that small differences in vertical position coupled with vertically stratified circulation significantly changed predicted dispersal kernels (Fiksen et al. 2007). Similarly, the onset of directional swimming in larval reef fish significantly increased the degree of expected connectivity for a given spawning source (Cowen et al. 2006). Moksnes et al. (2014) demonstrated that differences in vertical swimming behaviour of European shore crab (*Carcinus maenus*) likely reflected local adaptations to optimize settlement in preferred nursery habitat. Fisheries studies also invoke behavioural contributions to challenge traditional paradigms of source-sink dynamics. Until recently, the western Irish Sea seasonal gyre (WISSG) was assumed to primarily regulate dispersal and recruitment of Norwegian lobster (*Nephrops norvegicus*), largely because larvae were assumed to exhibit passive behaviour at a fixed depth (Hill et al. 1996). However, larvae of *N. norvegicus*, like many other meroplanktonic species, actively move vertically, and

incorporation of a biophysical dispersal simulation demonstrated a highly variable regulatory influence of the WISSG with significantly different predictions of dispersal outcomes between active and passive model formulations (Phelps et al. 2015). These studies exemplify a growing body of evidence that supports the need for comprehensive understanding of larval behaviour when modelling dispersal.

I chose American lobster (*Homarus americanus*), hereafter lobster, as a model species to evaluate variability in larval behaviour and its potential impact on dispersal and connectivity. Lobster span from North Carolina to Labrador, thus encompass a variety of environmental conditions across its geographic range (Cooper & Uzmann 1980). Indeed, across their range larvae lobster experience a range of temperatures (sea surface temperature ranging -2 : 28- C; Aiken & Waddy 1986) and circulation patterns. To date, much of the work on larval American lobster focusses on post-larval stages when larvae settle out of the pelagic zone for the remainder of their lives. While this stage represents a critical benthic-pelagic link, the majority of dispersal presumably occurs during the earlier wholly planktonic phase that may last for weeks, and realistic biophysical models must therefore consider associated behavioural parameters in biophysical models. Available data suggests that larvae are extremely capable swimmers (Ennis 1986) capable of vertical movement in response to light (Ennis 1975a) and temperature (Annis 2005). Swimming ability also varies among the four developmental larval stages (Ennis 1986, 1995). However, no previous study on larval swimming behaviour has provided direct estimates of larval swimming capacity or sources of variability (noting the exception of post-larval work by Rooney & Cobb 1991), which could contribute to a biophysical

model. Past studies simplify simulations of larval behaviour for this species to fixed positions in the water column (Chasse & Miller 2010, Incze et al. 2010, Xu & Schneider 2012) or directional swimming exclusive to the post-larval settlement stage (Katz et al. 1994). Despite evidence that the inclusion of even limited behaviour to the post-larval stages influences connectivity, no study has evaluated how changes in vertical swimming through larval ontogeny could influence dispersal.

American lobster represents the single most valuable fished species in Canada contributing ~ 40% of the total landed value of groundfish and shellfish fisheries in Atlantic Canada (DFO 2014b; data based on 2013). DFO bases assessment and management of lobster on management DUs referred to as Lobster Fishing Areas (LFAs) (DFO 2013a). Despite the value of the fishery, no comprehensive study to date has incorporated behaviour when evaluating the dispersal scales of larval lobster, bringing estimates of dispersal and connectivity provided by existing models into question. Despite reports of record landings of lobster through the majority of its range over the past decade (Steneck & Wahle 2013), the spatial and temporal dynamics of the species remain relatively poorly understood. A mismatch between the scales of management response and biological processes will threaten sustainability in the face of environmental change and high exploitation rates (Begg et al. 1999, Reiss et al. 2009).

The final two data chapters (Chapters 4 and 5) focus on larval American lobster behaviour, evaluating both capacity and variability. Chapter 4 utilizes controlled laboratory experiments to develop a null model of larval lobster behaviour. Swimming trials focus on both the horizontal and vertical domain in evaluating behavioural patterns in response to environmental stimuli (light and temperature; 3 levels each). I evaluate

variability in behaviour by repeating environmental manipulations across different ontogenetic stages (4 larval stages), and maternal (31 mothers; spanning 71-130 mm carapace length) and biogeographic (2 source locations) origins. I chose two geographically distant natal locations in coastal Newfoundland with significantly different thermal regimes. Through a reciprocal "transplant" of larvae into temperatures reflective of the two natal origins, I test whether larval behaviour varies with geographic origin and whether environment mediates this difference. Chapter 5 builds on the results of Chapter 4 by incorporating larval observations into a 3-dimensionsonal biophysical model and a 2-dimensional progressive vector model based on ADCP data from moorings in the study area, St. George's Bay, Nova Scotia. Specifically I use observations of swimming capacity and vertical position to develop potential behavioural 'rules' for larval lobster. I will then assign behavioral rules to simulated larvae in the biophysical model and allow them to disperse until reaching settlement age, hereafter 'settlement'. Using settlement location (LFA), pelagic larval duration, retention and dispersal distance as response variables, I evaluate how behaviour mediates dispersal and how active vertical movements alter dispersal patterns relative to passive fixed positions. I also test how behaviour interacts with release location (n = 4), horizontal diffusivity (0, 5, 25 $m^2 \cdot s^{-1}$), and time of release (July, August, and September) in setting settlement patterns. The focus of the biophysical model and aspects of its design are based in St. George's Bay. I chose St. George's Bay as a focal point because of high historical catches of larval lobster (Harding et al. 1982) and its position near the centre of the most productive Canadian east coast lobster fishing grounds. My thesis was part of three parallel studies on larval ecology also focused on St. George's Bay and nearby areas as part of the 'Population

Connectivity' theme within the Canadian Healthy Oceans Network (CHONe) (Lloyd et al. 2012b, a, Hrycik et al. 2013, Daigle et al. 2014b, a, Daigle et al. 2015).

Collectivity Chapters 4 and 5 provide the first comprehensive assessment of the movement ecology of larval American lobsters and its potential mediating influence on lobster dispersal and connectivity. The biophysical simulations also provide context for the potential match-mismatch of management zones to the spatial dynamics of this commercially important species.

Section 1.3 Comparison of approaches

Otolith fingerprinting and biophysical modeling offer two approaches to evaluating the spatial dynamics of marine species in a coastal setting. Each method offers inferences on the spatial history of an organism and can reveal insight into the connectedness of marine populations. However, the methods differ in the foundation of their output, inference, and the limitations of their use.

Otolith chemistry represents an endpoint, revealing potential patterns on where an organism has been (retrospective analysis; e.g. Gao 2002) and an approximation of the underlying spatial pattern, connectivity, or complexity of a system. The underlying biocomplexity and heterogeneity of the system and scale of sampling potentially limit the utility of otolith microchemistry in elucidating spatial structure and connectivity. Temporal stability in the heterogeneity in composition among sample locations further limits the utility of otolith microchemistry. If ephemeral environmental signals, such as an anomalous weather system (e.g. extended ice coverage or rain fall) produce differentiation, its long-term utility for delineating spatial patterns may be limited.

Assignment of fish to specific locations through different life history hinges on the accuracy and availability of an inventory of otolith composition from potential source locations (DiBacco & Levin 2000).

Biophysical models simulate how connectivity will likely develop as a product of the conditions an organism experiences during dispersal. The spatial context of the simulation, the accuracy of the physical aspect (e.g. circulation), and biological parameters utilized all limit the utility of biophysical models. Increasing evidence demonstrates that even small changes in release location can profoundly impact subsequent larval transport (e.g. Daigle et al. 2015, Phelps et al. 2015). This variability, in combination with behavioural considerations, increases variability in estimates of connectivity, and thus affects model outputs and inferences.

Scale limits validation of both otolith chemistry and biophysical models as tools to delineate spatial patterns. Otolith chemistry validation requires thorough assessment of the underlying heterogeneity in a system, and failure to do so may yield results with significant errors during mixture analysis or assignment (Campana 1999, Gillanders 2005b). Validation of biophysical models, though not necessary for every application, requires comparison of model output to field observations. Depending on the system and modeled species, dispersal ranges may be quite large, thus weakening the signal imparted by a spawning location relative to the underlying variability in a system.

As with any modelling approach, otolith chemistry and biophysical models are designed to provide insight into how specific variables influence spatial patterns and

biological processes of interest. Although this thesis aims to define better the application of these tools in the coastal environment, some influential variables remain unresolved. Importantly my thesis provides a comprehensive exploration of the variability associated with these biological processes, thus providing both an approach to improve accuracy and a guide to frame the precision of each connectivity tool.

Irrespective of potential limitations, otolith and biophysical modeling approaches provide pragmatic alternatives to genetic tools because they are less conservative (Reiss et al. 2009) and do not require multiple generations to produce a clear signal (Palumbi 2003), thus revealing spatial patterns that develop over short time periods and may prove more immediately relevant to management. The limitations of their application, and subsequent validation, can be circumvented with laboratory studies to validate assumptions and parameterize models. Both methods offer powerful insights that provide complementary information about connectivity during early life history stages that cannot be tested through conventional tagging techniques. Additionally both approaches can reveal unique information about potential environmental and behavioral consequences for connectivity.

1.4 Significance of study

Sustaining ocean health requires a broad management focus that extends beyond stock assessments and considers how to maintain ecosystem components (e.g. metapopulations) necessary for ecological processes. Maintenance of ocean resources requires healthy, intact ecosystems. Human activities increasingly threaten marine ecosystems, and no single strategy guarantees success in attaining all management goals.

However, management systems that include the consideration of the spatial dynamics of the population of interest can maximize chances of success. Well-designed management frameworks can help to mitigate threats posed by ocean industries that may adversely affect biodiversity (Cook et al. 2013) and modify ecosystem structure and function (Olsgard et al. 2008, Garcia et al. 2012).

Recent studies increasingly document greater genetic structure than previously recognized. The large homogenous ranges divided into population units inferred for some species (e.g. American lobster; Kenchington et al. 2009) are now known to display significantly genetic structure (Benestan et al. 2015). Studies also show surprisingly high genetic structure even in species with high dispersal potential throughout ontogeny (e.g. Atlantic cod; Ruzzante et al. 2000b). The importance of maintaining this genetic diversity and diversity of traits for reliance and conservation (Hilborn et al. 2003) presents a difficult problem to fisheries managers, who must manage a spatially dynamic and structured population with spatially and temporally static tools and frameworks (Botsford et al. 2009, Reiss et al. 2009).

Many commercially fished species depend on coastal habitats. In a recent meta-analysis of commercial landings data from the International Council for the Exploration of the Sea (ICES), Seitz et al. (2014) estimated that globally approximately 77% of fished species utilize coastal habitat for at least some part of their life history. Dynamic circulation, temperature, salinity and biological characteristics add challenge to efforts to evaluate spatial patterns and underlying complexity of marine species in coastal settings. Better understanding of sensitivity to dynamic conditions will improve tools such as otolith fingerprinting and biophysical modeling. Controlled laboratory studies help

improve these tools by refining and attributing variability to specific dynamic variables. Indeed, numerous recent studies evaluated the influence of environment on otolith chemistry (e.g. Elsdon & Gillanders 2002, Miller 2009, Reis-Santos et al. 2013) and the behaviour of larval organisms (e.g. Ennis 1975a, Chia et al. 1984, Ennis 1986, Schmalenbach & Buchholz 2010, Civelek et al. 2013). Although these studies provide important information, few studies incorporate their outputs into field-based applications, despite their potential to refine outputs.

My thesis bridges the divide between laboratory based experiments and field applications of connectivity tools. Specifically I utilize laboratory approaches to calibrate the design and guide the interpretation of both otolith fingerprinting and biophysical models. To test these tools I chose two commercially and culturally important species in Atlantic Canada. The use of connectivity tools to evaluate the spatial dynamics of each species has become more common, despite many untested assumptions. By improving aspects of design or testing these assumptions, I intend this thesis will not only provide a template for future use of otolith chemistry and biophysical modelling, but also punctuate the need for calibration and understanding the assumptions of each strategy when applying them to dynamic field conditions. Better tools will enable more informed decisions by managers about how best to apply spatially explicit management such as DUs or protected areas, in addition to optimizing use of source-sink dynamics for other management decisions. Particularly for MPAs, effective design requires knowledge of spatial distribution of production (Ludford et al. 2012, Stanley et al. 2015a), however

establishing a putative source and sink requires understanding underlying variability of spatial dynamics which characterize the system first (Chen et al. 2014).

1.5 Format

In summary, this thesis examines use of laboratory methods linked with biological and bio-physical models to improve the application of tools to evaluate spatial patterns in the coastal environment. The thesis is arranged in 6 chapters including this introduction and overview (Chapter 1*)*. Chapters 2-5 present the thesis research. These chapters were written as stand-alone manuscripts for publication, a strategy that necessarily results in some repetition of material among chapters. Chapter 2 was published in the ICES Journal of Marine Science (Stanley et al. 2015b) and Chapters 3-5 have been submitted to other journals for review.

In all chapters I attempted to develop methodologies and frameworks with broad applicability to other species and marine systems. Methods for developing environmental models of otolith composition (Chapter 2), and subsequent integration into field assessments (Chapter 3), could be easily applied to other fish species. Similarly, methods for measuring larval behaviour (Chapter 4*)* and developing biological parameters for comparison in biophysical models (Chapter 5) could be applied to other meroplanktonic species with swimming larvae. Chapter 6 provides an overall conclusion for Chapters 2-5 and highlights the key findings from each chapter, and the overall significance of this thesis to coastal connectivity science.

Chapter 2 – Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*)

2.1 Abstract

We evaluated the influence of environmental exposure of juvenile Atlantic cod (*Gadus morua*) to inform interpretations of natal origins and movement patterns using otolith geochemistry. Laboratory rearing experiments were conducted with a variety of temperature (~ 5, 8.5 and 12 °C) and salinity (~ 25, 28.5 and 32 PSU) combinations. We measured magnesium (Mg), manganese (Mn), strontium (Sr) and barium (Ba), expressed as a ratio to calcium (Ca), using laser ablation inductively coupled plasma mass spectrometry (ICP-MS), and stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes using isotope ratio monitoring mass spectrometry. Temperature and salinity significantly affected all elements and isotopes measured, with the exception of salinity on Mg:Ca. We detected significant interactions among temperature and salinity for Mn:Ca and Ba:Ca partition coefficients (ratio of otolith chemistry to water chemistry), with significant temperature effects only detected in the 32 and 28.5 PSU salinity treatments. Similarly, we detected a significant interaction between temperature and salinity in incorporation of $\delta^{13}C$, with a significant temperature effect except at intermediate salinity. These results support the contention that environmental mediation of otolith composition varies among species, thus limiting the ability of generalized models to infer life history patterns from chemistry. Our results provide essential baseline information detailing environmental influence on juvenile Atlantic cod otolith composition, punctuating the importance of laboratory validations to translate species-specific otolith composition when inferring *in situ* life histories and movements.

2.2 Introduction

The application of otolith geochemistry to resolve natal habitats or to reconstruct life histories assumes otolith chemistry corresponds to local environmental conditions and ambient water chemistry, and that these conditions vary to impart a unique geochemical signal (Campana 1999). Physiological and environmental factors both mediate assimilation of elements and isotopes into otoliths (e.g. Thorrold et al. 1997b, Elsdon & Gillanders 2002). Prediction of where otolith composition differences might exist, or the reconstruction of fish movements and environmental histories, requires predictable relationships between environmental variables and otolith chemistry (Elsdon et al. 2008, Reis-Santos et al. 2013). Laboratory studies that have quantified specific relationships between select environmental factors and otolith composition (Hoff & Fuiman 1995, Bath et al. 2000, Elsdon & Gillanders 2002, 2003, Martin & Wuenschel 2006, Miller 2011, Reis-Santos et al. 2013) highlight a lack of generality across among different species (Gillanders 2005b, Reis-Santos et al. 2008, Barnes & Gillanders 2013). For instance, Sr:Ca ratios in otoliths vary considerably among species in the absence of differences in dissolved Sr:Ca in ambient water (Campana 1999), presumably resulting from inter-specific differences in metabolic traits (e.g. blood plasma composition; Kalish 1991, Campana et al. 2000, Miller 2011).

Ambient concentrations of elements and isotopes in seawater may also co-vary with temperature and salinity (Epstein & Mayeda 1953, Elsdon & Gillanders 2003, Reis-Santos et al. 2013, this study). Retrospective assignment of adults to nursery habitats or environmental conditions during the juvenile period has been accomplished through

geochemical analysis of material close to the otolith core, corresponding to the juvenile period (Thorrold et al. 2001, Gillanders 2005b, Farmer et al. 2013). Determining the effects of temperature and salinity on otolith elemental and isotopic incorporation will enhance our ability to predict where significant differences might occur and interpret life history movements (Elsdon & Gillanders 2002). Although use of otolith signatures does not require explicit understanding of all details influencing incorporation (Campana et al. 2000), the significant influence of environmental factors adds complexity to the interpretation of geochemical concentrations in isolation of environment (Martin & Wuenschel 2006).

Atlantic cod (*Gadus morhua*) is a demersal finfish species native to the North Atlantic, spanning from Greenland to Cape Hatteras, North Carolina to as far east as the Baltic Sea. Spawning occurs in both inshore and offshore environments, between late winter and early spring. Pelagic eggs develop into larvae that later metamorphose into benthic juveniles inhabiting offshore areas as well as shallow inshore regions, often associating with complex habitat (Laurel et al. 2003). The large latitudinal and depth range of Atlantic cod exposes juveniles to a gradient of temperatures and salinities (Dalley & Anderson 1997), making them ideal candidates for studies on environmental effects on otolith elemental incorporation. Atlantic cod have received considerable research attention, in particular efforts to understand the structure of remaining fragmented populations in the Northwest Atlantic. The pelagic egg and larval phase imparts a large dispersal potential, mediated by spawn timing and ambient conditions (Bradbury et al. 2000, Stanley et al. 2013). Genetic (Ruzzante et al. 2000b), tagging (Rose

et al. 2011), and otolith chemistry techniques (Campana et al. 1999, Jamieson et al. 2004, D'Avignon & Rose 2013) have successfully delineated spawning aggregation spatial structure, but fall short of providing information on critical juvenile habitat and potential links to adult populations.

The use of otolith chemistry for retrospective evaluation of environmental history, or to aid in delineating nursery habitat, requires a baseline understanding of the influence of environment on otolith composition. Controlled laboratory experiments provide a vehicle to evaluate relationships between otolith composition and the environment. Interactive effects of temperature and salinity (e.g. Miller 2011) necessitate orthogonally designed experiments to fully evaluate environmental drivers on otolith chemistry (Elsdon & Gillanders 2002). The objective of this study was to evaluate the influence of temperature and salinity on the biogenic incorporation of trace elements in the otoliths of juvenile Atlantic cod, a commercially important species that spans a wide range of these environmental parameters. Specifically, we measured concentrations of magnesium (Mg), manganese (Mn), strontium (Sr) and barium (Ba), expressed as a ratio to calcium (Ca), and stable isotopes of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$); past studies show some form of environmental mediation in incorporation of all of these elements. We utilized controlled laboratory conditions to determine the relative and interactive effects of temperature and salinity on elemental and isotopic concentrations of juvenile cod otoliths.

2.3 Methods

2.3.1 Experimental Design

Juvenile cod were obtained from a common broodstock provided by Atlantic Genome Canada and the Dr. Joe Brown Aquatic Research Building (JBARB) at the Ocean Sciences Centre (OSC), Memorial University of Newfoundland (MUN). Newly hatched fish were reared at ambient conditions (~ 11 °C and 32 PSU) for up to 137 days before they were distributed among the experimental treatments. Forty fish (~ 4 cm standard length) were moved to each experimental aquaria (40 L) and acclimated to desired temperature treatment by adjusting cold room temperatures by 2 °C every 2 days, in order to slowly acclimate fish to the new thermal regime from the common start point of 11 °C. When desired temperature treatments were achieved, salinities were manipulated by 2 PSU every two days, until the new salinity regimes were reached from the common start point of 32 PSU. Once acclimation and treatment levels were achieved, fish were immersed in seawater treated with Alizarin Red-S (ARS) (600 $Mg \cdot l^{-1}$) at a pH adjusted to 7.0, for 24 hours. Staining in Alizarin Red induces a fluorescent tag that clearly indicated the start of the experiment and the relevant otolith material to sample (Figure 2.1; Beckman & Schulz 1996). A standardized finfish pelletized diet (EWOS ®) was fed to all fish to minimize any dietary effects of food on otolith chemistry (Walther & Thorrold 2006, Reis-Santos et al. 2013). Throughout the experiment we fed fish to saturation *ad-libitum* three times daily and maintained at a 12-hour light cycle.

Three experimental temperature treatments of approximately 5, 8.5 and 12 °C, hereafter referred to as low, medium, and high temperatures, were maintained in three separate cold rooms at the OSC. For each temperature treatment we used three separate

salinity treatments of 25, 28.5 and 32 PSU, hereafter referred to as low, medium, and high salinities. Note here we consider 32 PSU high relative to the other treatments, but representative of what we expect the fish would experience in full salinity seawater. Each temperature by salinity treatment was replicated three times for a total of 27 experimental aquaria, and nine combinations. Salinities were achieved through dilution of filtered seawater from the JBARB (32 ±0.25 PSU) using non-chlorinated well water from the Marine Institute of Memorial University. Set salinity treatments were pre-mixed and stored in each cold room for 24 hours prior to use in tanks to avoid the need for any acclimation during daily water exchanges. Partial water exchanges (50%) were performed daily along with siphoning any excess food, waste, or dead fish. Temperature and salinity were checked for consistency daily using a YSI-55 probe (± 0.01 °C and 0.1 PSU). In addition, dissolved oxygen, pH, and ammonia levels were monitored every second day with the YSI probe and ammonia test meters. To minimize ammonia levels, each aquarium was equipped with a Bio-Wheel filter and ammonia filter pads.

2.3.2 Otolith preparation and geochemical analysis

Fish were exposed to experimental treatments for a total of 90 days (14 July – 12 October 2007). Upon completion of the experiment, we removed the fish and recorded standard length. Both sagittal and lapillal otoliths were removed, cleaned with ultrapure water, air dried, and stored in acid-washed glass vials. We later mounted otoliths on glass microscope slides and polished them using 0.3 μm lapping film. Mounted otoliths were cleaned again with a nylon brush, triple rinsed in ultrapure water and sonified for 2 min. We then air dried otoliths in a laminar flow hood, prior to transfer to clean petri dishes,

and transport to the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility for analysis. One lapillus per individual was randomly selected for laser ablation and one sagitta was randomly selected for $\delta^{13}C$ and $\delta^{18}O$ analysis.

We measured otolith elemental composition using a Thermo Finnigan *Element2* inductively coupled plasma mass spectrometer (ICP-MS) coupled with an ArF excimer 193-nm laser ablation system. We chose to quantify five elements (^{25}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, and ^{138}Ba,) that were consistently higher in the otolith samples than in HNO_3 blanks during preliminary analyses and have shown potential for environmentally mediated otolith incorporation. We lasered along a line parallel to the otolith edge within a common growth band and outside of the Alizarin mark (~ 500 μm). Laser repetition rate was set at 5 Hz for all analyses, with a scan speed of 5 μm s^{-1}. We used a certified reference material (CRM) consisting of powdered otoliths (Yoshinaga et al. 2000), dissolved in 2% ultrapure HNO_3 (SeaStar®) and diluted to a Ca concentration of 40 $\mu g \cdot g^{-1}$, to correct for instrument bias and drift following Thorrold and Swearer (2009). External precision was estimated by analyzing a second otolith CRM (Sturgeon et al. 2005), also dissolved in 2% HNO_3 (SeaStar®) and diluted to a Ca concentration of 40 $\mu g \cdot g^{-1}$, periodically throughout the laser analyses (n = 55). Analytical accuracy was determined from the concentrations of Japanese Otolith and NRC standards, averaged across all samples. Accuracy exceeded 98% for all elemental otolith constituents measured, including isotopic ratios described later. We calculated limits of detection (LOD) as 5 times the mean intensity of blanks (2% HNO_3), run every 10 assays for each otolith constituent. Any measurements below the respective LOD were excluded from

analysis. We conducted all statistical analyses using otolith elemental compositions converted to molar values and standardized to calcium concentrations ($Me:Ca_{otolith}$). Estimates of external precision based on the relative standard deviation values of the second CRM were 4.2% for Mg, 17.9% for Mn, 0.40% for Sr, and 1.4% for Ba.

We measured $\delta^{13}C$ and $\delta^{18}O$ values in sagittal otoliths of each individual by milling otoliths with a MicroMill sampler (New Wave Research) or a handheld dental drill under a stereomicroscope until we obtained 50 to 200 μg of near edge material. Stable isotopes were measured using an isotope ratio monitoring mass spectrometer according to methods outlined by Ostermann and Curry (2000). We report values of both isotopes relative to Vienna Pee Dee Belemnite (VPDB ‰). Long-term precision estimates based on the use of NBS-19 were ±0.07 for $\delta^{18}O$ and ±0.03 for $\delta^{13}C$ (Ostermann & Curry 2000).

2.3.3 Water analyses

We used ICP-MS to measure the chemical composition of water samples taken from each experimental treatment (n=27) at the mid-point of the experiment (day 32). Note that water samples were taken prior to adding them to fish cultures. Water intake for the OSC and JBARB comes from approximately 5 m depth with a very stable salinity (±0.25 PSU, yearly variance) and temperature (± 1 ^{o}C, weekly variance) (Danny Boyce, JBARB facilities manager, Memorial University of Newfoundland, St. John's Canada, A1C 6N3, personal communication). Low variance in water condition indicates, particularly during the experimental period, a relatively stable intake water mass, with no expected major changes in ambient water chemistry during the course of the experiment.

Because water for all treatments was obtained from a common filtered and stable source, we assume samples collected represent the average conditions experienced over the course of the experiment aside from any temperature or salinity effects. For each treatment, a 50-ml aliquot of water was sampled, acidified with ultrapure nitric acid, and subsequently analyzed for elemental composition. Samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista Pro). Methods for analysis were adapted from EPA Method 200.7 (ICP-OES). Elemental calibration standards were prepared from 1000 mg l^{-1} reference solutions and original stocks are NIST-Traceable. Precision estimates are not provided because only one water sample from each treatment was analyzed. Elemental concentrations were standardized to and expressed as element to calcium ratios ($Me:Ca_{water}$), which were used in all subsequent statistical analyses.

2.3.4 Growth

Upon experiment completion, we recorded standard length for each fish. Because all fish came from the same fertilization event, and were harvested at the same time, we used standard length of the fish as measure of somatic growth. We also measured otolith deposition using the Alizarin stain as a marker for the beginning of the experiment. Otolith length was defined as the length of a transect radiating out from the beginning of the alizarin stain to the outer edge of the otolith. In addition, we measured pre-experimental growth from the otolith core to the beginning of the alizarin stain (Figure 2.1). Pre-experimental otolith length provides an index scoring the relative size of individuals placed into experimental treatments.

2.3.4 Statistical Analysis

Elemental ($Me:Ca_{otolith}$ & $Me:Ca_{water}$) and isotope data were inspected for normality using Q-Q plots and clear outliers (i.e., more than two times the standard deviation from the mean of a given metric) were removed. In total, less than 3% of observations met these exclusion criteria. Partition coefficients provide a complementary metric to compare otolith incorporation rates across experimental treatments accounting for both $Me:Ca_{otolith}$ and $Me:Ca_{water}$ (Reis-Santos et al. 2013), and are especially useful for comparisons among species and studies (Martin & Wuenschel 2006). We calculated partition coefficients (D_{me}) for each element by dividing the measured otolith calcium ratio ($Me:Ca_{otolith}$) by the observed water ratio ($Me:Ca_{water}$) of the treatment (Morse & Bender 1990). The majority of field studies only provide baseline information based on observed otolith composition because the ambient water chemistry is often missing and therefore partition coefficients cannot be calculated (but see, Thorrold et al. 1998). For this reason, we present analyses of otolith composition ($Me:Ca_{otolith}$) and partition coefficients (D_{me}) in tandem.

Statistical analyses were conducted in R-stats version 3.2.0 (R Development Core Team 2015). Differences in growth, water chemistry, otolith chemistry, and partition coefficients, as a function of experimental treatment, were analysed as general mixed-effects models (LME) using the "*lme4*" package in R (Bates et al. 2014). Response variables (i.e. D_{me}) were $\log_{10}$ transformed and treated as continuous variables in response to our categorical fixed treatments, temperature and salinity. To account for growth effects, we constructed a temperature by salinity growth model, using the same mixed-effects model outlined previously, and employed the residuals as an index of growth

variability which accounts for correlations with environment. Growth residuals were incorporated into LME models as continuous covariates. Replicate tanks, within each treatment, were treated as a nested random categorical factor for all models. We did not report the non-significant random tank effect because it was unimportant to the statistical question posed in the analysis (Bolker et al. 2009). Each element and isotope was analyzed individually, thus creating 6 separate statistical tests. To control for family-wise error rates and multiple comparisons, the significance of each test was adjusted using a *Benjamini-Hochberg* (Benjamini & Hochberg 1995) correction and compared to a significance level set at $\alpha = 0.05$. In instances where we detected significant differences, post-hoc pairwise tests using the '*multcomp*' package in R determined which treatments differed.

For all mixed-effects models we found no significant replicate tank effect. All p-values exceeded 1.2 except otolith $\delta^{18}C$ which approximated 0.08.

2.4 Results

2.4.1 Rearing conditions

Experimental conditions were consistent throughout the trials for low (5.0 ± 0.2 °C and 25.1 ± 0.1 PSU; Mean ± SD), intermediate (8.6 ± 0.3 °C and 28.5 ± 0.2 PSU; Mean ± SD), and high (12.2 ± 0.3 °C and 31.8 ± 0.2 PSU; Mean ± SD) treatment levels (Figure 2.2). All water was sourced from a common intake and filtration system, but variation in ambient water chemistry occurred as a function of experimental conditions (Table 2.1). Salinity significantly affected Me:Ca_{water} ratios (Table 2.2), however, we detected no temperature, temperature-salinity interactions, or tank replicate effects in

$Me:Ca_{water}$. Mg:Ca and $Sr:Ca_{water}$ were positively correlated and Mn:Ca and $Ba:Ca_{water}$ were negatively correlated with treatment salinity (Figure 2.3).

2.4.2 Otolith Chemistry

Temperature significantly influenced elemental-calcium ($Me:Ca_{Otolith}$) and isotope ratios (Tables 2.2 & 2.3 respectively; Figure 2.4). Salinity significant influenced on all elements and isotopes tested, with the exception of magnesium ($Mg:Ca_{Otolith}$). We detected no interactive effects for any elemental ratio or for either stable isotope variables. Both Mg:Ca and Mn:Ca were significantly higher at the warmest temperatures. Mg:Ca and Sr:Ca ratios increased with salinity, whereas Mn:Ca and Ba:Ca ratios decreased (Figure 2.4), corresponding to trends observed in $Me:Ca_{Water}$ (Figure 2.3). Similarly, temperature and salinity significantly affected isotope ratios. We did not find significant interactive effects of temperature and salinity on $\delta^{18}O$ ratios, but did find a significant interaction on ratios of $\delta^{13}C$ (Table 2.3). Both $\delta^{13}C$ and $\delta^{18}O$ associated positively with salinity across all temperature treatments. $\delta^{13}C$ values were significantly lower at the low temperatures compared to intermediate and high temperature treatments, except at the intermediate salinity treatment (thus the significant interaction between temperature and salinity). Conversely, we observed significantly higher $\delta^{18}O$ in the low temperature treatment compared to intermediate and high temperatures (Figure 2.4).

2.4.3 Partition Coefficients

Ranges of elemental partition coefficients, D_{Me} (Table 2.1), were similar to those reported in previous studies (Martin et al. 2004, Martin & Thorrold 2005, Martin & Wuenschel 2006, Miller 2011, Reis-Santos et al. 2013). Estimates of D_{Me} for all elements

showed a significant influence of temperature and a similar effect for salinity for all elements except D_{Mg}. We found significant interactive effects between temperature and salinity for D_{Mn} and D_{Ba} (Table 2.2, Figure 2.5). D_{Mg} showed a weakly significant positive association with temperature only. Temperature significantly influenced D_{Mn} only at the highest salinity treatment. D_{Mn} associated positively with salinity for all temperature treatments, especially at the highest temperature. Salinity influenced D_{Sr} coefficients, particularly at low temperatures, where the intermediate salinity treatment had a significantly lower mean D_{sr} value than low or high salinity treatments. D_{sr} associated negatively with temperature, with the largest mean D_{Sr} in the lowest temperature treatments (Figure 2.5). For D_{Ba}, we observed a temperature effect only in the high salinity treatment where coefficients were significantly greater than the low temperature treatment. Barium partition coefficients tracked positively with salinity in all temperature treatments with highest coefficients associated with the highest salinity. We observed temperature effects in D_{Ba} only in the high salinity treatment.

2.4.4 Growth

We found no significant difference among experimental treatments in pre-experimental otolith length, confirming that all individuals were similar in size before experimental rearing (ANOVA: $F=0.99$ $p=0.38$, $F=0.44$ $p=0.64$, for temperature and salinity respectively). Somatic and otolith growth were highly correlated (Pearson $r=0.58$, $p<0.0001$) and temperature affected both variables significantly, with lowest growth in the coldest temperature treatment (Figure 2.6). Collectively, otoliths grew proportionately faster than the fish themselves, resulting in proportionally smaller otoliths

in faster growing fish (log linear model: slope = 1.4 t=12.7, p=<0.00001). Salinity did not significantly affect growth rate singularly, but we did observe a weakly significant interaction between temperature and salinity for somatic growth (Table 2.4). In low temperature treatments, growth was significantly higher at intermediate salinities.

Mixed-effects models revealed significant relationships for Me:Ca and D_{Me} with Mg, Mn, and Sr, as well as ratios of $\delta^{13}C$, with treatment growth residuals (Tables 2.2 and 2.3). All estimated slopes were positive except for D_{Sr} and Sr:Ca, and we observed no significant relationships between growth residuals and Ba:Ca, D_{Ba} and $\delta^{18}O$. These trends mirror directionality and significance of correlative relationships among otolith constituents and growth across treatments (Supplementary Table 2.1).

2.5 Discussion

Otolith geochemistry provides potentially powerful methodology for identifying natal habitats and inferring environmental histories of juvenile Atlantic cod, potentially allowing individuals in adult populations to be linked to specific juvenile or natal nursery habitat. Reliable prediction and evaluation of fish environmental history requires, however, an understanding of how the environment mediates incorporation of ambient elements and isotopes into fish otoliths. Broadly speaking, the influence of temperature and salinity on otolith elemental composition remains ambiguous, with laboratory studies (e.g. Bath et al. 2000, Elsdon & Gillanders 2002, 2003, Miller 2011, Reis-Santos et al. 2013) highlighting strong species-specific relationships (Reis-Santos et al. 2008, Melancon et al. 2009), likely attributable to variable physiology (Miller 2011). Indeed, there are few examples of consistent relationships between otolith chemistry and

environment among fish species (Campana 1999). Laboratory validations thus provides an important and necessary step in evaluating biotic and abiotic influences on otolith incorporation rates (Kalish 1991, Miller 2011) for specific elements and isotopes prior to field application (Reis-Santos et al. 2013). Our experiment represents an important contribution towards understanding the application of otolith microchemistry to environmental reconstructions of juvenile Atlantic cod (*Gadus morhua*) and similar coastal marine fishes.

Our experiments demonstrate significant influences of both temperature and salinity on otolith composition of juvenile Atlantic cod (*Gadus morhua*). Gradients of temperature, salinity, and ambient chemical conditions ($Me:Ca_{water}$), driven by variation in upwelling and mixing of marine and freshwater inputs (Reis-Santos et al. 2013) often characterize coastal nursery habitats in which fish reside or move. Our experiment included a range of temperatures experienced by juvenile Atlantic cod in the Newfoundland inshore environment (Dalley & Anderson 1997, Craig & Colbourne 2004), therefore providing a realistic template on which to test the relationship between environment and otolith composition.

2.5.1 Otolith chemistry

Both temperature and salinity significantly affected almost all of the $Me:Ca_{Otolith}$ and D_{Me} variables. Only $Mg:Ca_{otolith}$ and D_{Mg} were influenced by temperature alone. Previous laboratory experiments evaluating the effects of environment on elemental incorporation found significant effects of both temperature and salinity both with (Secor et al. 1995, Elsdon & Gillanders 2002, Barnes & Gillanders 2013) and without (e.g.

Martin & Thorrold 2005, Martin & Wuenschel 2006) an interaction. Significant interactions between temperature and salinity brings into question generalities drawn from assessments where uncontrolled factors such as temperature or salinity in coastal environments may add environmental heterogeneity and bias otolith signals (Elsdon & Gillanders 2002).

Temperature and salinity both significantly influenced Sr:Ca ratios and partition coefficients. We observed highest $Sr:Ca_{otolith}$ values in the highest salinity treatments (~32 PSU). The result was expected as $Sr:Ca_{Otolith}$ commonly occurs in proportion to ambient availability (Farrell & Campana 1996, Campana et al. 1999) and measurements of water chemistry showed the highest $Sr:Ca_{Water}$ ratios at the highest salinity across all temperature treatments. By calculating partition coefficients, we were able to examine the effect of temperature and salinity on $Sr:Ca_{Otolith}$ after accounting for differences in $Sr:Ca_{Water}$. While several studies found a positive effect of temperature on D_{Sr} (e.g. Bath et al. 2000, Martin et al. 2004), we found the opposite, with highest D_{Sr} values in the low temperature treatment, similar to trends reported for larval Pacific cod, *Gadus marcrocephalus* (DiMaria et al. 2010), and Atlantic cod (Townsend et al. 1995). At low temperatures fish apparently have a reduced ability to discriminate Sr incorporation into the otolith (Townsend et al. 1995), which mirrors our finding of higher Sr:Ca and D_{Sr} at the lowest temperature treatment. We found a dome-shaped effect of salinity on Sr partition coefficients, with lowest D_{Sr} values at intermediate salinities. Martin et al. (2004) also noted a significant effect of salinity (and hence dissolved Sr concentrations in the ambient water) on D_{Sr} and suggested that mutual inhibition of Ca and Sr ions across

intestinal membranes may have generated this result. Our results are more complicated, as this inhibition would need to be complex and non-linear to generate the relation between salinity and D_{Sr} that we observed. Correspondingly Brown and Severin (2009) reviewed published otolith work and noted that marine species in general often exhibit equivalent or greater variation in $Sr:Ca_{Otolith}$ ratios relative to diadromous and freshwater species, despite less variable ambient concentrations; these results suggest an equivocal contribution of $Sr:Ca_{Water}$ levels to $Sr:Ca_{Otolith}$. Strontium is typically used as a chemical tracer of salinity, often defining transitions between freshwater and marine habitats (e.g. Bradbury et al. 2008a). Studies that successfully developed otolith strontium and salinity relationships often utilized a larger gradient than used in our study (e.g. Martin & Wuenschel 2006), and potentially spanned a greater range than that used as coastal juvenile cod habitat. The use of otolith strontium as an environmental tracer in Atlantic cod appears tenuous in isolation, as applied in other species (e.g. Townsend et al. 1995), though in combination with other elements might provide a contextual relationship to environment and a tracer for ambient Sr:Ca chemistry.

Barium incorporation correlated positively with temperature and negatively with salinity. $Ba:Ca_{Otolith}$ ratios were significantly lower at the lowest temperature treatment with decreasing levels at increasing salinity, as seen in previous studies with black bream (Webb et al. 2012). As expected, $Ba:Ca_{Water}$ did not differ significantly across temperature treatments, but did decrease significantly with salinity. Controlling for ambient water chemistry a different pattern emerges, with a significant temperature by salinity interaction influence on D_{Ba}. We observed higher D_{Ba} coefficients and detected

temperature effects only at the highest salinity. Previous studies reported interactive influences of temperature and salinity on otolith Ba:Ca ratios in Chinook salmon (Miller 2011) and European seabass (Reis-Santos et al. 2013). Changes in $Ba:Ca_{otolith}$ and D_{Ba} patterns may be a product of water chemistry. Indeed, manipulations of ambient barium uniformly showed a clear influence on $Ba:Ca_{otolith}$ ratios (Miller 2009, Collingsworth et al. 2010). Facilitation between Ba and Sr ions may also explain our observations. Previous studies highlighted elevated incorporation of Ba with increased Sr ratios (de Vries et al. 2005). Though our study did not manipulate Sr concentrations directly, we did observe the higher dissolved Sr concentrations, and lowest dissolved Ba concentrations, in the highest salinity treatment. A complementary hypothesis to Sr facilitation proposes that increased ambient water concentrations of Ba may inhibit incorporation of Ba into otoliths, based on the fact that Ba approaches proportional incorporation with the ambient water at low concentrations (Bath et al. 2000, de Vries et al. 2005).

Magnesium may offer promise as temperature proxy for juvenile Atlantic cod because only temperature significantly affected incorporation, measured by both Mg:Ca and D_{Mg}. Increasing temperature led to increasing Mg:Ca ratios in otoliths, both pooled across and within salinity treatments. This result confirms trends reported by Barnes and Gillanders (2013) and Elsdon and Gillanders (2002), who also found a significant positive relationship between Mg:Ca and temperature but no effect of salinity. Mg:Ca is believed to be primarily under biological control (Martin & Thorrold 2005), with otolith Mg concentration regulated by physiological fractionation between blood and endolymphatic fluids surrounding fish otoliths (Melancon et al. 2009). Temperature-mediated control of

$Mg:Ca_{otolith}$ ratios observed in our study and others may represent a product of thermal influence on the fractionation of Mg from blood to endolymphatic fluids (Barnes & Gillanders 2013). Responses of otolith Mg:Ca ratios to temperature vary across taxa, with reports of non-significant (Martin & Thorrold 2005, Martin & Wuenschel 2006) and negative effects of temperature (Fowler et al. 1995). Variation in physiological response to temperature and its impact on fractionation likely contributes to among-species differences (Barnes & Gillanders 2013).

Temperature and salinity significantly increased otolith manganese incorporation. Both $Mn:Ca_{Otolith}$ and D_{Mn} were positively associated with temperature, with highest values in the warmest treatment pooled across salinity treatments. The influence of salinity was more varied with significantly lower $Mn:Ca_{Otolith}$ and higher D_{Mn} ratios at higher salinities only when the temperature exceeded 5 ^{o}C. Considering partition coefficients, we found a significant temperature and salinity interaction on D_{Mn}, similar to previous work with juvenile spot, *Leiostomus xanthurus* (Martin & Thorrold 2005). Temperature had a significant positive effect on D_{Mn} in high salinity treatments. As in Martin and Thorrold (2005), we found greater differentiation in D_{Mn} across salinity treatments at the warmest temperatures. Differences between Me:Ca and D_{Me} are often attributed to water chemistry variation across treatments (Martin & Wuenschel 2006), and indeed we observed a significant negative association between $Mn:Ca_{Water}$ and salinity as previously documented (Martin & Thorrold 2005). However, past studies have not reliably linked ambient ratios of Mn ($Mn:Ca_{Water}$) to otolith chemistry (Elsdon & Gillanders 2003, Collingsworth et al. 2010). The signal-to-noise ratio of Mn is high

relative to other elements, given sensitivity to changes in water chemistry and low otolith concentrations. Nonetheless, our observations of inverted trends with $Mn:Ca_{Otolith}$ and D_{Mn} potentially indicate limitation, however, without mediation of ambient conditions we cannot fully partition the influence of variable physiology and ambient availability on Mn incorporation. Despite a lack of consensus on the mechanisms underlying concentrations in fish otoliths, Mn has proven useful in discrimination analyses (Thorrold et al. 1998, Reis-Santos et al. 2008, D'Avignon & Rose 2013). For Atlantic cod, Mn could potentially be used as a useful tracer for temperature; however, our results suggest that reliable reconstructions would require some prior knowledge of salinity and clear variation in concentration (e.g. among geographic areas).

Stable Carbon and Oxygen isotope chemistry offers a useful tool for reconstructing environmental histories of aquatic organisms because both isotope systems vary geographically in coastal and ocean waters (Thorrold et al. 1997a). Carbon isotopes ($\delta^{13}C$) in otoliths reflect a mixture of ambient dissolved inorganic carbon (DIC) and dietary carbon (Kalish 1991, Schwarcz et al. 1998) sources in a ratio that is likely mediated by physiology (Thorrold et al. 1997a). Reported otolith ratios between ambient DIC and metabolic carbon vary from approximately 20% (Weidman & Millner 2000) to 30% (Hoie et al. 2004) for Atlantic cod. We found a non-linear effect of temperature on $\delta^{13}C$ values, with decreases between intermediate and high temperature treatments as expected (Weidman & Millner 2000) but with the lowest $\delta^{13}C$ values found at the coolest temperatures (~ 5 °C). We held juvenile cod in the low temperature treatment at the bottom of their expected thermal range (Brander 1995). Correspondingly, somatic and

otolith growth were significantly reduced in the low temperature treatments. The result is, nonetheless, similar to that of Hoie et al. (2003) who observed higher $\delta^{13}C$ values than low growth rate fish in larval and early juvenile Atlantic cod (<21 mm) with higher growth rates and presumably higher metabolism. Like Hoie et al. (2003), our observations cannot be attributed to diet, because we did not vary feeding among treatments, and all cultures were fed to saturation, although consumption nonetheless varied slightly among treatments. Previous studies reported behavioural differences both in respiration rate (McConnaughey et al. 1997) and swimming alterations of metabolic rate (Bjornsson 1993, Hoie et al. 2003) that correlated negatively with $\delta^{13}C$. We lacked a direct measure of either index but would not expect either behaviour to increase at lower temperatures. Considering only the intermediate and high temperature treatments, where we observed no significant difference in size, $\delta^{13}C$ declined on average -0.14 ‰/°C ($p=0.001$, $r^2=0.37$), similar to rates derived from seasonal otolith records of wild caught Atlantic cod ~-0.16 ‰/°C (Weidman & Millner 2000). This rate is similar to that previously reported for bearded rock cod (Kalish 1991), Atlantic croaker (Thorrold et al. 1997a), as well as various species of foraminifera and mollusks (Grossman & Ku 1986) (-0.18,-0.22, -0.11, and -0.13 ‰/°C, respectively).

Otolith $\delta^{18}O$ decreased with temperature, confirming patterns previously reported for Atlantic cod (Hoie et al. 2004) and other biogenic aragonites (Grossman & Ku 1986). Past studies documented negative associations of otolith $\delta^{18}O$ and temperature for a variety of species, and demonstrated the reliability of Atlantic cod as an indicator of

temperature to within 1 °C (Weidman & Millner 2000) when adequately constraining the $\delta^{18}O$ of the ambient water.

Despite evidence linking salinity to variable isotope incorporation in fish otoliths (e.g. Schwarcz et al. 1998, Elsdon & Gillanders 2002), no previous studies tested salinity effects on otolith isotope concentrations in cod. We found a significant positive association between otolith isotopes and salinity. The positive association between salinity and $\delta^{18}O$ confirms previous studies documenting proportional otolith incorporation with ambient conditions (Thorrold et al. 1997a) and a positive relationship between water $\delta^{18}O$ and salinity (Gao 2002).

2.5.2 Relationships with growth

Somatic and otolith growth rate can influence incorporation of elements and isotopes in fish otoliths (Hoie et al. 2003, Martin & Thorrold 2005). Fish with faster growth rates generally have proportionally smaller otoliths (Worthington et al. 1995). In particular, past work found a negative correlation between otolith size and growth rate in juvenile Atlantic cod (Otterlei et al. 2002); our study shows that otoliths grow proportionally faster (~1.4) than fish length, confirming this trend. Diet and changes in physiology often associated with growth rate and size, influence carbon in particular (Hoie et al. 2003) as our data also showed. Somatic growth rate might be more important for larval and juvenile fishes where more variable growth is expected (Martin & Wuenschel 2006). Our experiment revealed strong correlations between somatic growth and temperature (Bolland 2008), which precluded a full evaluation of the combined effects of somatic growth with environmental condition resulting from limited variation

individual growth within a treatment. A three-way temperature, salinity and feeding (growth) experiment similar to the nested design employed by Hoie et al. (2003) would most clearly elucidate the effect of variable growth rate on otolith incorporation (Martin & Wuenschel 2006).

Though growth was not controlled orthogonally to environment in our experiment, we nonetheless observed variation in growth within treatments. Residuals from a temperature by salinity growth model offer a tool to evaluate the effect of growth while accounting for environmental correlates. Our study found significant positive relationships between growth and otolith magnesium and manganese (Me:Ca and D_{Me}), similar to observations of D_{Mn} in Atlantic cod (Limburg et al. 2011) and other groundfish species (Limburg et al. 2014). Strontium (Sr:Ca and D_{Sr}) and growth were significantly and negatively related, as reported in juvenile striped bass (*Morone saxatilis*) (Secor et al. 1995). Incorporation of barium (Ba:Ca and D_{Ba}) was growth independent, as reported in adult Atlantic cod (Thorisson et al. (2011) and juvenile spot croaker (*Leiostomus xanthurus*) (Bath et al. 2000). Similarly, we observed an overall negative but non-significant relationship between oxygen ($\delta^{18}O$) incorporation and growth, suggesting growth independent incorporation, mirroring patterns previously reported for juvenile Atlantic cod (Hoie et al. (2003). Carbon ($\delta^{13}C$) ratios and growth were significantly and positively related, echoing similar trends in previous studies (Thorrold et al. 1997a). At low temperatures, growth was highest at intermediate salinities, where we also observed the highest $\delta^{13}C$ values. This relationship with growth likely drove the significant interaction between temperature and salinity on $\delta^{13}C$ incorporation. As with $\delta^{18}O$, the

positive association between $\delta^{13}C$ and growth mirrors patterns observed for temperature and juvenile Atlantic cod (Hoie & Folkvord 2006). Indeed all observed relationships between growth and otolith chemistry mirror temperature patterns. Collectively our results support the assertion that variation in physiology and otolith deposition associated with growth influence trace element incorporation (Secor et al. 1995), further highlighting the need for experimental studies that nest growth within temperature.

2.5.3 Statistical considerations

Logistical constraints unfortunately forced us to nest salinity treatments within one replicate of each temperature level. This design limitation potentially limits inferences regarding temperature, because of unknown, and unquantifiable, biases associated with the temperature replicates and inflated probability of Type I error. To address this issue, and justify the use application of temperature 'replicates' within our statistical models, we minimized the likelihood any artifacts associated with the temperature replicates. First, temperature controlled rooms shared a common design with standardized bench tops, aquaria, water filtration, and aeration systems. All water was sourced from common inputs and mixed to the ascribed salinities outside the temperature controlled rooms. Within each room we randomized the position of aquaria (n=9). Acknowledging that a fully orthogonal temperature by salinity design would have been ideal, we believe our approach to minimize any variability not associated with temperature permitted the statistical approaches used and inferences drawn. The agreement of our results with previously published work detailing temperature influences

on juvenile Atlantic cod otolith chemistry (e.g. Hoie et al. 2004, Hoie & Folkvord 2006) strongly supports this assertion.

2.5.4 Conclusion

Ultimately, otolith geochemical composition provides a template to discern environmental histories of juvenile Atlantic cod, potentially providing novel information about this critical period. Analyses of otolith composition provides one method to assign juvenile residency in Atlantic cod, offering a feasible alternative to tagging studies that are logistically difficult or impossible in larval and juvenile fishes given their small size and relatively high mortalities (Thorisson et al. 2011). Spatial and temporal differences in otolith chemistry among geographic regions may reflect ambient water chemistry and environmental condition, noting the relatively small effect that diet alone has on otolith incorporation for most fish (Hoff & Fuiman 1995, Milton & Chenery 2001, Walther & Thorrold 2006, Webb et al. 2012, Woodcock et al. 2012), with some notable exceptions (Buckel et al. 2004). The wide range of environmental conditions experienced by Atlantic cod in nearshore nursery habitat necessitates a comprehensive understanding of temperature and salinity effects on otolith chemistry. Moreover, these complex environments demand a diverse suite of otolith trace elements and isotopes to ensure successful reconstruction. Results from our study provide a comprehensive assessment of temperature and salinity effects on a substantial suite of both elemental and isotopic constituents in juvenile Atlantic cod otoliths. Temperature and salinity both significantly affected all elements and isotopes we measured except Mg, which was apparently incorporated into otoliths independent of salinity. Collectively our results highlight the

potential utility of otolith geochemistry to identify change and reconstruct environmental life histories of Atlantic cod, but they also highlight key considerations and limitations of the application when considering environmental variables in isolation.

2.6 Tables

Table 2.1 Means, standard deviations (sd) and ranges for each element and isotope *Gadus morhua* otolith constituent measured across all treatments. Trace elemental data is standardized as a ratio of calcium; units for elements are per mol Ca, Isotopic ratios of $\delta^{18}O$ and $\delta^{13}C$ are expressed in ‰ relative to VPDB, and partition coefficients are ratios of otolith chemistry to ambient water chemistry.

Parameter	Mean	Sd	Range	Units	n
$\boldsymbol{Me:Ca_{Water}}$					
Mg	5.31	0.57	(5.06-5.53)	Mol	24
Mn	5.99	2.60	(2.26-9.50)	µmol	24
Sr	9.40	0.23	(8.87-9.74)	mmol	24
Ba	22.98	14.8	(5.23-44.25)	µmol	24
$\boldsymbol{Me:Ca_{Otolith}}$					
Mg	20.19	11.15	(7.27-61.7)	µmol	278
Mn	2.62	1.05	(1.02-6.07)	µmol	273
Sr	3.08	0.76	(1.68-5.05)	mmol	273
Ba	2.49	0.79	(1.32-4.54)	µmol	273
$\delta^{18}O$	-3.68	0.79	(-5.3--1.87)	‰	116
$\delta^{13}C$	-0.57	0.98	(-2.57-1.46)	‰	116
$\boldsymbol{D_{Me}}$					
Mg	3.81	2.1	(1.43-11.3)	$*10^{-6}$	269
Mn	0.50	0.27	(0.129-1.72)	-	267
Sr	0.28	0.08	(0.18-0.53)	-	265
Ba	0.18	0.14	(0.033-0.73)	-	269

Table 2.2 Results of linear mixed effects models comparing water chemistry ($Md:Ca_{Water}$), otolith chemistry ($Me:Ca_{Otolith}$) and elemental partition coefficients (D_{Me}) as a function of temperature (T) and salinity (S) and their interaction (x), with replicate tank controlled as a random variable. Residuals from the temperature x salinity growth relationship were evoked as a continuous covariate. To control for multiple comparisons p-values are adjusted according to the *Benjamini-Hochberg* transformation.

			Mg		**Mn**		**Sr**		**Ba**	
	Term	df	F	P	F	P	F	P	F	P
$Me:Ca_{Water}$	T	2	1.8	0.354	5.4	0.042	0.5	0.879	0.3	0.904
	S	2	238.0	**<0.001**	194.5	**<0.001**	324.4	**<0.001**	1233	**<0.001**
	TxS	4	0.2	1.000	1.9	0.337	0.6	0.879	0.2	0.924
$Me:Ca_{Otolith}$	T	2	6.8	**0.003**	14.2	**<0.001**	27.7	**<0.001**	27.5	**<0.001**
	S	2	1.3	0.343	14.4	**<0.001**	6.2	**0.004**	17.2	**<0.001**
	G	4	4.7	**0.046**	15.0	**<0.001**	31.6	**<0.001**	0.3	0.728
	TxS	9	0.1	0.984	0.6	0.799	0.1	0.984	1.8	0.192
D_{Me}	T	2	7.2	**0.002**	34.8	**<0.001**	28.0	**<0.001**	25.9	**<0.001**
	S	2	0.3	0.888	117.6	**<0.001**	4.2	**0.029**	742.4	**<0.001**
	G	4	4.7	**0.049**	15.0	**<0.001**	31.6	**<0.001**	0.3	0.606
	TxS	9	0.1	0.974	2.7	**0.041**	0.1	0.974	2.6	**0.046**

Table 2.3 Results of linear mixed effects models comparing otolith isotopes Carbon and Oxygen ratios among temperature (T) and salinity (S) treatments with replicate tank controlled as a random variable. Residuals from the temperature x salinity growth relationship were evoked as a continuous covariate. To control for multiple comparisons p-values are adjusted according to the *Benjamini-Hochberg* transformation.

	Source of Variation	df	$\delta^{18}O$ F	$\delta^{18}O$ p	$\delta^{13}C$ F	$\delta^{13}C$ p
$Me:Ca_{Otolith}$	T	2	61.39	**<0.0001**	43.72	**<0.0001**
	S	2	23.23	**<0.0001**	21.70	**<0.0001**
	G	4	0.02	0.914	0.95	**0.030**
	T x S	9	1.72	0.208	4.67	**<0.0001**

Table 2.4 Results of linear mixed effects models comparing somatic and otolith growth as a function of temperature (T) and salinity (T). Tank replicate treated as a random factor.

Source of variation	df	Somatic F	Somatic p	Otolith F	Otolith p
T	2	102.4	**<0.0001**	34.0	**<0.0001**
S	2	1.7	0.23	2.9	0.06
T x S	4	5.3	**0.001**	1.7	0.16

2.7 Figures

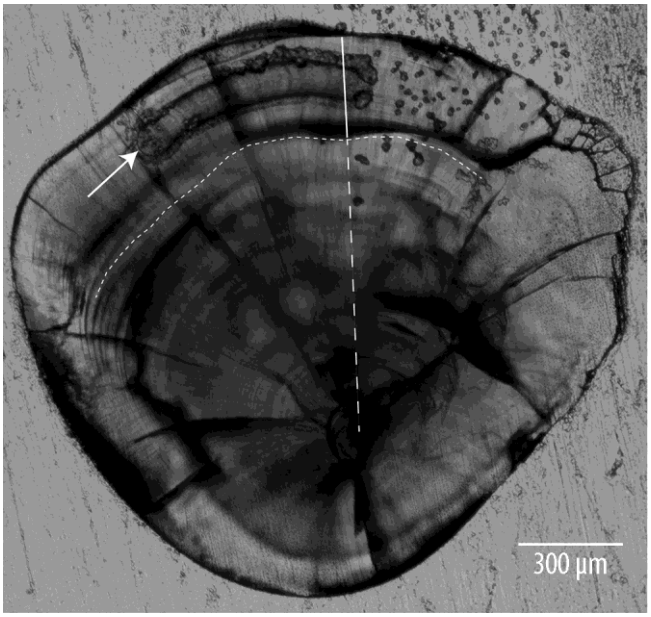

Figure 2.1 Polished juvenile *Gadus morhua* sagittal otolith showing laser raster (arrow), oltolith growth (solid line), pre-experimental growth (long dashed line) and alizarin tag (short dashed line) denoting beginning of the experiment.

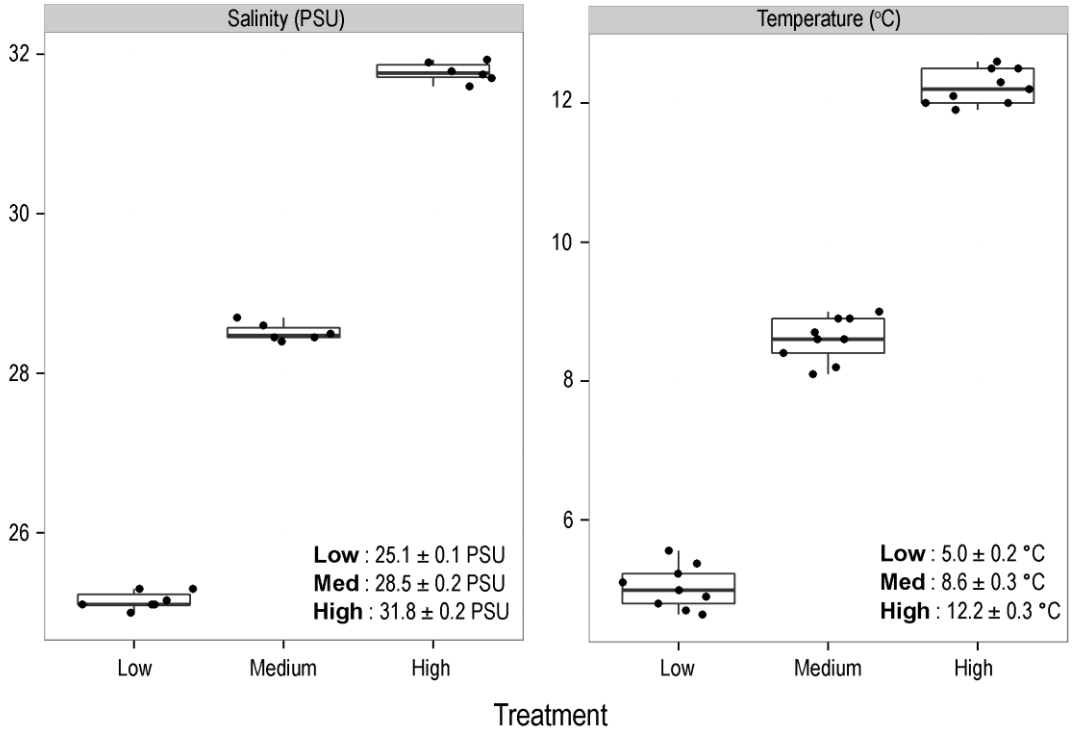

Figure 2.2 Summary of experimental treatments conditions. Means values are reported ± 1 standard deviation.

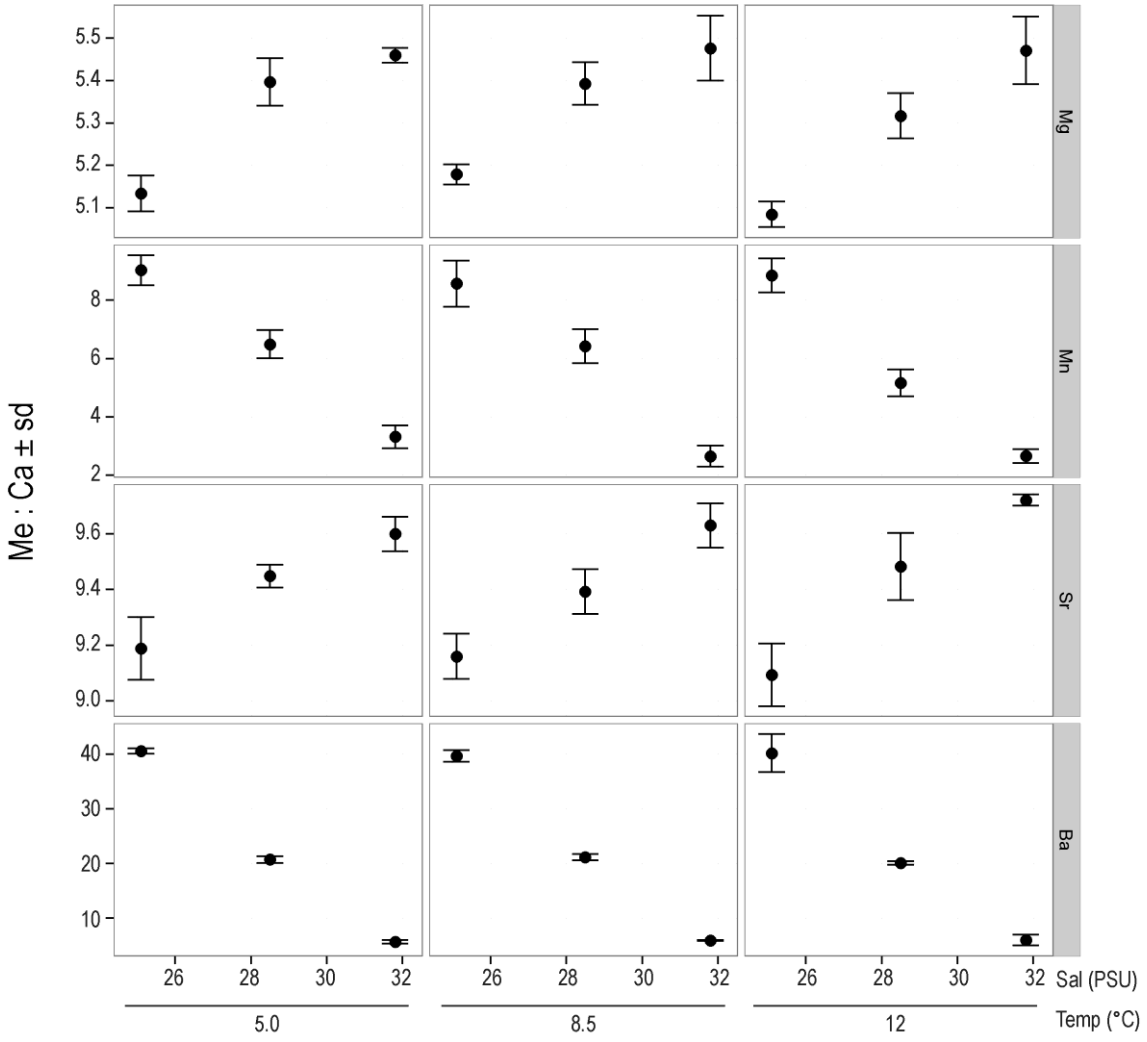

Figure 2.3 Mean ratios of Me:Ca_{water} among temperature and salinity experimental treatments. Mg:Ca values are in mol·mol^{-1}, Mn:Ca and Ba:Ca are µmol·mol^{-1}, and Sr:Ca is mmol·mol^{-1}.

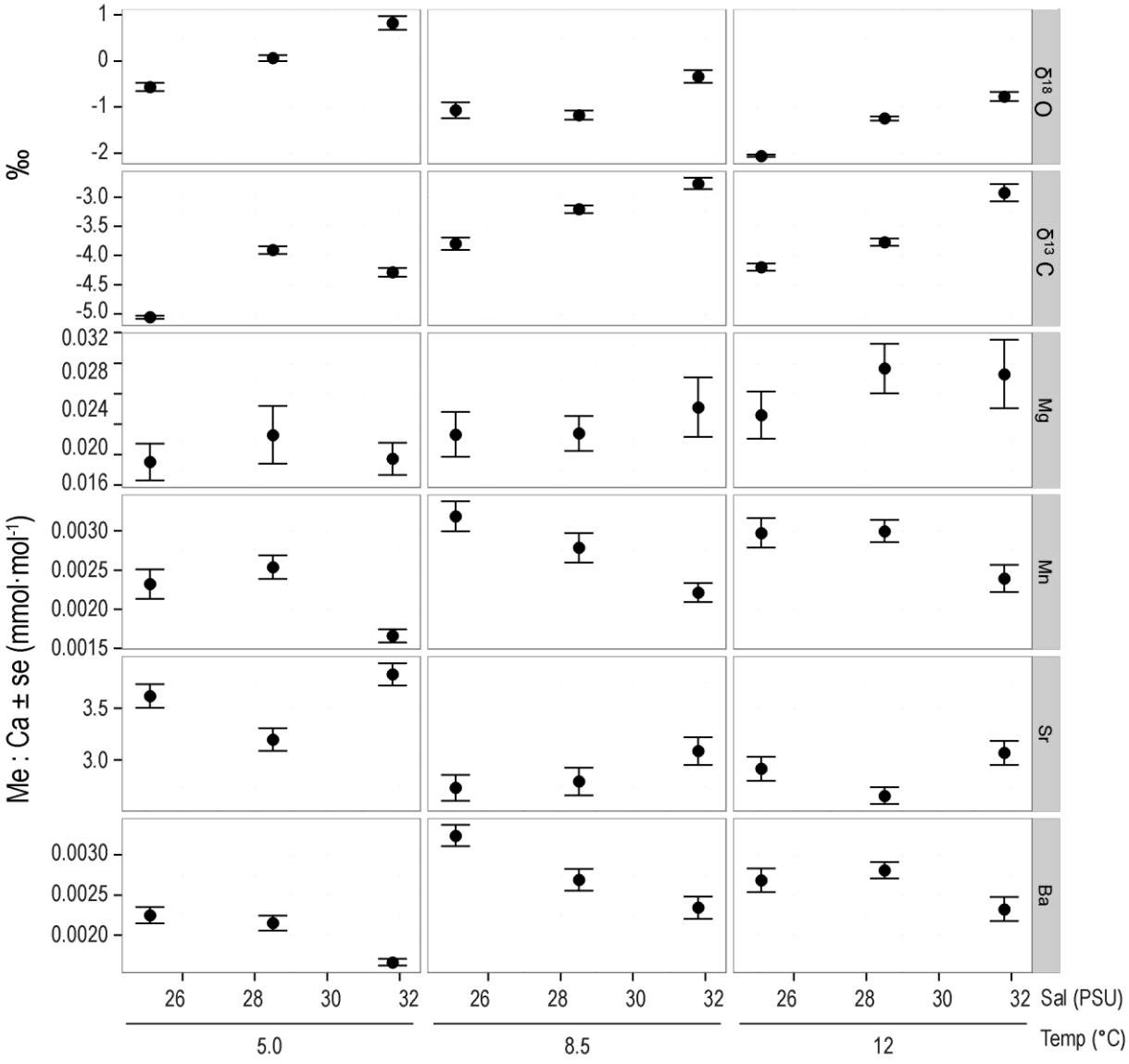

Figure 2.4 Mean Me:$Ca_{Otolith}$ and isotopic ratios for temperature and salinity treatments (n=3 respectively) of juvenile *Gadus morhua* otoliths. Isotopes in ‰ relative to VPDB. Error bars represent ± 1 standard error.

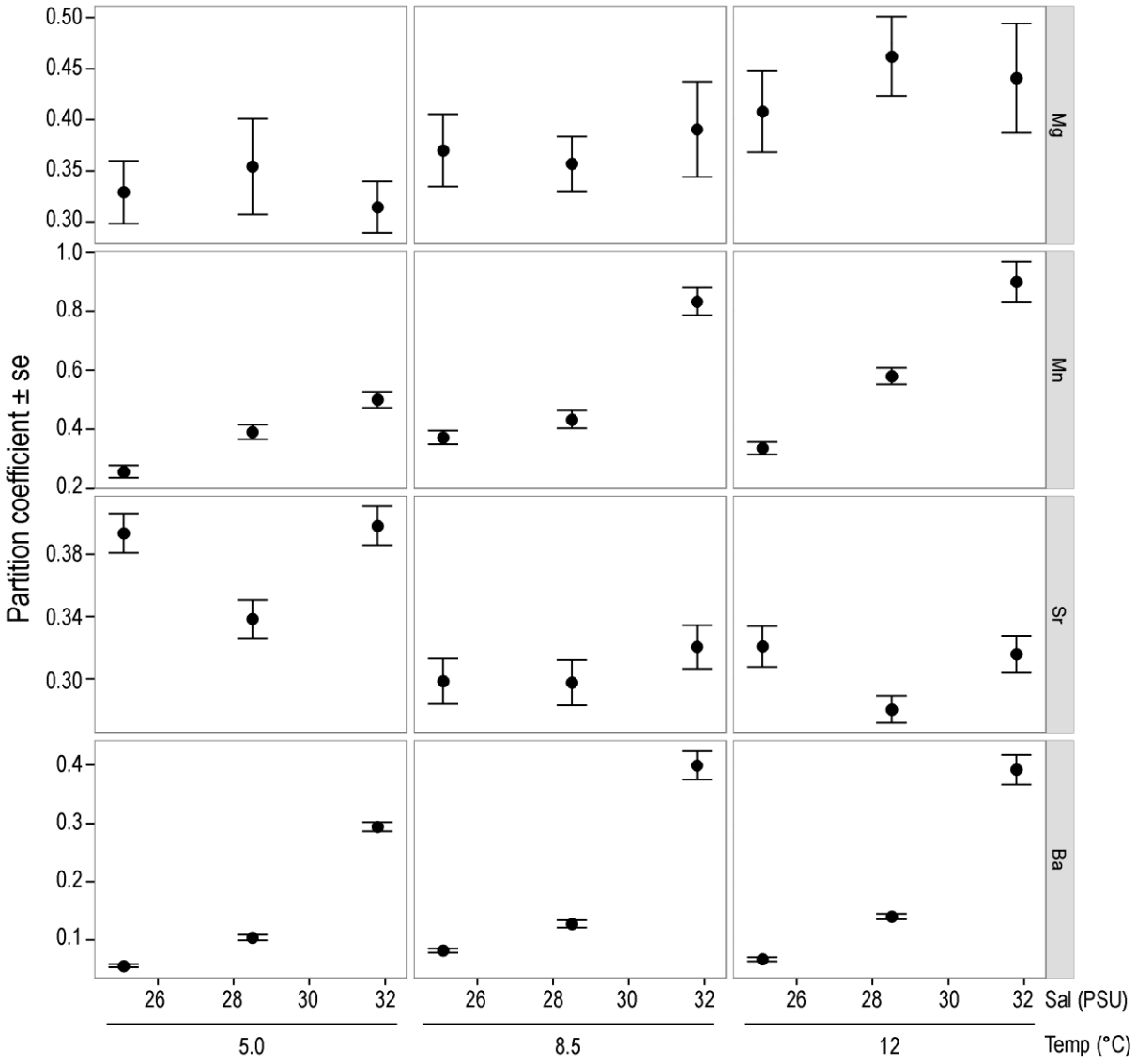

Figure 2.5 Mean partition coefficients (D_{me}) for juvenile *G. morhua* otoliths reared under temperature and salinity experimental treatments. Mean D_{mg} values are multiplied by 10^5.

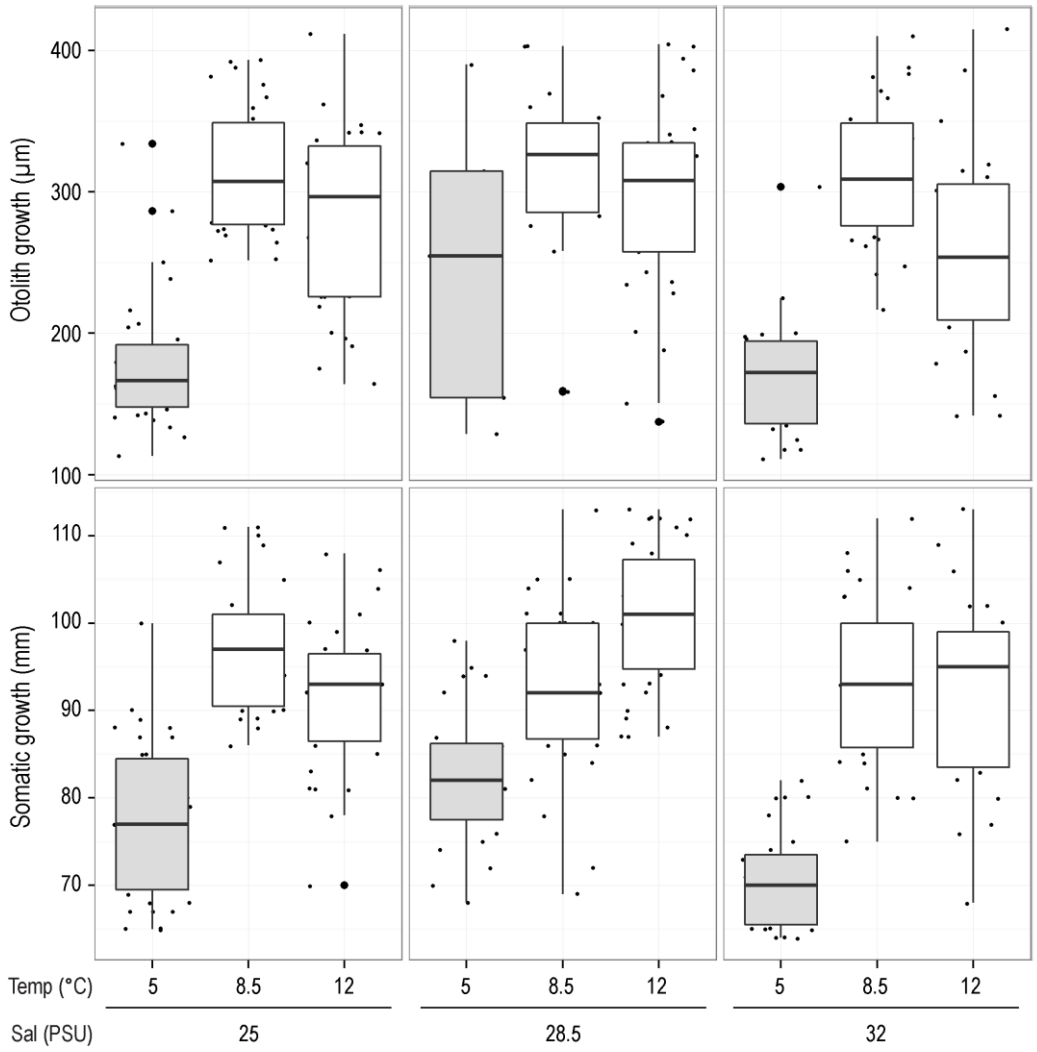

Figure 2.6 Summary of somatic and otolith growth throughout the experiment. Boxplot fill colours denote results of within treatment Tukey's post-hoc tests ($\alpha < 0.05$).

Chapter 3 – Regional variation in otolith geochemistry of juvenile Atlantic cod, *Gadus morhua*, in coastal Newfoundland

3.1 Abstract

We examined spatial variation in otolith geochemistry as a natural tag in juvenile Atlantic cod to resolve geographic patterns during early life history. Individuals from 54 inshore sites spanned five embayments in eastern Newfoundland. Otolith composition differed at all spatial scales and related inversely to spatial scale. Classification analysis revealed increasing discrimination at coarser spatial scales: site (26-58%), bay (49%), and coast (76%). Assignment success declined by ~10% per added site with increasing sampling sites per bay, demonstrating fine-scale (<100 km) variation. When we partitioned environmental variability from observed otolith chemistry using predictive models, assignment success improved by 56, 14 and 5% for site, bay, and coast, respectively. Our results demonstrate environmental influence on spatial structure of otolith chemistry and illustrate the importance of resolving baseline variability in otolith chemistry when conducting assignment tests. Collectively our results describe the potential utility of juvenile otolith composition in evaluating contributions of sub-populations to the Northwest Atlantic cod stock and highlight important limitations imposed by environmental variation at scales less than 100 km.

3.2 Introduction

The extent of dispersal and survival to successful reproduction collectively determine the geographic scale of natural populations (Cowen et al. 2006, Hastings & Botsford 2006, Pineda et al. 2010). Together these processes dictate ecological connectivity and the evolutionary persistence of populations and species (Hilborn et al.

2003, Hastings & Botsford 2006). Accordingly, the successful management of aquatic resources requires identification of population links, or paths of recruitment, and their relative contributions to population persistence (DiBacco et al. 2006, Botsford et al. 2009, Grant et al. 2010). Such knowledge facilitates stock identification (e.g. Cadrin et al. 2005), marine reserve design (e.g. Sale et al. 2005, Almany et al. 2009, Berglund et al. 2012), and the protection of marine biodiversity (Ruzzante et al. 2006). However, the multitude of physical and biological processes that potentially influence the distributions of marine populations with dispersive larval and adult stages complicates understanding of connectivity among habitats and geographic regions (Pineda et al. 2010). Future successful management of marine ecosystems requires the development of methodologies that permit estimates of marine dispersal and connectivity at the scale of individual population components.

Multiple empirical approaches have been developed to quantify connectivity and dispersal in marine environments (see reviews in Thorrold et al. 2002, DiBacco et al. 2006, Levin 2006, Botsford et al. 2009, Leis et al. 2011). Molecular genetic approaches effectively test population divergence, individual assignment, or parentage (e.g. Bradbury & Bentzen 2007, Jones et al. 2007, Hedgecock 2010), but their effectiveness requires stable isolation or extremely small population sizes (Saenz-Agudelo et al. 2009). Alternatively, markers in calcified structures (e.g. otoliths) may distinguish population structure within large numbers of individuals and may therefore provide effective metrics of dispersal, even in high gene flow scenarios (Campana 1999, Thorrold et al. 2001, Elsdon et al. 2008). Fish otoliths are metabolically inert calcified structures that grow

continuously throughout development, and integrate at least some elements from the surrounding water into the calcium carbonate matrix in proportion to ambient concentrations. In regions with sufficient spatial variation in water chemistry and environmental conditions (e.g. water temperature and salinity), analysis of otolith geochemical composition can provide a natural marker of both time and space to determine differences in spatial use by marine populations. The identification of species and habitats that provide fine-scale, geochemical-based otolith tags will significantly advance understanding of marine dispersal and connectivity, and resolve fish movement in a way not possible with other approaches (e.g. Thorrold et al. 2001).

Atlantic cod, *Gadus morhua*, a common demersal species, occupies coastal and continental shelf environments throughout the North Atlantic. After a 1-2 month pelagic egg and larval phase (Pepin & Helbig 1997), larvae metamorphose into benthic juveniles, primarily inhabiting shoal waters near the coast (Bulatova 1963, Laurel et al. 2003). Adults move in variable patterns (Rose et al. 2011), often migrating many hundreds of kilometers (Rose 1993, D'Avignon & Rose 2013), yet an estimated 44% of all known Atlantic cod spawning groups do not migrate extensively and are characterized as small, inshore groups (Robichaud & Rose 2004).

Our study evaluates the potential use of otoliths as a natural tag to discern fine-scale natal patterns of Atlantic cod in coastal Newfoundland. Specifically, we evaluate how environmental variability within an assessment unit can influence the application of assignment tests using otolith chemistry over fine geographic scales (10-100 km). We develop this evaluation using spatial sub-sampling and a complementary laboratory

rearing study (Stanley et al. 2015b; Chapter 2), asking whether biogenic variation in otolith geochemistry in field populations provides a sufficient signal to differentiate among otolith compositions of fish from different natal sites and which factors might influence this distinction. Previous population studies based on adult Atlantic cod otoliths (Campana et al. 1994, D'Avignon & Rose 2013) and genetics (Hutchinson et al. 2001, Bradbury et al. 2011b, Neat et al. 2014) distinguished among spawning stocks separated by large spatial scales (>1000 km). However neither approach provided a mechanism to link these aggregations with juvenile nursery habitat. Our study builds on this body of work by determining variability in otolith geochemistry of newly settled juvenile fish at fine spatial scales (within and among coastal embayments) and thus evaluates the potential for future assignments of adults to critical coastal nursery habitats. Our objectives are to: (1) measure spatial distributions of juvenile cod in coastal Newfoundland and their association with known spawning locations; (2) examine spatial heterogeneity in geochemical composition of juvenile (age-0) cod otoliths from five embayments in eastern Newfoundland; and (3) quantify the success with which juveniles may be assigned, based on otolith geochemistry, to known site, bay, or coast of capture, and (4) evaluate how small-scale geographic sampling and environmental variation influence assignment power. Quantification of discrete nursery signatures represents the first step in developing geochemical signatures to measure connectivity and dispersal in marine systems at finer spatial scales (<100 km) than previous studies.

3.3 Materials and Methods

3.3.1 Sample collection

To collect juvenile cod, we deployed a 25-m seine from a small boat in shallow water (usually <10 m); two people then hauled the seine onshore. The stretched mesh size of the beach seine was ~10 mm, retaining fish as small as 20 mm standard length. Each haul swept an area of approximately 880 m^2 (16 m alongshore by 55 m offshore), sampling only the bottom two meters of the water column. We collected juvenile fish using two hauls in adjacent but non-overlapping locations at each site in rapid succession. In total we sampled 55 sites (Figure 3.1) spanning approximately 800 km of coastline in eastern Newfoundland, Canada. Sites were classified based on the embayment (n=5) and coast of capture (n=2). Coasts were classified as north and south of the Avalon Peninsula in eastern Newfoundland, where St. Mary's and Placentia Bay constitute 'southeast' coast sites (Figure 3.1). All sites were sampled between October and November of 2007. We collected a maximum of 50 juvenile cod from each site, measured total length of all individuals, and preserved them in 95% ethanol. We included only sites where we captured a minimum of five individuals in further analyses. Salinity was recorded at each site using water collected at the surface and a YSI® 55 probe (± 0.1 PSU).

3.3.2 Otolith preparation and geochemical analysis

Juveniles 80 mm in length or less were selected for otolith extraction, assuming that size range would represent new (age-0) recruits. Both sagittal and lapillar otoliths were removed, cleaned with ultrapure water, air dried, and stored in acid-washed glass vials. All otoliths were then mounted on glass microscope slides and polished to the core using 0.3 µm lapping film. Mounted otoliths were cleaned again with a nylon brush, triple

rinsed in ultrapure water and sonified for 2 min. Otoliths were then air dried in a laminar flow hood, transferred to clean petri dishes, and transported to the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility for analysis. We selected one lapillus per fish at random for laser ablation and one sagitta at random for $\delta^{13}C$ and $\delta^{18}O$ analysis. Sagittae were selected for $\delta^{13}C$ and $\delta^{18}O$ analysis because they provided sufficient material for analysis. We chose lapilli for laser ablation because their clearly distinguishable annuli helped us standardize sampling of otolith material among individuals. We selected one lapillus and sagitta at random from each fish for analysis.

We measured otolith elemental composition using a Thermo Finnigan *Element2* inductively coupled plasma mass spectrometer (ICP-MS) coupled with an ArF excimer 193-nm laser ablation system. We chose to quantify five isotopes (^{25}Mg, ^{55}Mn, ^{138}Ba, ^{88}Sr, ^{43}Ca,) that were consistently higher than blank replicates during analyses. Assays, based on a 50-µm spot size, consisted of a 250-µm raster along the edge of the otolith. Laser repetition rate was set at 5 Hz for all analyses using a dwell time of 60 s and a scan speed of 5 µm s^{-1} for spot and line analyses, respectively. The ICP-MS method consisted of 38 passes per analysis with a dwell time of 0.1 sec per isotope on each pass and a total dwell time of 3.8 sec per isotope during each analysis. Otolith sampling targeted the outer edge of the otolith to measure elemental composition laid down in the early larval period. Otoliths were randomized for analysis to eliminate potential bias associated with temporal changes in instrument precision. To correct for instrument bias and drift we used certified reference material (CRM) consisting of powdered otoliths (Yoshinaga et al. 2000), dissolved in 2% HNO_3 (SeaStar ®) and diluted to a Ca concentration of 40 □g•g^{-1},

following Thorrold and Swearer (2009). External precision was estimated by analyzing a second otolith CRM (Sturgeon et al. 2005), also dissolved in 2% HNO_3 (SeaStar ®) and diluted to a Ca concentration of 40 µg•g^{-1}, periodically throughout the laser analyses. We determined analytical accuracy from concentrations of Japanese Otolith and NRC standards, averaged across all samples. We found that accuracy exceeded 98% for all otolith constituents measured, including isotopic ratios described below. We calculated limits of detection (LOD) as five times the mean intensity of blanks (2% HNO_3), run every ten assays for each otolith constituent. Any measurements below the respective LOD were excluded from analysis. We conducted all statistical analyses using otolith elemental compositions converted to molar values and standardized to calcium concentrations (Me:Ca). External precision estimates, based on the relative standard deviation values of the second CRM, were 0.40% for Sr:Ca, 1.4% for Ba:Ca, 4.2% for Mg:Ca and 17.9% for Mn:Ca.

We measured $\delta^{13}C$ and $\delta^{18}O$ values in sagittal otoliths of each individual by milling otoliths with a MicroMill sampler (New Wave Research) or a handheld dental drill under a stereomicroscope until we obtained 50 to 200 µg of near-edge material. Stable isotopes were measured using an isotope ratio mass spectrometer and techniques outlined by Ostermann and Curry (2000). We report values of both isotopes (‰) relative to Vienna Pee Dee Belemnite (VPDB). Long-term precision estimates based on the use of NBS-19 are ±0.07 for $\delta^{18}O$ and ±0.03 for $\delta^{13}C$ (Ostermann & Curry 2000).

3.3.3 Statistical analysis

Fish otoliths likely incorporate trace elements, such as Mg, Mn, Ba, and Sr, as substitutes for calcium (Campana 1999), therefore we expressed all elemental concentrations as ratios to Ca (Me:Ca). Data for each element and isotope were assessed for normality by visual inspection and log transformed as necessary. We examined the utility of geochemical signatures for spatial assignment to multiple geographic scales with quadratic discriminant function analyses (QDFA) using the 'MASS' package in *R* (Venables & Ripley 2002). Assignment success of each QDFA was assigned using jack-knifed cross validation with a set training subset of 7 randomly chosen individuals per site. To evaluate the influence of small-scale heterogeneity on classification success, we generated all possible unique combinations of sites for bays with at least four sample sites (Bonavista, Trinity, and Placentia Bay), and ran a QDFA for each subset. Here we consider embayment as the scale of interest because it is generally the scale at which management delineates coastal stocks and the smallest scale at which we can evaluate the potential influence of sampling spatial coverage on assignment success. We limited our comparison of classification success to four sites per bay or fewer because that was the number available for all sites except Placentia Bay. Based on changes in assignment success we evaluated *a-priori* hypotheses about the interpretation of assignment at this scale relative to the spatial coverage of sampling within embayments, inferring that: 1) no significant change signifies that spatial heterogeneity in otolith chemistry contributes little to patterns observed, 2) significant reduction signifies significant fine-scale variability within embayment, which mediates assignment success, and 3) significant improvement

suggests low variability among sites within an embayment and that more samples improve the estimation of the multi-elemental centroid and thus assignment success.

To describe the utility of the assignment tests we compared the proportion of successfully assigned individuals at each spatial scale and data source to the proportion expected if samples were randomly distributed among sites, using contingency tables and Chi-square proportional comparisons in R.

Complementary laboratory experiments (see Stanley et al. 2015 for details) tested the relationship between environment and juvenile cod otolith chemistry based on a controlled temperature (5-12 °C) by salinity (25-32 PSU) experimental design that reflected the range of conditions expected of juvenile habitat sampled in this study (Supplementary Table 3.1). Experiments revealed significant environmental influence on otolith chemistry for all elements and isotopes tested (Stanley et al. 2015b; Chapter 2). From these analyses we developed a predictive mode that employed canonical ordination and a redundancy analysis (RDA) based on Euclidean distance dissimilarity matrices (see Legendre and Anderson 1999). The redundancy model enables prediction of baseline scaling relationships between multivariate dependant and independent environmental variables, in our case, otolith chemistry as a function of temperature and salinity. As with field otolith data, otolith constituent estimates were checked for normality and log-scaled prior to fitting the RDA model. Models were fitted using RDA functions available as part the Vegan community ecology package available in R (Stephens et al. 2010). Using predicted compositions from this model, we ran QDFAs based on residual differences (composition observed in the field minus predicted otolith chemistry for each

constituent), and compared assignment success. Here we assumed the model would capture the baseline influences of variable environmental conditions observed among sample sites and therefore could help to partially elucidate the 'residual' variation in otolith chemistry within the system. Based on changes in assignment success, we evaluated *a priori* hypotheses about the influence of abiotic and biotic variables on otolith chemistry where we infer: 1) no significant change signifies that environmental heterogeneity contributes little to patterns in otolith chemistry or the model and-or inputs were not suitable, 2) significant reduction signifies that most of the discrimination signal could be attributed to environmental heterogeneity, and 3) significant improvement signifies a robust residual signal exists, external to environmental heterogeneity, and reflects local abiotic and biotic complexity. We explored changes in assignment at three different spatial scales: site, bay, and coast of origin, basing inputs of salinity for the redundancy model on measurements averaged from multiple locations within the sample sites from ~1 m depth at the time of fish sampling. Sampling did not coincide with significant rain events and therefore we assume our data provide a relative index of salinity conditions among sample sites. Temperature inputs were collected from sea surface temperature (SST) satellite telemetry measurements (NOAA ETOPO1 1 minute global relief) taken within 25 km of each sample site and averaged for two months prior to collection (see Supplementary Table 3.1), representing the approximate period sampled by the otolith assays and well before fish were likely to disperse from nursery areas in their second year (Shapiera et al. 2014). Surface measurements were assumed to represent

reasonable proxies of benthic juvenile habitat, given that we anticipated little vertical temperature-salinity structure over the depths sampled (< 5 m).

We examined general associations between otolith chemistry and environment observed in the field for each otolith constituent in relation to water salinity, surface temperature, and location of sample site. Correlative relationships were tested in R using permutational Pearson correlations according to Legendre (2005). Associations between otolith chemistry and standard length were explored using a mixed effects model with the sample site treated as a random factor. To control for family-wise error rates and multiple comparisons, we based the significance of each test on Benjamini-Hochberg adjusted p-values (Benjamini & Hochberg 1995) using the p-value adjustment methods available in base R (R Development Core Team 2015).

All statistical analyses assume deposition of otolith material during the juvenile period in the sampled nursery habitat. Micro-milling the edge of the otolith ensured we measured elements and isotopes recently deposited during the juvenile life history. Home ranges for age-0 cod have been estimated at less than 2.5 km^2 (Clark & Green 1990) and 3.5 km^2 (Shapiera et al. 2014) in coastal Newfoundland, considerably less than the spatial scales tested, suggesting that between-site movements are highly unlikely.

3.4 Results

3.4.1 Spatial distribution of juveniles

Juvenile cod abundance varied significantly across the survey area and juveniles occurred at only 32% of the 54 sites surveyed (Table 3.1). Catches averaged 32 juvenile $cod.tow^{-1}$; highest catches were observed along the northern side of Trinity Bay and in

southern Bonavista Bay (Figure 3.2), where we collected as many as 500 juveniles.tow^{-1}. Fish ranged from 30 to 100 mm in standard length. Placentia Bay and St. Mary's Bay cod were ~10 mm smaller on average than other bays (ANOVA, df_{num}=1, df_{den}=325,, F=78.25, p<0.001), but were not different from each other (ANOVA, df_{num}=1, df_{den}=175,df= 1 F=0.028, p=0.87) (Figure 3.3). Placentia Bay cod displayed a bimodal size frequency distribution with significantly higher average size for sites at the mouth of the bay near Spanish Room (ANOVA, df_{num}=1, df_{den}=126, F=11.95, p=0.001, n=129; Figure 3.1).

3.4.2 Otolith geochemistry

Otolith chemistry varied within and among sites (Figure 3.4) for all elements. The largest differences among samples sites for otolith constituents were observed for Sr:Ca, Mn:Ca, Ba:Ca and δ^{13}C, particularly between bays and coasts of capture (Figure 3.5).

We tested individual relationships between otolith geochemical constituents with juvenile cod length and environmental factors and found significant patterns in only seven of thirty possible combinations. Body length was significantly related to δ^{13}C (r=0.03, p<0.0001) and weakly related to Mg:Ca (r=-0.002, p=0.046). Significant environmental associations were found between salinity and both Ba:Ca (r=0.2, p=0.001) and Sr:Ca (r=-0.17, p=0.002). We also observed significant, albeit weak, correlations with the latitude of each sample site for δ^{18}O (r= -0.19, p<0.0001), Sr:Ca (r=0.21, p<0.0001), and Ba:Ca (r=-0.16,p=0.003). No significant correlations were observed between any otolith constituent and temperature or longitude.

3.4.3 Classification success

We evaluated the impact of low numbers of individuals in several samples on observed patterns. First, we examined the scale of correct assignment using all sites, excluding Bristol's Hope, where we sampled only 5 individuals. The QDFA of elemental and isotope data produced correct assignments ranging from 5 to 48% per site, with highest correct assignment for the Colinet Island samples (Table 3.2). For misassigned samples, over 54% were correctly assigned to bay and 78% to coast of origin. Assignment to an individual site might have been partially limited by the number of available samples. Overall, we had less than 20 individuals with complete isotopic and elemental data for the majority of sites (Table 3.1), which potentially limited the ability of discrimination tests to differentiate among sites.

We also evaluated assignment success for pooled sites both at the natal bay and coast scale. Because St. Mary's Bay contained only one sample (Colinet Island), we pooled it with adjacent Placentia Bay. Correct assignments using elemental data alone varied from 24 to 77% for each of the four bay groupings, with lowest values for Conception Bay and highest values for Placentia Bay (Table 3.3). Correct assignments to the north and south coast were 88% and 61% respectively (Table 3.3). Assignments based on isotope ratios revealed similar patterns of relative assignment success as the element data, with lowest bay-scale assignments for Conception Bay (27%) and highest for Placentia Bay (77%). Correct assignments to the north and south coast were 91 and 58%, respectively (Table 3.3). Combining elemental and isotopic data generally improved assignments on average, however, in some cases assignment success decreased with the

inclusion of isotopic data compared to elemental data alone (Table 3.3). Correct assignment estimates with all data averaged ~50% and ranged from 26% (Trinity Bay) to 75% (Placentia Bay). Correct assignment to the north and south coast were 66 and 86% respectively, using both elemental and isotopic data. Collectively, assignment success decreased with the scale of resolution (Table 3.4). Irrespective of the analysis scale or data, fish length had no significant effect on assignment success (Table 3.4).

The impact of small-scale heterogeneity was apparent in Placentia Bay sites which varied widely in correct assignment success (5 to 42 %). Placentia Bay fish also displayed a bimodal size distribution, with inner bay fish 10 mm smaller on average than those from the outer, more exposed site (Spanish Room) (ANOVA: $df_{num}=1, df_{den}=92$, $F=11.95$, $p=0.001$). By separating Placentia Bay into inner and outer bay samples, assignment success increased in the outermost site by ~10%. When we repeated the bay-scale QDFA with Placentia Bay size modes separated, the inner bay (small individuals) samples correctly assigned with 93% accuracy and the outermost site increased to 42% accuracy.

To evaluate the influence of among-sample site heterogeneity on bay-scale assignment, we generated all possible permutations of sites for bays with four or more sampling sites (Table 3.1). Correct assignment declined with increasing number of samples, on average by 10% for every within-bay sample added (Linear regression: slope = -10.21, $r^2 = 0.38$, $p<0.0001$), ranging from 90-100% with one sample per bay, to ~60-70% with four samples per bay (Figure 3.6).

The multivariate predictive model (RDA) based on results from Stanley et al. (2015) was highly significant (ANOVA, df_{num}=1, df_{den}=305, F=55.023 p<0.00001), with an adjusted coefficient of determination (r^2) of 0.49, partitioned into 0.30 and 0.19 for temperature and salinity, respectively (see Supplementary Figure 3.1 for model summary plot). Though all redundancy axes were significantly related to otolith geochemistry (RDA_1 - ANOVA, df_{num}=1, df_{den}=305, F=80.73, p<0.0001; RDA_2 - ANOVA, df_{num}=1, df_{den}=305, F=29.306, p<0.0001), variability and similarities between the intermediate and warm temperature conditions likely reduced the explained variance (49% of the model).

Distribution of residuals (Figure 3.7) differed in overall pattern and magnitude from that of the raw otolith chemistry (Figure 3.4). Classification success based on residuals (observed otolith chemistry minus predicted otolith chemistry from RDA model) improved assignment success at all scales (Tables 3.2 and 3.3, Figure 3.8). The majority of observed changes in assignment success (residuals vs. observed) were greater than zero, averaging (± 1 standard error, n) 57% (± 5.9, 17), 14% (± 6.9, 5) and 5% (± 0.3, 2) at the site, bay, and coast respectively (Figure 3.8). Assignment to Bonavista Bay using only isotopic data was the only classification that significantly decreased post-correction (~ 40% decrease) (Table 3.5). The addition of length to the assignment analysis did not significantly change assignment success (ANOVA: df_{num}=1, df_{den}=4, F= 0.004, p=0.95) (Figure 3.8).

Though the relative assignment success varied among sites and spatial scales, chi-square proportional comparisons revealed that the majority of tests were significantly

greater than classification based on a random assignment of fish among all sites (Tables 3.2 and 3.3).

3.5 Discussion

Successful management and conservation strategies for exploited marine species hinge upon understanding the extent of dispersal and connectivity in marine organisms (Sale et al. 2005, Laurel & Bradbury 2006, Botsford et al. 2009). Characterization of dispersal kernels and connectivity matrices requires measurements of movements that integrate across life history stages (Hastings & Botsford 2006). Our evaluation of otolith geochemistry as a natural tag using a suite of elements and stable isotopes showed significant spatial variation in juvenile Atlantic cod among sites in coastal Newfoundland. Individuals successfully classified to bay of capture with 54% accuracy on average, or to coast (south versus north) with 78% accuracy on average, which significantly exceeded the accuracy rates possible using techniques that require either large spatial scales (morphometrics - Templeman 1981, genetics - Ruzzante et al. 2000a, parasite loading - Khan et al. 2011), anadromy (e.g. Avigliano et al. 2014) or by random chance (20% for bays [n=5] and 50% for coasts [n=2]). Otolith geochemistry also varied significantly at finer spatial scales, with assignment success to the correct embayment and coast exceeding 80% for some sites (93% in inner Placentia Bay), highlighting the potential for fine-scale environmental variability to limit assignment success. Indeed, we found an inverse relationship between successful discrimination and the number of sample sites within an embayment, suggesting that unresolved variation could lead to misclassifications. Similarly, when we accounted for small-scale variation in

environmental conditions among sites with a laboratory-based predictive model, assignment success improved significantly across all spatial scales tested. Our work adds to a growing literature that documents significant spatial variation in otolith geochemistry of coastal marine fishes (Clarke et al. 2009). The presence of significant spatial variation in otolith geochemistry and subsequent relatively high rates of spatial discrimination support its potential as a tool to delineate movements and spatial patterns of Atlantic cod in coastal Newfoundland, at scales considerably smaller than previously tested (see Campana et al. 1999, D'Avignon & Rose 2013 for examples using adult cod). We posit the potential role for geochemical approaches in the conservation and management of this species, and emphasize the complexities imposed by natural environmental variability at small spatial scales, often overlooked by assignment studies.

Many studies have used otolith composition to track movement patterns across species and habitats (Campana 2005, Elsdon et al. 2008). Studies of variation in otolith composition have successfully discriminated among fish from local rivers (e.g. Kennedy et al. 2002), estuaries (e.g. Thorrold et al. 1998, Bradbury et al. 2008c), reefs (Swearer et al. 1999), coastal regions (Campana 1999, Warner et al. 2005, Correia et al. 2012) and spawning aggregations (Thorisson et al. 2011, D'Avignon & Rose 2013), with varying degrees of accuracy. Our geochemical data provided levels of successful classification comparable to previous coastal applications. Clarke et al. (2009) reported classification accuracies of 70-77% for Atlantic silversides along the eastern United States using a similar suite of elements and stable isotopes. On a broader scale, Rooker et al. (2008) documented >88% correct classification of bluefin tuna to nursery locations throughout

the Atlantic and D'Avignon and Rose (2013) documented accuracies of 66-78% for Atlantic cod over broad spatial scales (> 500 km) in the northwest Atlantic. Continuous distributions of individuals, spatial-temporal variability in environment, and relatively weak spatial heterogeneity in otolith composition pose substantive challenges to assignment of coastal marine fishes on small scales. Though variable within the scale of assignment, our results nonetheless suggest variability in juvenile Atlantic cod otolith chemistry at small spatial scales (>100 km), which suggests the utility of retrospective analysis at smaller scales than previously applied (e.g. bay or coast), which are particularly relevant to management (e.g. DFO 2013b).

We observed variation at the scale of sample site for most elements and stable isotopes. The largest differences among our samples were in Sr:Ca, Mn:Ca, Ba:Ca and $\delta^{18}O$, which provided much of the discriminatory power, particularly among bays and coast of capture. The identification of strong Sr:Ca, Ba:Ca and $\delta^{18}O$ differences among sites, and their significant correlation with ambient salinity, supports the hypothesis that concomitant variability in water chemistry potentially generates regional composition in otolith chemistry through direct mediation (Elsdon & Gillanders 2004, Stanley et al. 2015b, Chapter 2). River runoff typically has lower Sr:Ca and $\delta^{18}O$ values, and higher Ba:Ca values, than seawater (Gillanders 2005a). Previous work documented consistently lower salinity at the head of Placentia Bay (Bradbury et al. 2000), near an important spawning and nursery location where we noted high assignment success. In addition to elemental analysis, stable isotopes have been used extensively to reconstruct environmental histories, trophic interactions, and movement patterns in marine fishes

(Weidman & Millner 2000, Rooker et al. 2008, Jones & Campana 2009).The fractionation of $\delta^{13}C$ and $\delta^{18}O$ in otolith aragonite is also well-studied in relation to biological and hydrographic variation (e.g. Thorrold et al. 1997a). Stable oxygen isotopes are thought to be deposited close to isotopic equilibrium with ambient water, and thus reflect a combination of $\delta^{18}O$ of the ambient water and water temperature at the time of deposition (Thorrold et al. 1997a, Weidman & Millner 2000, Jamieson et al. 2004, Jones & Campana 2009). The correlation we observed here between $\delta^{18}O$ and latitude confirms a similar pattern reported for the eastern Atlantic (Weidman & Millner 2000) linking $\delta^{18}O$ to bottom temperature and to relationships we evaluated in the lab (Stanley et al. 2015b, Chapter 2). Warmer temperatures characterize the southern portion of the survey area and the inner reaches of embayments such as Placentia Bay (Bradbury et al. 2000), potentially contributing to observed otolith variation within and among bays.

The spatial scale of analysis can clearly impact assignment success in geographic comparisons. We observed significant variation in otolith composition at multiple spatial scales, with improved classification success at increasing spatial scale. We also observed several examples of significant small-scale variation, where individual sampling sites classified with a high level of success. These sites included western Trinity Bay, where the cod spawning -overwintering aggregation represents one of the largest remaining in eastern Newfoundland (Rose et al. 2011, Stanley et al. 2013). Despite these few specific sites with high classification success, overall classification to sampling site was relatively poor (~42% overall) with, for example, only 8% success for the Spanish Room site, our most exposed sample site. Poor assignment to the Spanish Room site was a function of

highly variable otolith composition that prevented reliable assignment in the discrimination analysis, which likely reflected reduced stability in environmental conditions. Similarly, Fodrie and Levin (2008) noted lower assignment success rates for halibut at exposed sample sites relative to halibut collected within embayments and Jamieson et al. (2004) noted higher variability in Atlantic cod otolith $\delta^{13}C$ in offshore relative to inshore habitats. Poor assignment in such sites may suggest errors in characterization, reducing assignment success collectively for all sites. Nonetheless, the majority of misclassifications (including Spanish Room) assigned successfully to embayment of capture, resulting in classification success at the bay and coast scales. Importantly, analysis restricted to the two dominant spawning areas in eastern Newfoundland - the head of Placentia Bay and northwestern Trinity Bay - produced classification successes of 77% and 85%, respectively.

Accurate assignment of individuals, populations, or locations normally requires sampling all possible geographic sources in a region because failure to do so may result in significant errors during mixture analysis or assignment (Campana 1999, Gillanders 2005b). We addressed this concern by evaluating the relationship between number of samples examined and assignment accuracy for the embayment of capture. The significant declines in correct assignment we observed with increasing number of samples are consistent with increased misassignments when spatial variation in otolith composition is accurately described. When comparing assignment success at different spatial scales we used different training datasets (same number but with different allocations). We considered this limitation a tradeoff for the need to incorporate the

variability nested within the system, as highlighted by declining assignment success with an increased number of sample sites. Indeed, the degree to which baseline variability has been quantified remains a challenge for assignment studies but the consequences of an incomplete baseline are potentially significant.

We are confident that our experimental design effectively sampled most juvenile habitat within the study region. Elevated juvenile density and survival in Atlantic cod occur in structured habitats, primarily eelgrass (Laurel et al. 2003). Our survey targeted eelgrass habitats using published records of cod sampling in nearshore Newfoundland (Methven et al. 1998), which allowed us to sample the dominant potential nursery areas on the north and south coast respectively. Moreover, the overall spacing of sample sites at ~70 km between bays and <30 km within bays should resolve much of the inherent spatial variation, given relatively limited expected movement in age-0 cod (Laurel et al. 2004, Ryan et al. 2012). Interestingly, the geochemical similarities we observed between distant sites within our study area (e.g. outer Placentia and eastern Trinity Bay) reduced our classification success. This observation suggests that geochemical similarities among distant sites could easily lead to inference of movement among unconnected sites in the absence of extensive sampling to characterize accurately the probability of assignment. Environmental conditions experienced by fish significantly influence the rate of incorporation of otolith constituents in addition other mediating factors that characterize the ecosystem (e.g. genetics, diet, ambient water chemistry). Work with juvenile black bream (Elsdon & Gillanders 2002), chinook salmon (Miller 2011) and juvenile Atlantic cod (Hoie et al. 2004, Stanley et al. 2015b, Chapter 2), all document significant and

complex interactive influences of temperature and salinity on incorporation rates of elements and isotopes into otoliths. The observation of consistent decreased assignment success as sites were 'added' to each bay during discriminant analysis likely relates to environmental heterogeneity within the scale of assessment that reduced discrimination. Spatial-temporal variability in environmental conditions characterizes many coastal environments (Craig & Colbourne 2004), especially at the small spatial scales and short time periods (~ 2 months) such as those investigated in our analysis.

To further illustrate the impact of environmental heterogeneity we partitioned the variance in otolith chemistry attributable to environmental conditions using a predictive laboratory model and observations of environmental conditions in the field. Residual discriminant analyses significantly increased assignment success for all spatial scales we tested. Increased assignment success suggests that even with removal of environmental influence (temperature and salinity), a residual 'signal' remains that presumably reflects the remaining abiotic and biotic variables that characterize the system. This residual signal appears robust with discrimination among some areas greatly improving. High assignment success to sites such as inner Placentia Bay, for example, could be attributed to terrestrial influences (e.g. Palmer & Edmond 1992), as these areas are characterized by higher riverine runoff than other sample sites and thus create the potential for a land-based signal. Collectively, these results describe how environmental variability at small scales (10-100 km) could potentially diminish or accentuate spatial pattern signal characteristic of the system.

The inability of conservation and management plans to evaluate significant small-scale spatial complexity of coastal fish species presents a clear threat to the persistence and stability of coastal stocks (Hilborn et al. 2003, Botsford et al. 2009). Our survey area was located within multiple management areas for Atlantic cod that are often subject to different management decisions. Placentia Bay is thought to support both resident and migratory stocks, which are each subject to local commercial harvest (Robichaud & Rose 2004, Mello & Rose 2005). The maintenance of the inshore spawning aggregations will be critical to the stability and potential rebuilding of cod stocks (Hutchinson 2008). The application of otolith chemistry to connect adult fish to natal habitats represents an important approach for evaluating the spatial and source-sink dynamics of this species. Clearly, the application of approaches such as otolith geochemical analysis which can at least partially resolve some fine-scale spatial dynamics, as we have shown here, may be critical to successful conservation and management of rebuilding stocks with complex population structure.

3.5.1 Summary

We observed spatial variation in otolith composition of juvenile cod from coastal Newfoundland and describe the potential of this variation to partially resolve the spatial structure of juvenile habitat at scales smaller than previously available. Moreover we demonstrate how variation in environmental conditions at scales less than 100 km could confound assignment through mediation of otolith composition and the alteration of natural spatial variance, stressing the importance of resolving baseline variability in otolith chemistry when conducting assignment tests. Natural geochemical tags recorded

in otolith geochemistry provide an important tool to facilitate the management and protection of genetic diversity in this commercial species, which until recently (see trends reported in DFO 2013b) was considered at risk of commercial extinction in Canadian waters (Bradbury 2010).

3.6 Tables

Table 3.1 Sample sites and sample sizes for otolith geochemical analysis of Atlantic cod from coastal Newfoundland. See Figure 1, for geographic positions. Abbreviations used in subsequent plots are shown in parentheses.

ID	Location name	Bay	Site Code	N element	N isotope
1	Great Brule	Placentia Bay (PB)	GB	20	19
2	St. Bernard's Cove		BC	14	14
3	Woody Island		WE	17	16
4	Spanish Room		SR	19	12
5	Harbour Buffett		HB	22	13
6	Colinet Island	St Mary's Bay (SMB)	CI	33	32
7	Harbour Grace	Conception Bay (CB)	HG	20	19
8	Holyrood		HR	18	18
9	Bristol's Hope		BH	5	5
10	Little Mosquito Cove	Trinity Bay (TB)	LMC	20	18
11	Smith Sound		SS	19	19
12	Dildo		DL	20	20
13	Tappers Cove		TC	18	17
14	Cannings Cove	Bonavista Bay (BB)	CC	19	17
15	Bread Cove		BC	20	20
16	Piper's Cove		PC	20	20
17	Indian Bay		IB	20	20
			Total	**324**	**299**

Table 3.2 Proportion of juveniles correctly assigned to sampling site, bay, and coast of origin using otolith elemental composition and a quadratic discriminant function analysis. Analyses presented are product of observed field chemistry and residual chemistry (observed – predicted) from laboratory environmental corrections. Note proportions flagged with * denote assignment tests that were not significantly greater than random at $\alpha = 0.01$. Sample size (n) included.

ID	Location name	n	Bay	Raw	Residual
1	Great Brule	**20**	Placentia Bay	0.42	0.79
2	St. Bernard's Cove	**14**		0.28	1.00
3	Woody Island	**17**		0.27	1.00
4	Spanish Room	**19**		0.08*	0.63
5	Harbour Buffett	**22**		0.17	1.00
6	Colinet Island	**33**	St Mary's Bay	0.48	1.00
7	Harbour Grace	**20**	Conception Bay	0.32	1.00
8	Holyrood	**18**		0.28	1.00
10	Little Mosquito Cove	**20**	Trinity Bay	0.28	0.83
11	Smith Sound	**19**		0.26	0.63
12	Dildo	**20**		0.35	0.95
13	Tappers Cove	**18**		0.38	0.50
14	Cannings Cove	**19**	Bonavista Bay	0.24	0.47
15	Bread Cove	**20**		0.25	0.35
16	Piper's Cove	**20**		0.32	1.00
17	Indian Bay	**20**		0.45	0.95
			Average	**0.30**	**0.82**

Table 3.3 Proportion of juveniles correctly assigned to pooled sites using otolith geochemical composition (O), elements (E) or isotopes (I) and a quadratic discriminant function analysis with cross validation to the bay or coast of capture. Analyses conducted using observed (Obs) and residuals from predicted laboratory composition (Lab). Note proportions flagged with * denote assignment tests that were not significantly greater than random at $\alpha = 0.01$. Sample size (n) included.

Location	**n**	**E_{Obs}**	**E_{Lab}**	**I_{Obs}**	**I_{Lab}**	**O_{Obs}**	**O_{Lab}**
Placentia	92	0.77	0.76	0.77	0.75	0.75	0.75
Conception	38	0.24*	0.65	0.27*	0.41	0.32*	0.62
Trinity Bay	77	0.32*	0.38	0.26*	0.44	0.27*	0.48
Bonavista	79	0.59	0.66	0.51	0.11*	0.6	0.62
Average	**--**	**0.48**	**0.61**	**0.45**	**0.43**	**0.49**	**0.62**
South	125	0.61	0.66	0.58	0.62	0.66	0.67
North	194	0.88	0.9	0.91	0.87	0.86	0.89
Average	**--**	**0.75**	**0.78**	**0.75**	**0.75**	**0.76**	**0.78**

Table 3.4 Results of analyses of variance (ANOVA) examining differences in assignment success as a function of spatial scale, addition of length, and lab standardization. Post-hoc Tukey's tests presented are significant at $\alpha = 0.05$ level.

Index	df	MS	F	p	Post-hoc
Scale	2	2333	12.040	0.0004	Site < Bay = Coast
Length	1	1085	1.421	0.2360	
Standardization	1	9208	14.77	0.0002	Standardization > Raw

3.7 Figures

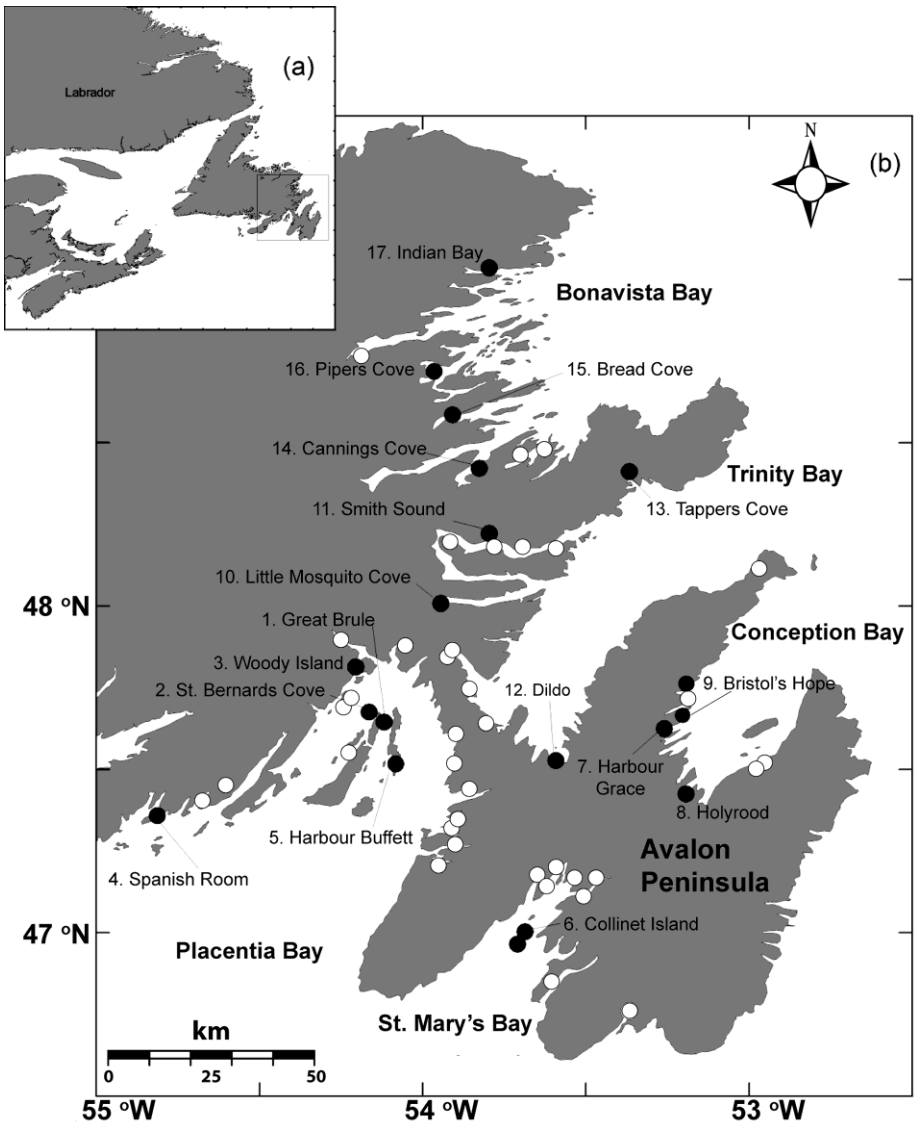

Figure 3.1 Map of surveyed locations for juvenile Atlantic cod in coastal Newfoundland 2007. Inset shows location of eastern Newfoundland with respect to eastern Canada. Black dots represent sites where sufficient numbers (N=17) and white dots represent sites with insufficient numbers (N=38) of juvenile Atlantic cod were available for discrimination analysis. See Table 1 for sample size information.

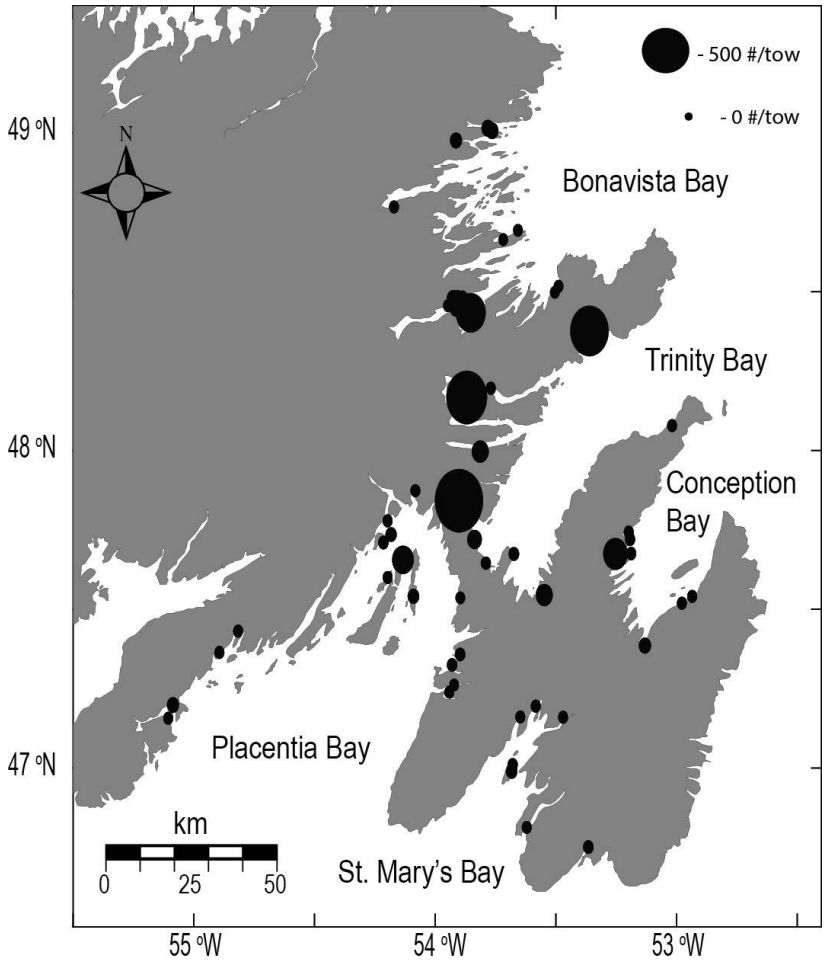

Figure 3.2 Map of juvenile cod abundance from a beach seine survey of eastern Newfoundland in 2007.

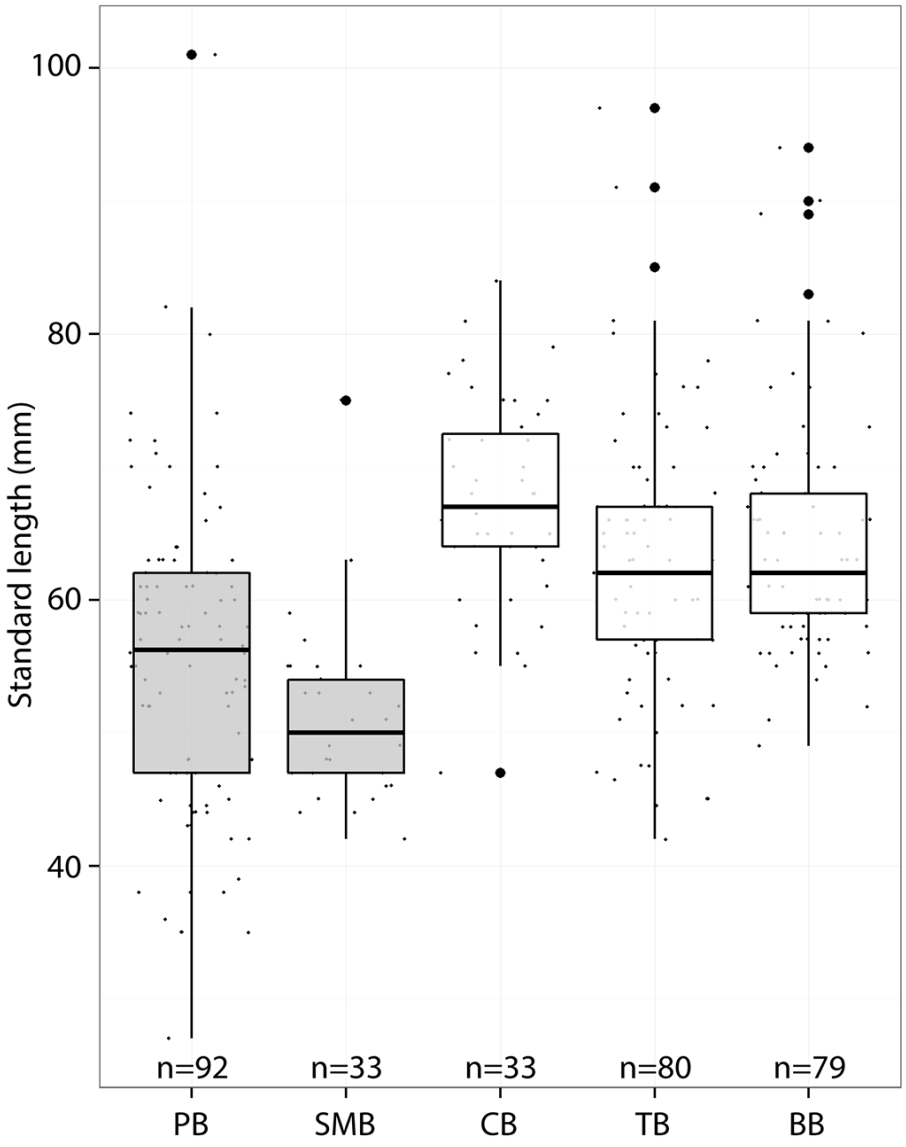

Figure 3.3 Boxplots of juvenile length from each of the five bays surveyed. Box fill colours denote results of ANOVA Tukey's post-hoc tests for differences in length among bays at the α=0.05 level. Raw data presented as points underlying the boxplots. Sample sizes n represent the number of fish captured at that location.

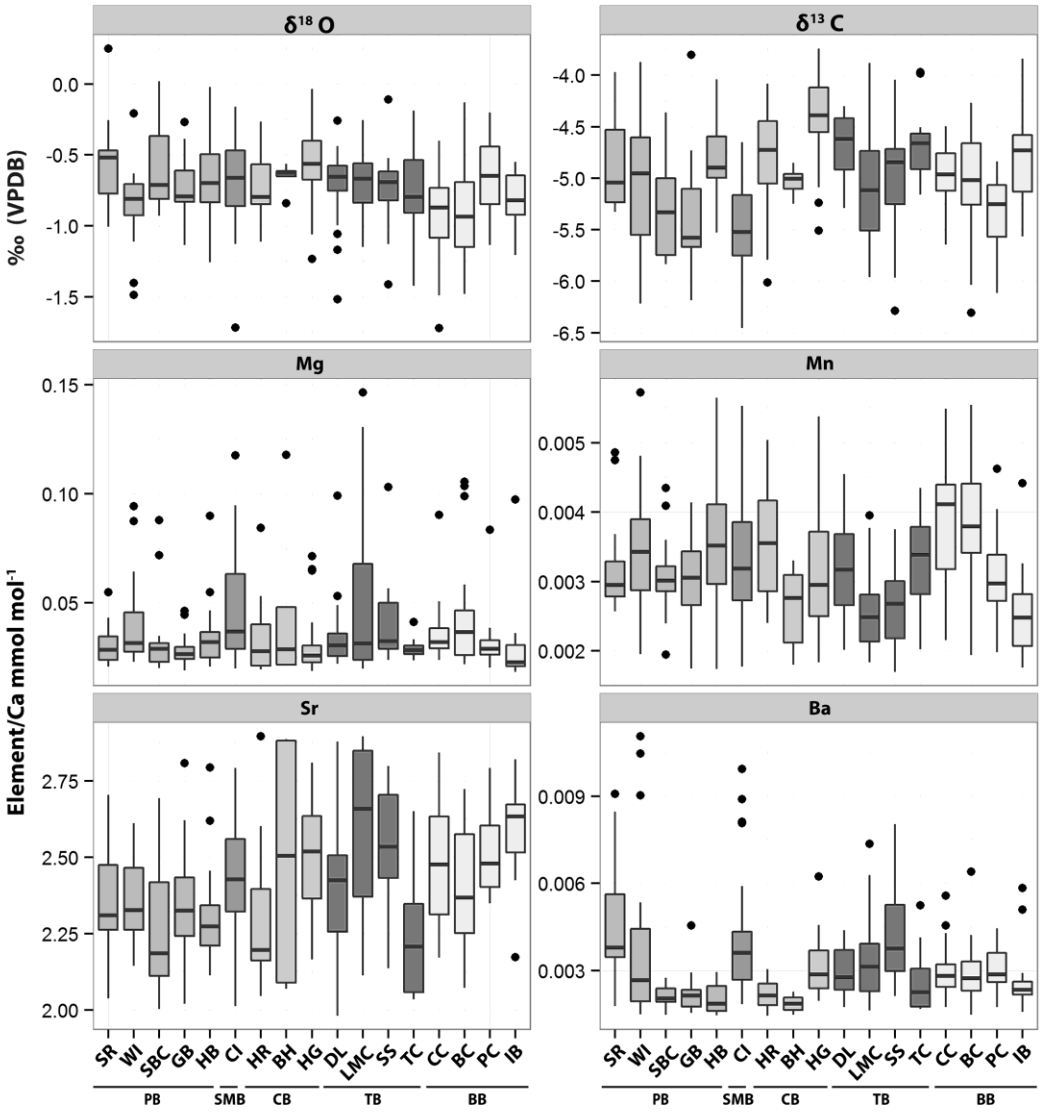

Figure 3.4 Boxplots of measured otolith elements and isotopes per sample site. Center-points of each box represent the median value, edges of the box the 25th and 75th percentiles, the whiskers indicate the 95th and 5th percentiles. Closed dots represent outliers outside the 90th percentile range. Refer to Table 3.1 for key to abbreviations.

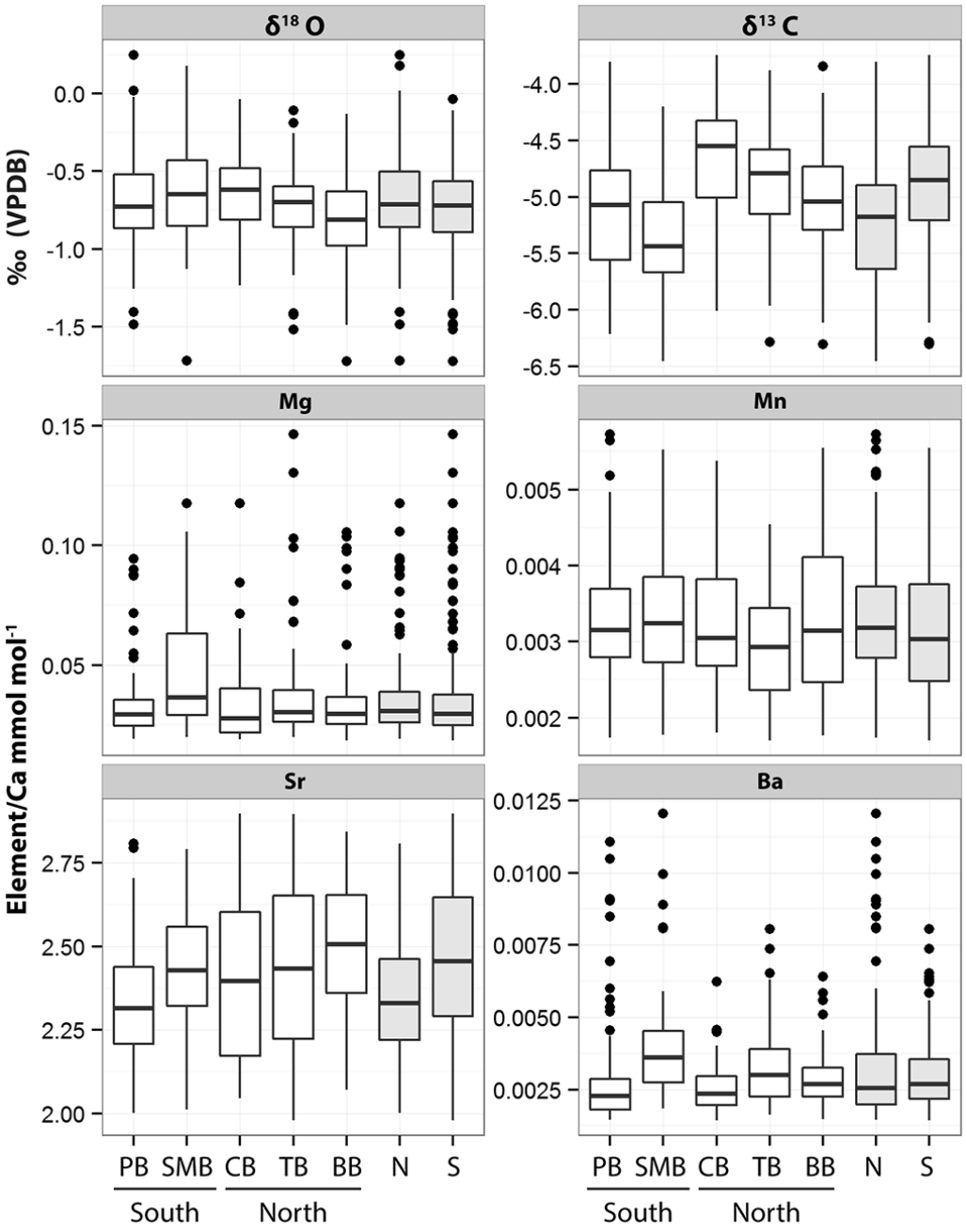

Figure 3.5 Boxplots of measured otolith elements and isotopes per bay and coast of capture. North (N) and South (S) coastal data are shaded grey. Center-points of each box represent the median value, edges of the box the 25th and 75th percentiles, the whiskers indicate the 95th and 5th percentiles. Closed dots represent outliers outside the 90th percentile range. Refer to Table 3.1 for key to abbreviations.

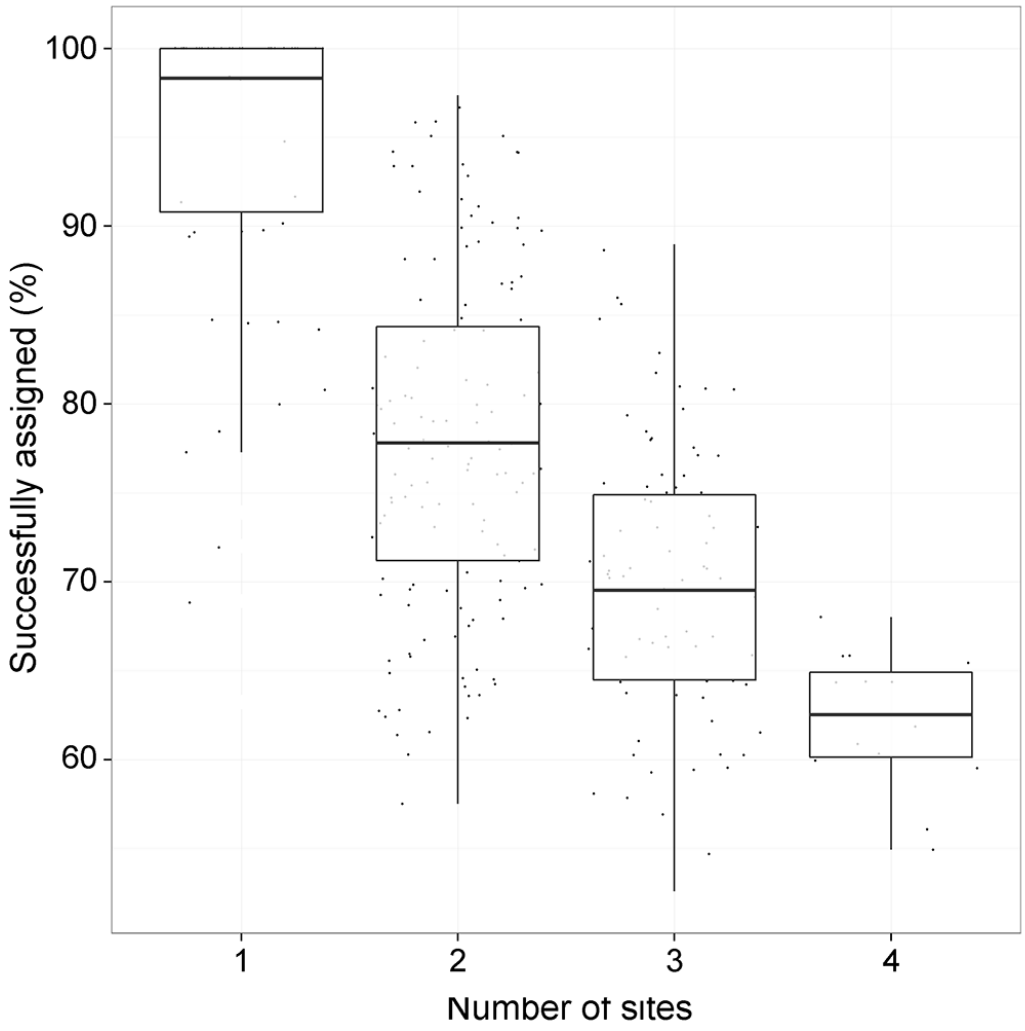

Figure 3.6 Discriminant function assignment success aggregated as a function of the number of sites within each bay. Analysis was restricted to bays, Bonavista, Trinity and Placentia, with 4 or more sites. Raw data presented as points underlying the boxplots.

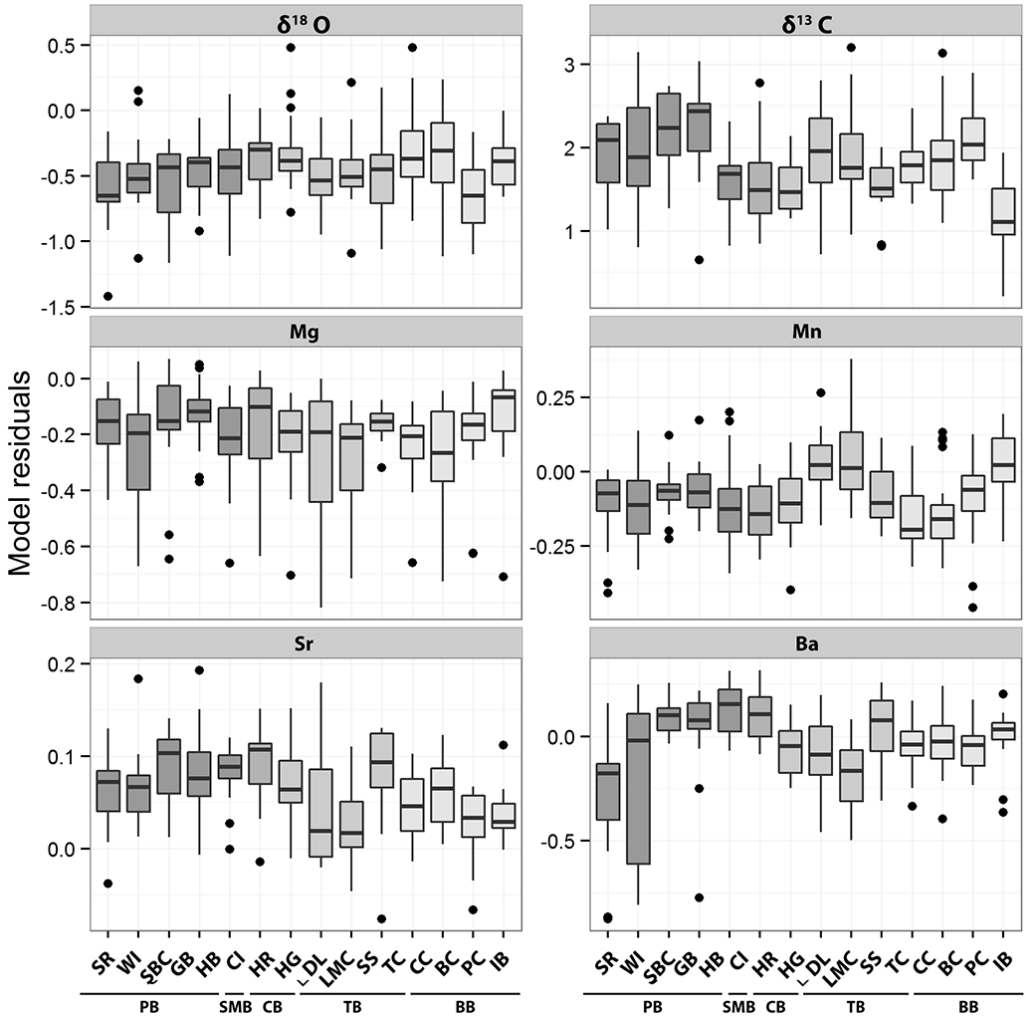

Figure 3.7 Boxplots of showing the residual otolith composition, elements and isotopes, calculated as the observed otolith chemistry minus predictions based on observed environmental conditions and a laboratory predictive model. Center-points of each box represent the median value, edges of the box the 25th and 75th percentiles, the whiskers indicate the 95th and 5th percentiles. Closed dots represent outliers outside the 90th percentile range. Refer to Table 3.1 for key to abbreviations.

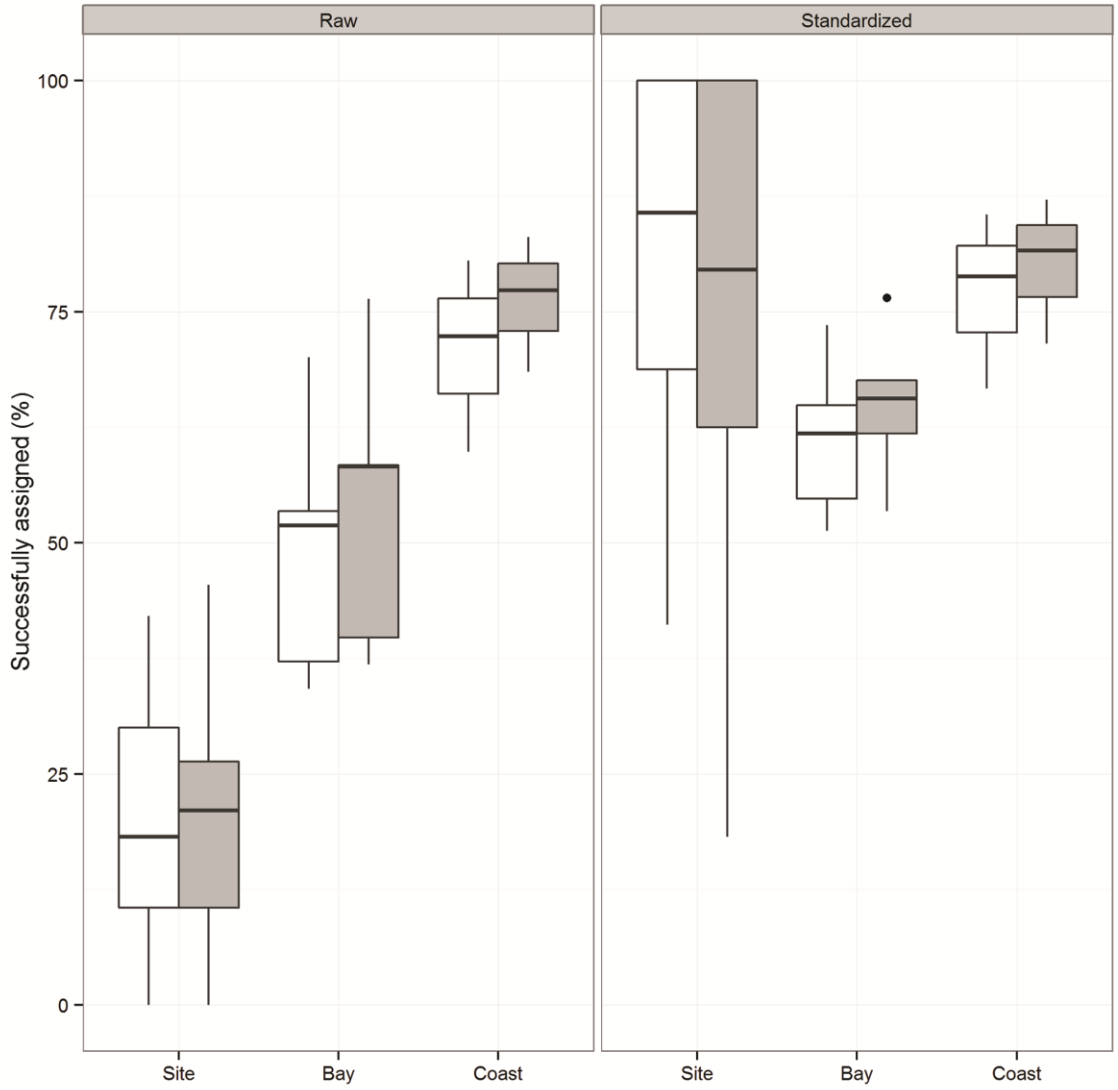

Figure 3.8 Discriminant function assignment success as a function of scale for raw data and laboratory corrected residuals. White boxes are assignments using geochemistry data while grey boxes represent data using a combination of fish length and geochemistry.

Chapter 4 - Biogeographic, ontogenetic, and environmental variability in larval behaviour of American lobster (*Homarus americanus*)

4.1 Abstract

Through laboratory observations we evaluated the influence of natal origin, ontogeny, and environment (light, temperature) on the vertical and horizontal swimming behaviour of larval American lobster (*Homarus americanus*). We quantified several behavioural indices including vertical water position, average swim speed, and linearity, and measured how these factors interacted to determine horizontal diffusivity. Larval swimming behaviour varied significantly for all behavioural indices as a function of natal origin, ontogeny, temperature, and light. Across treatments, post-larvae exhibited fastest swim speeds (5.96 $cm \cdot s^{-1}$), horizontal diffusivities (9.97 $cm^2 \cdot s^{-1}$), and highest position in the water column compared to earlier stages. We observed significantly lower (~60%) swimming speed and vertical position in intermediate developmental stages compared to early and late stages. Within stage I larvae, swim speed averaged 2.1 $cm \cdot s^{-1}$ with a horizontal diffusivity of 3.31 $cm^2 \cdot s^{-1}$, and although swim speed did not vary with maternal origin we observed a significant interaction between origin and temperature. Larvae from different geographic regions, reared in common garden conditions, differed in behavioural response to temperature; warm origin larvae swam significantly faster (~25%) in warm water, as did cold origin larvae in cold water (~ 43% faster), relative to each other. Our results detail potential novel sources of variability in larval behaviour and provide one of few direct measures of swimming behaviour of larval American lobster. Collectively, our results provide a biological-behavioural context to parameterize bio-

physical models with broad applicability to meroplanktonic species, and a powerful tool to improve accuracy of dispersal models and advance understanding of larval transport.

4.2 Introduction

The geographic scale of pelagic dispersal in marine organisms reflects passive and active processes (Sponaugle et al. 2002), where larval behaviour and physiology interact with the physical flow environment to determine displacement. Most studies fall short, however, in evaluating 'active' or behavioural components of dispersal (Metaxas & Saunders 2009) relative to efforts to model physical flow fields, despite evidence that horizontal swimming (Gerlach et al. 2007) and vertical placement (Sulkin 1984, Bellwood & Fisher 2001, North et al. 2008, Butler et al. 2011) strongly influence dispersal outcomes (Fiksen et al. 2007, Phelps et al. 2015). Better understanding of physical and biological components of dispersal will improve resolution and accuracy of coupled bio-physical models, and in turn better resolve scales of connectivity (Hastings & Botsford 2006), enabling more targeted conservation and marine management applications (Incze et al. 2010).

For meroplanktonic marine macro-invertebrates and fish (reviewed by Cowen & Sponaugle 2009), the larval period provides a vector for colonizing new habitats, connecting distant populations and sustaining metapopulations through source-sink dynamics (Cowen et al. 2007). Correspondingly, the planktonic stage plays a critical role in defining the geographic scale and abundance of meroplanktonic populations. Distributions of marine meroplankton often vary with depth (reviewed by Cohen & Forward 2009, Lloyd et al. 2012b) and, whereas flow differences can partially explain

distributions, a growing body of work suggests that behavioural response to various environmental stimuli influences broad-scale dispersal, often through vertical position (Sulkin 1984, Botsford et al. 1994, Shanks et al. 2002). Such behaviour is especially important in negatively buoyant species that would otherwise sink. Indeed, swimming abilities of many meroplanktonic species meet or exceed typical vertical flow speeds in nature (Metaxas & Saunders 2009). Behaviour and response to stimuli by meroplankton can thus influence dispersal and, eventually settlement, profoundly (Botsford et al. 1994), especially in the presence of heterogeneous vertical and horizontal flow structure (Queiroga & Blanton 2005, Fiksen et al. 2007). Some combination of exogenous conditions (e.g. temperature and light) and endogenous changes in larval behaviour (e.g. kinetic response to stimuli; Sulkin 1984, Boudreau et al. 1991) may influence position in the water column.

Although numerous studies illustrate the influence of environment and ontogeny on meroplankton behaviour, few evaluate variability in larval behaviour among populations and in response to environmental conditions. Larval fish studies link variability in swimming to latitudinal gradients among species (Leis et al. 2013), but intra-specific variation in response to different environments remains unresolved (but see Guan et al. 2008). Observations of larval shore crabs (*Pachygrapsus crassipes*) highlight intra-specific variation in swimming behaviour among different coastal (Miller & Morgan 2013) and tidal environments (Moksnes et al. 2014), highlighting potential influence of intra-specific variation in behaviour on larval transport trajectories. For species with pelagic durations that span weeks to months, population differences in behaviour and

dispersal can lead to genetic differences (Bertness & Gaines 1993, Stobutzki 1998) and potentially to local adaptation (Yamahira & Conover 2002, Moksnes et al. 2014). For example, variation in larval green crab (*Carcinus maenas*) dispersal has been linked to heritability of migration rhythm within the context of variable tidal flow (Zeng & Naylor 1996a). Given the potential influence of larval behaviour on dispersal, evaluating regional variation and potential local adaptation across populations illustrates the limitations of applying singular, simplified behavioural metrics across an entire species range.

Current understanding of the importance of environmental stimuli and sources of behavioural variability in meroplankton remains limited (Metaxas 2001), and many studies treat larvae as passive drifters (Siegel et al. 2008, Metaxas & Saunders 2009). To date, most larval behavioural work has focused on settlement, despite the relatively short timescale of settlement behaviour relative to the pelagic period. The inability to track individual larvae, or cohorts of individuals, directly for extended periods of time *in situ* limits evaluation of behavioural responses to stimuli, particularly for species with relatively low abundances and poorly-known vertical distributions. Some studies have inferred relative meroplankton behaviour *in situ* by documenting changes in distribution relative to physical features in the water column (Lloyd et al. 2012b). Laboratory studies offer an effective tool to evaluate and directly measure behaviour in response to defined biological or physical variables, providing an empirical estimate of behavioural scope. Laboratory studies can generate behavioural observations that inspire hypotheses that can be evaluated empirically in the field or simulated numerically using biophysical models (Metaxas 2001).

Larval behaviour defines the capacity to respond to a cue, or to change and/or maintain position in the water column, capture prey, or evade predation. Laval behaviour can counteract passive sinking, either through active swimming in response to salinity (Scarratt & Raine 1967), circulation, or light direction (Shirley & Shirley 1988), or by changing activity levels in response to specific light conditions (e.g. Utne-Palm & Stiansen 2002, Jekely et al. 2008), temperatures (Civelek et al. 2013), and water column features (Daigle & Metaxas 2011), all of which influence vertical position in the water column and thus dispersal potential.

Multiple studies demonstrate larval behavioural responses to temperature (Fuiman & Batty 1997, Stanley et al. 2012, Leis et al. 2013) and light cues (Thorson 1964, Jekely et al. 2008, Cohen & Forward 2009), as well as variation in behaviour through larval development (e.g. Sulkin et al. 1980, Utne-Palm & Stiansen 2002). Moreover, ontogenetic changes in swimming mode, size, morphology, and sensory sensitivity influence behaviour through altered interactions with the fluid environment and response to stimuli. Ontogenetic vertical migrations have been described for multiple meroplanktonic species, where developmental stage and/or swimming capacity mediate vertical distributions (e.g. Ennis 1975b, Gallager et al. 1996, Harding et al. 2005). For example, changes in behavioural scope corresponded with changes in swimming mode and size among different larval stages of American lobster, where substantial increases in locomotive capability occur following key morphological developments (Ennis 1986, 1995). Similarly, response to stimuli can vary at relatively short temporal scales, where

changes in phototactic response and overall activity level have been observed within stages of larval American lobster (Ennis 1975a).

We chose American lobster, (*Homarus americanus*), as a model species to study biogeographic variation in dispersal potential because of its broad range, with populations spanning the east coast of North America from North Carolina, USA to Labrador, Canada (Cooper & Uzmann 1980). Through this range, dispersing larvae may experience a wide range of environmental conditions, both in physical forcing (circulation) and temperature (sea surface temperature ~ -2 to 28 °C; Aiken & Waddy 1986). Because planktonic stages are extremely capable swimmers, with swim speeds estimated at ~20 $cm \cdot s^{-1}$ during the post-larval stage (Cobb et al. 1989), they span other invertebrates and fishes in terms of potential active contributions to dispersal. This behavioural capacity, coupled with an expected interaction between temperature and behaviour (i.e. Fuiman & Batty 1997), suggests that the range of thermal habitats across the species range may impart a selective gradient, potentially leading to biogeographic differences in swimming behaviour and response to environment.

We investigated patterns and variability in larval American lobster swimming behaviour in response to environment and natal origin. To examine the influence of natal origin, we compared stage I larvae sourced from distant natal origins, separated by ~ 900 km of coastline. Consistent differences (~ 5-6 °C) in sea surface temperature between these sources offer an opportunity to evaluate biogeographic variation and potential interactions with environment. Given their relatively low numbers in nature and the difficulty of direct observation *in-situ*, we designed a series of laboratory experiments to

observe and quantify variation in vertical and horizontal larval swimming behaviour under controlled conditions. Specifically, we compared vertical position, swim speed, and swimming path among experimental temperatures. We then extended those observations with experiments that added variation in light to different ages and stages of lobster larvae in order to document ontogenetic change in behaviour expected through development. The methodologies we present offer a mechanism to observe and quantify behaviour for a wide range of marine meroplankton, which then can be used to evaluate natal and environmental influence in natural populations and greatly improve representation of realistic behavioural contributions in biophysical models.

4.3 Methods

4.3.1 Larval culture

During June 2012, local fishermen collected 26 ovigerous female lobsters (71-136 mm carapace length) from Port au Choix (PaC, n=17) and Red Harbour (RH, n=9), Newfoundland and Labrador, Canada (Figure 4.1) using standard commercial traps under Department of Fisheries and Oceans Canada (DFO) experimental licence NL-1339-12. Females were transported to the Ocean Sciences Centre of Memorial University of Newfoundland and maintained in individual flow through containers under ambient surface seawater intake conditions (7-15 °C). Females were fed a ration of squid twice weekly for the duration of spawning.

American lobster typically begin to release larvae shortly after dark (Ennis 1975b). To optimize collection of larvae immediately after eclosion, we employed a reverse 12-hour light cycle by turning on full spectrum UV bulbs from 7 pm to 7 am in

covered tanks. Tanks were checked twice daily and, upon hatching, we removed larvae using a fine-meshed net. Larvae from each mother were then transferred to separate 4 l holding containers maintained in a shared water bath of filtered ambient sea water (12-16 °C). On the second day post-hatch, larvae were transferred to the Joe Brown Aquaculture Research Building (JBARB) and held in shared 50 l plankton kreisel tanks filled with filtered flow through seawater maintained at 15 °C (±0.5 °C). Logistical constraints only permitted the use of one rearing temperature and so we selected 15 to be the approximate mid-point between surface temperatures which characterize the pelagic environments of each natal origin. Larval cultures were held under a 12-hour light cycle using artificial light at concentrations less than 50 larvae l^{-1}. The tanks were aerated strongly to reduce cannibalism, and we fed larvae daily *ad libitum* live enriched *Artemia salina* cultured at the JBARB.

4.3.2 Natal origin

Two evaluate whether behaviour varies spatially and in response to environment, we chose two natal origins which were geographically separated and characterised by differences pelagic thermal environmental conditions (Figure 4.1). Port aux Choix is among the highest latitude fishing ports in the lobster's range and correspondingly has a relatively cooler pelagic thermal environment. Red Harbour is located within Placentia Bay which is characterized by some of the warmest summer surface water temperatures in the province (Craig & Colbourne 2004). We obtained temperature data from Department of Fisheries and Oceans Canada Sea Surface Temperature Database using the Pathfinder 5 satellite telemetry data at a 4 km resolution, spatially aggregated to lobster

management areas (LFAs). Estimated sea-surface temperatures for Port au Choix (LFA 14a) were significantly colder than Red Harbour (LFA 10) (ANOVA: df=1, MS=139622, F=22975, $p<0.0001$). Overall temperatures ranged from 8.6-14.8 and 12.5–18.4 °C for Port au Choix and Red Harbour, respectively.

In order to examine population-level differences in behaviour, we held larvae from different natal sources in separate containers prior to experiments. The reduced swimming behaviour exhibited by later stages (see below) and the challenge of rearing many cultures simultaneously combined with larval mortality led us to pool later stage larvae (II-III) of a common age from different natal sources and maternal origins once we had completed experiments with the first zoeal stage. These pooled individuals were then fed cultures with frozen adult *A. salina* (~ 10 g per culture).

Our first series of experiments focused on newly hatched stage I larvae only. Stage I larvae display a well-documented phototactic response for 24 hours post-eclosion (Hadley 1905, Ennis 1975a), which we exploited to demonstrate the influence of natal origin and temperature on swimming ability. In order to observe vertical distributions we used three 24-litre clear polyurethane vertical chambers (Figure 4.2a) filled with filtered sea water maintained at temperature treatments of 10, 15 or 20 °C (± 1.25 °C). Newly hatched larvae were collected from each mother (n=26) and immediately acclimated to the experimental conditions for 2 hours before beginning the experiment. Preliminary observations noted no appreciable change in behaviour for acclimation periods longer than 2 hours. Once acclimated, groups of 10 randomly selected larvae from a given maternal origin were placed in the chamber. We demarked a 50-cm observational window

(30 – 80 cm; Figure 4.2a) in the middle of the chamber to measure vertical swimming velocity ($cm \cdot s^{-1}$). After introduction, we attracted larvae below the measurement window using a bottom light source, and once all larvae had moved to the bottom 20 cm of the chamber we switched on top lighting. Larvae were individually timed using a tablet-based multi-timer to determine how long it took to traverse the 50-cm segment. We repeated this procedure 5 times for a total of 50 unique measurements from each mother. For the analysis, we included only those observations in which larvae demonstrated a clear phototactic response, defined as swimming actively towards the light over a 3-minute period.

Using digital calipers (±0.1 mm), we measured maternal carapace length as the posterior edge of the eye socket to the posterior margin of the carapace parallel to the longitudinal axis. For each maternal source, 100 larvae were chosen at random and imaged under a dissection microscope. Larval carapace length was measured following the same morphological boundaries in ImageJ calibrated to the nearest mm (±0.01 mm).

4.3.3 Horizontal swimming behaviour

A second series of experiments evaluated variation in swimming behaviour within stage I (intra-ontogenetic) and among stages I-IV (inter-ontogenetic) as a function of natal origin, light intensity, and temperature along the horizontal plane of displacement. In these experiments, we recorded swimming within a 75-l horizontal arena (Figure 4.2b) lined with a matte white vinyl film to maximize contrast for observation. Videos were taken using a digital camera (Axis 221 Day and Night Network Cameras, model no. 0221-01-04, Axis Communications, Lund, Sweden) affixed 200 cm above the

experimental arena with a resolution of ~ 10 pixels mm^{-1} and a frame rate of 30 frames per second (fps). Four broad spectrum daylight incandescent lamps (Exo-Terra® 25 w) were positioned in at the corners of the arena, 75 cm above water level to control lighting (Figure 4.2b). Larval swimming was observed under light intensities of ~1200, 600, and 30 (± 20) lx measured using a VWR Traceable® Light Meter (hereafter full, intermediate, and low light, respectively). To vary light intensities we used a paper light array which altered the relative intensity of surface light (lux).

We utilized three temperature treatments of 10, 15 and 20 °C (± 1.25 °C) for each lighting condition, controlling temperature using a heater/chiller unit connected to a titanium heat exchange below the observational arena. The phototactic directional behaviour of newly hatched stage I larvae was used to compare the influence of natal origin (biogeographic variance) and temperature on larval swim speed. We conducted experiments with stage I larvae at 24, 48 and 72 hours post-hatch to document diminishing phototactic response with age and ontogeny (sensu Ennis 1975a). Past observations of stages II and III showed significant behavioural changes for ~24 hours post-molt, where larvae briefly display strong phototaxis (Hadley 1905, Ennis 1975a), which diminishes for the duration of the development stage. To ensure that our observations were representative of behaviour for the majority of the zoeal stage, we utilized stage II and III larvae 48-72 hours post-molt. Finally, we utilized stage IV post-larvae within 48 hours of molting from stage III.

We transferred larvae from holding tanks to a water bath of the same temperature as the treatment in darkness for 1 hour prior to observation, randomizing the order of

temperature and light treatment sequences for each stage. We used natal origin as an experimental factor only for newly hatched stage I lobster, given that subsequent stages were pooled among origins during rearing. For each observation, 10 larvae were placed in the observational arena and filmed for 5 minutes. In total we recorded 220 stage I larvae 24 hours post-hatch (n= 120 and 100 for Port au Choix and Red Harbour, respectively), 100 stage I larvae 48 and 72 hours post-hatch, and 50 stage II-IV larvae.

Videos were uploaded to ImageJ® for analysis. We cleaned image sequences using background subtraction and binary thresholding, which resulted in images of dark larvae swimming against a white background. Additional image smoothing and de-speckling algorithms in ImageJ removed video grain associated with low light conditions. Cartesian coordinates of larvae for each frame (recorded at 30 fps) were then extracted using the Computer Assisted Sperm Analysis ImageJ plugin (CASA; Wilson-Leedy & Ingermann 2007). We then aggregated and analysed larval track coordinates in *R* 3.1.1 (R Development Core Team 2015).

4.3.4 Vertical distribution

The third series of experiments examined the four pelagic larval stages, hereafter stages I-IV, to evaluate the influence of lighting condition and ontogeny on vertical distributions. We evaluated vertical distributions using the same observational chambers and collection protocols outlined above. Experiments were undertaken during a fixed daytime period (~10:00-14:00 hrs) to eliminate any potential artifacts associated with endogenous rhythm. We then selected actively swimming, healthy, individuals at random from the cultures each morning between 08:00 and 10:00 h and acclimated them for 1

hour to experimental conditions before beginning a trial using the same light intensities and temperatures described for experiments on horizontal swim speeds. Larval distributions were observed under full, intermediate, and low light treatments according to methods described above. Light irradiance peaked between 300-600 nm, which spans the spectral sensitivity reported for American lobster (Goldsmith 1978) and follows prior experiments with lobster larvae (Rodriguez et al. 2000). For a given trial (n_{trial}=10), we placed a group of 20 larvae of a given age or development stage in each observational chamber ($n_{chamber}$=3). After transfer to the observational chamber, we exposed groups of larvae to the three lighting conditions, interspersed with periods of darkness, assigned in random order for 30 minutes. Larval positions were recorded in 10-cm vertical segments within the 120-cm chamber. Between each light treatment we allowed larvae to position themselves for a period of 30 minutes in darkness without light cues.

4.3.5 Statistics

We employed mixed effect models to evaluate the influence of experimental treatment on the measured behavioural indices from video data and vertical swimming trials, using the lme4 R package version 1.17 (Bates et al. 2014). We treated natal origin, ontogeny, and light as fixed categorical effects, temperature as a continuous fixed effect, and video sequence (5 per treatment), mother (n=27 for vertical swim speeds), or larval group (10 per treatment in vertical position trials) as random effects, to deal with the non-independence between multiple larvae in the same video sequence and preventing over-estimating confidence in our estimates of the fixed effects (Rose & Rowe 2015). Vertical distributions were analysed using the same mixed model approach accounting for non-

independence among larvae by assigning observational sequence as a random effect. We then calculated marginal means and confidence intervals for the fixed effects based on parametric bootstrapping the mixed effects models (Bates et al. 2015). All indices except linearity were $\log_{10}$ transformed to meet assumptions of normality and homogeneity of variance; linearity was logit transformed, as it ranges between 0 and 1. For coordinate-based indices, we analysed only paths whose length exceeded 1800 frames, or one minute, for stages I and IV. Stage II and III paths were considerably shorter and we therefore included paths exceeding 900 frames, or 30 seconds. Variable path lengths and rules defining inclusion of paths, produced an unbalanced design that necessitated the use of mixed effect models and type III sums of squares (using the lme4 package). Post-hoc comparisons were tested using '*glht*' tests in the multcomp package 1.2.12 in R (Hothorn et al. 2008, R Development Core Team 2015).

Several behavioural indices were extracted from video-derived coordinate data. Average swimming velocity swimming velocity (V) was calculated as:

Equation 1
$$V = \frac{\sqrt{(x_i - x_{i+n})^2 + (y_i - y_{i+n})^2} \cdot \text{fps}}{n}$$

where *x* and *y* are Cartesian coordinates, *i* is a counter of sequential coordinate pairs, n is the number of frames over which velocity is averaged, and fps is the frame-rate (30 fps for our analysis). Swimming velocity was calibrated to $cm \cdot s^{-1}$ utilizing a pixel per cm coefficient (~ 0.01 $cm \cdot pixel^{-1}$).

Linearity (LIN) is an index of the tortuosity of a larval swimming path in a fixed window of time (Codling et al. 2008, Civelek et al. 2013), calculated as the cumulative

distance travelled from each frame to its location after *n* steps (the displacement distance) divided by the sum of the per-frame distances traveled in *n* steps (the path distance), averaged over all *m* of the frames in the observed path:

Equation 2 $$\mathrm{LIN} = \left(\sum_{i=1}^{m} \frac{\sqrt{(x_i - x_{i+n})^2 + (y_i - y_{i+n})^2}}{\sum_{j=0}^{n-1} \sqrt{(x_{i+j} - x_{i+j+1})^2 + (y_{i+j} - y_{i+j+1})^2}}\right) m^{-1}$$

For this study we set n=450 frames, representing 15 seconds of footage.

Finally, we estimated each larva's long-term diffusion coefficient based on its horizontal movement, given its observed behaviour in the trial (hereafter referred to as its diffusivity). The diffusion coefficient is the long-term constant rate at which the squared displacement of an individual from its starting point increases, assuming individuals follow a random walk without a preferred direction and with finite variance of step lengths (Turchin 1998). It takes into account the combined effects of movement speed and the tortuosity of the movement path to determine how individuals will spread out horizontally in the water, assuming constant movement over time. We estimated diffusivity by assuming individuals followed a correlated random walk (Kareiva & Shigesada 1983), then calculated what the long-term diffusion coefficient would be if they followed that random walk. For each larval path, we first subsampled the paths down to one frame a second to reduce correlations between turn angles in each step, then calculated the mean sine (*s*), cosine (*c*) of the relative turn angles of the path, and the mean (*l)* and variance (σ^2) of step lengths. The diffusivity (in $cm^2 \cdot sec^{-1}$) was then calculated as (Kareiva & Shigesada 1983):

Equation 3: $$\text{Diffusivity} = \sigma^2 + l^2 + 2l^2\left(\frac{c - c^2 - s^2}{1 - 2*c + c^2 + s^2}\right)$$

We extracted several behavioural indices from the vertical column experiments. For the vertical distribution trials, weighted mean depth, calculated as an arithmetic mean, or centre of mass (COM), using counts per 10 cm depth interval (n=12), was treated as a continuous response variable for a group (n=10) of larvae in a given light treatment and tank. Vertical swim speed was measured as the time (t) for a larva of a given maternal origin to swim the length of the swimming window (d) towards surface light ($cm \cdot s^{-1}$).

Relationships between maternal size and larval swim speed were developed using vertical swimming experiments and stage I larvae. We utilized an ANCOVA comparing the relationship between maternal size (carapace length) and vertical swimming speed among natal locations.

Behaviour indices are reported as means ± 1 standard deviation in text.

4.4 Results

4.4.1 Biogeographical variation

Linear mixed effects models and Tukey's post-hoc comparisons revealed consistent temperature by natal origin interaction effects on swim speed of newly emerged stage I larvae (Table 4.1). Vertical swim speeds recorded for warm-origin larvae (Red Harbour) were significantly faster than cold-origin larvae (Port au Choix) at 15 and 20 °C (0.44 and 0.75 $cm \cdot s^{-1}$ or 35 and 36%, faster respectively at $\alpha<0.001$, Figure 4.3). Cold-origin lobsters swam 0.1 $cm \cdot s^{-1}$ (~7%) faster in the coldest 10 °C treatment but did not differ significantly from warm-origin lobsters ($p=0.764$, Figure 4.3). Swimming in the

horizontal plane mirrored patterns in the vertical swimming trials. Temperature, natal origin, and their interaction influenced stage I swim speed significantly. At the coldest temperatures (10 °C), cold-origin larvae swam significantly faster (~1.5 $cm \cdot s^{-1}$ or 43%) than warm-origin larvae ($p<0.001$). At the warmest temperatures (20 °C) warm-origin larvae swam significantly faster (~0.5 $cm \cdot s^{-1}$ or 24%) than cold-origin larvae ($p<0.05$). Swim speeds at the intermediate temperature treatment (15 °C) did not differ significantly (Table 4.1; Figure4. 3).

The observed linearity of swimming varied with temperature and natal origin under 'full' lighting conditions. Collectively, larvae exhibited more directed (~10%), straight, movement at 10 °C than warmer temperature treatments ($p<0.001$, Table 4.1). Across treatments, warm-origin larval swimming was 10% straighter than cold-origin larvae, largely driven by significantly straighter swimming at 15 and 20 °C (5 and 24% respectively, Table 4.1; Figure 4.3).

As with swim speed, linear mixed effects models revealed a significant interaction between natal origin and temperature on diffusivity ($cm^2 \cdot s^{-1}$) of swimming larvae. Diffusivity at the lowest temperature treatment was 31% higher for cold-origin larvae compared to warm-origin larvae, and higher overall when pooling larvae from both natal origins. We observed no significant difference at 15 °C, but warm-origin larvae displayed significantly higher diffusivity (57%) at 20 °C compared to cold-origin larvae (Table 4.1; Figure 4.3).

4.4.2 Maternal variation

Swim speed did not vary with maternal size pooled across natal origins ($F=0.291$, $p=0.591$, $r^2 = 0.21$), but did vary with the interaction between maternal carapace length and origin (MS =0.876, $F=4.02$, $p=0.049$), which produced a significant positive relationship for warm-origin (0.02 $cm \cdot s^{-1}$ per mm carapace length, $p=0.049$) and a non-significant relationship for cold-origin (0.01 $cm \cdot s^{-1}$ per mm carapace length, $p=0.27$) larvae (Figure 4.4). Average vertical swim speed of larvae was unrelated to the average carapace length of larvae from a given maternal origin ($F = 1.03$, $p = 0.324$, $r^2=0.122$).

4.4.3 Intra-ontogenetic variation

We measured within-stage variation in swimming behaviour in both vertical and horizontal planes, using different ages of stage I larvae at 24, 48, and 72 hours post-hatch. Age and the interaction between age and lighting condition significantly influenced stage I distribution in the water column (Table 4.2). Vertical center of mass (COM) differed among light treatments only for larvae within 24 hours post-hatch, or 0 day old individuals ($p<0.001$), with larvae higher in the water column with more available light (28.6 (± 15.3 cm), 35.04 (± 12.1cm), and 45.7 (± 19.1 cm) for full, intermediate and low light respectively; Figure 4.5). Overall, 0 day old larvae were significantly higher in the water column compared to 1 and 2 day old larvae (25.6 and 45 cm, respectively) across lighting treatments ($p<0.001$; Figure 4.5), with the magnitude of difference generally declining with light level. 1 and 2 day old larvae were more evenly distributed through the water column, and did not vary significantly among light treatments. We observed a small difference in vertical position among light treatments in only one instance; COM estimated for 1 day old larvae at intermediate light were ~15 cm higher than at full and

low light, however this difference was not significant (p=0.18 and 0.26, respectively, Figure 4.5).

In the horizontal plane, larval age had a small but significant effect (p=0.0218) on swim speed, with fastest swim speeds observed for larvae within 24 hours post-hatch, irrespective of treatment (Table 4.2; Figure 4.6). These larvae exhibited swim speeds of 2.21 (±1.27) $cm \cdot s^{-1}$, which was 12 and 14% faster than 48 and 72 hours post-hatch, respectively. Swim speed generally decreased with temperature, but a significant interaction between light and temperature yielded non-significant relationships with temperature at full light (Figure 4.6). Linearity varied with age, although the pooled differences were small, with 24 hours post-hatch larvae swimming straighter (average 2 and 5%, greater than 48 and 73 hours post-hatch). A significant three-way interaction between age, light, and temperature yielded decreasing linearity with temperature only under low light conditions and significantly lower linearity at low light and 20 °C across all ages (Table 4.2; Figure 4.6). As with swim speed, diffusivity was highest for larvae 24 hours post-hatch, on average 3.81 (±2.7) $cm^2 \cdot s^{-1}$, which was 16 and 24% more diffuse than larvae 48 and 72 hours post-hatch, respectively. Diffusivity decreased with temperature across all light treatments and was highest in low and intermediate light treatments in temperatures below 20 °C, but no differences were significant (Table 4.2; Figure 4.6).

4.4.5 Inter-ontogenetic variation

Vertical distributions, measured by centre of mass, varied significantly among larval stages, lighting treatments, and their interaction (Table 4.3; Figure 4.7). Overall,

Stage IV larvae had the shallowest COM (37 ± 11cm) followed by stage I (50 ±22 cm) and stages II and III larvae (115 ± 1.2 cm). Light treatment did not influence stage II and III distributions, which were absent above 100 cm depth, compared to stages I and IV where distributions differed significantly among light treatments, explaining the significant interaction (Table 4.3; Figure 4.7). Stage IV larvae demonstrated a strong affinity for the upper water column with 76% (± 6.2%) of larvae residing in the upper third of the water column under full and intermediate light conditions. Vertical distribution of stage IV larvae varied among lighting conditions with a significantly shallower COM (31.2 ± 7.2 cm) for larvae under lighted treatments compared to the low light treatment (48.5 ± 11.3 cm) (Table 4.3; Figure 4.7). For all lighting treatments, most stage IV larvae (> 50%) were found in the upper one third of the water column. Stage I larvae exhibited the most variable distribution in the water column (Figure 4.7) with standard deviations of COM 2-3 times greater than those of later stages. Stages I and IV both exhibited the shallowest COM at intermediate light, which was significantly higher than in low light ($p<0.001$; Figure 4.7).

Swim speed, characterized as average of a given path, varied as a function of larval stage, lighting condition, and the two-way interactions between stage and both temperature and light (Table 4.3). Overall, ontogeny influenced swim speed most, with stage IV larvae swimming significantly faster 4.17 (±1.24) $cm \cdot s^{-1}$, than preceding stages, 1.92 (±1.46), 0.92 (±1.33), and 0.67 (±1.12) $cm \cdot s^{-1}$, 54, 78, and 84% faster, for stages I-III, respectively (Figure 4.8). Significant interactions between stage and temperature (Table 4.3) yielded decreased swim speed with increasing temperatures for stage I larvae

except at full light, as observed in intra-ontogenetic trails, and increased swim speed with temperature for stage IV, except at low light. No significant relationships were observed between swim speed and temperature for intermediate stages II and III. Swim speed varied significantly with light for stages I and II, with highest speeds in the low light treatment. Swim speeds decreased in stage IV larvae with light for temperatures less than 20 °C. Light conditions did not influence stage III swim speed ($p=0.9$; Figure 4.8).

Swimming behaviour, as measured by linearity of swimming path, varied as a function of ontogeny, lighting, and temperature condition (Table 4.3; Figure 4.8). Overall across treatments stages I and IV swam significantly straighter, 38 (±8) and 44 (±5) %, respectively, than intermediate stages II (16 ±0.05 %) and III (17 ±5 %). Temperature significantly influenced linearity of stage I larvae only, where linearity decreased by ~10% for every 5 °C (-8 ± 4%; Figure 4.8), averaged across lighting treatments. No significant three-way interactions were found between temperature, light and larval stages.

Behavioural diffusivity varied as a function of ontogeny and experimental treatment. As with swim speed, stages I and IV exhibited significantly higher diffusivity (3.31 (± 6.0) and 9.98 (± 17.4) $cm^2 \cdot s^{-1}$, respectively), than intermediate stages II and III, (0.74 (±6.9) and 1.55 (±11.4) $cm^2 \cdot s^{-1}$, respectively). Diffusivity measures did not differ significantly between stages II and III or among treatments (<15%). Although we found no significant two-way interactions between larval stage and either temperature or light, we did observe a significant three-way interaction (Table 4.3). Stage I diffusivity decreased significantly with temperature and was significantly higher at low light

treatments for temperatures less than 20 °C. Stage IV diffusivity increased with temperature at full and intermediate light treatments, and overall, was lowest at intermediate light for temperatures less than 20 °C (Figure 4.8).

4.5 Discussion

Ours is the first study to describe and quantify expressly biogeographic, onotogenetic, and environmental influence on the behaviour of all developmental stages of larval American lobster, *Homarus americanus*. We demonstrate considerable variability in larval behaviour in response to environment, both between natal origin and among larval stages. Collectively our results challenge the suitability of singular descriptors to describe behaviour across a species range and among ontogenies. Behaviour of the pelagic meroplanktonic stages of benthic organisms plays a key role in defining the dispersal potential and connectivity of a species. The relatively strong larval behavioural capacity of crustacean species such as *H. americanus*, and their responsiveness to environmental stimuli (Chia et al. 1984), underscores the need to quantify their potential behavioural contributions to dispersal. Across their home range, American lobster larvae experience diverse environmental conditions, particularly temperature, potentially leading to a wide range of dispersal outcomes that may be augmented by interactions between environment and behaviour. Incorporation of behaviour into biophysical models can strongly influence predicted dispersal and connectivity potential of species (Katz et al. 1994, Butler et al. 2011), and biological inputs thus influence model precision (Metaxas 2001). Realistic biological input should consider whether the application of one simple description of behaviour over an

ontogenetic or biogeographic range represents reality, and how variability might influence dispersal. A resolution of the key scales of dispersal requires realistic parameterization and evaluation of the influence of meroplankton behaviour, whether considering how vertical movement may limit large-scale dispersal (Metaxas & Saunders 2009) or small-scale horizontal movement may influence settlement decisions (e.g. Snelgrove & Butman 1994).

4.5.1 Biogeographic variation

American lobster inhabit coastal waters ranging from North Carolina to Labrador, exposing larvae to a relatively diverse range of thermal habitats (Aiken & Waddy 1986). The strongly seasonal waters of coastal Newfoundland represent the northern limit of the species and expose larval life history stages to thermal habitats that span from relatively warm (13-17 °C) to some of the coldest summer surface temperatures (6-12 °C) within the species range. We found differences in behaviour and environmental correlates of behaviour, between recently hatched stage I lobster from two locations that differ in summer temperature ranges. Swim speeds of larvae from the northern limit of the lobster fishery, Port au Choix (cold-origin) were markedly faster (~43%) in cold temperature treatments and slower in warm water treatments relative to larvae hatched from mothers from comparatively warmer Red Harbour. In warm water treatments the inverse was true, with warm-origin larvae swimming faster (~23%) at warm temperatures. Swim speeds from warm-origin larvae were positively related to temperature, though this relationship was weaker than the negative relationship observed for cold-origin larvae. Similarly,

diffusivity and linearity of movement for cold-origin larvae decreased with temperature whereas warm-origin larvae demonstrated no clear temperature relationship.

Behavioural trait variability may reflect plasticity or genetic differences resulting from consistent selection for a given trait expression. For example, plasticity in larval response to tidal circulation was suggested as a mechanism to help larvae reach favourable nursery habitat in coastal and offshore populations of shore crab *Pachygrapsus crassipes*, where genetic differences are not expected (Miller & Morgan 2013). Conversely, oceanographic conditions that strongly influence dispersal may restrict gene flow and lead to local adaptation in populations of acorn barnacles (*Semibalanus balanoides*) (Bertness & Gaines 1993). Our analysis illustrates plasticity in response to temperature as illustrated by variability of swimming behaviour among temperature treatments, particularly for cold water origin larvae. Behavioural plasticity, however, does not necessarily explain different, and in some cases inverted, response to temperature between natal origins. Our common garden approach suggests a potential underlying genetic or maternal effect component to behavioural differences between our populations, analogous to cogradient variation. Cogradient variation is expected when diversifying selection favours different phenotypes in different environments (Marcil et al. 2006). The two natal regions we compared differed significantly in thermal regimes in the summer-early fall months when pelagic larvae occur. The relative differences in temperature we used reflect those expected within the respective natal origins, and led to corresponding behavioural differences in response to environment, similar to observations on activity costs of cold- versus warm-origin larval Atlantic cod (*Gadus morhua*) (von

Herbing & Boutilier 1996). If adaptation to local temperatures results in improved capacity for vertical migration under local conditions, the result could be improved retention and/or survival and thus potentially cogradient variation. Additionally, differences in the response to temperature among regions could be related to acclimation during development. Cold-water organisms are often associated with an upregulation of metabolic enzymes, leading to a relatively higher metabolism at warmer temperatures than would be expected of individuals acclimated to warmer temperature (McGaw & Whiteley 2012). Given the extended brood times associated with American lobster, differences in environmental conditions among regions will likely influence the embryonic development of lobster. The stronger response to temperature observed for stage I Port au Choix lobster larvae, thus is likely in part due to the difference in physiology associated with embryonic development and known physiological thermal response of this species (e.g. Spees et al. 2002).

Preliminary work on American lobster populations in Newfoundland suggested limited genetic structure (Kenchington et al. 2009), however, that study did not encompass our sample sites. More recent expanded coverage suggests significantly greater genetic structure (Benestan et al. 2015) in Atlantic Canada than previously reported. Given the high abundance of lobsters in some environments and large dispersal potential (e.g. Incze et al. 2010), larval behaviour presumably plays some role in the discreteness of genetic structure. An early life history contribution may be particularly important in Newfoundland, where limited adult movement places the majority of genetic dispersal potential on the larval phase (Kenchington et al. 2009). Further studies

evaluating the selection potential, either through varying recruitment or survival, are needed to fully evaluate how variable response to temperature might influence genetic variation.

In many species, larger females are thought to have greater reproductive resources and therefore produce larger eggs that hatch into correspondingly larger and presumably more behaviourally capable larvae. American lobster generally fit this maternal- to egg-size paradigm, though relationships vary greatly (Ouellet & Plante 2004). Our observations add an additional biogeographic-behavioural consideration to the larger female – larger egg size relationship. We found that swim speed scales positively with maternal size for larvae from Red Harbour but not for Port au Choix. Though variable (r^2 < 0.25), our results demonstrate a biogeographic trait difference, as observed in larval swim speed in response to temperature. Understanding the influence of maternal size is particularly important for fisheries with high exploitation rates that impart a size-selective influence on the population, such as American lobster.

4.4.2 Swimming behaviour

We observed significant *intra-ontogenetic* variation in swimming behaviour in response to environmental treatments. Previous observations of stage I larvae reported significant differences in vertical position and taxis during the first 24 hours post-hatch compared to successive days within the first zoeal stage of American lobsters (Hadley 1908, Ennis 1975a). Our study confirms this observation, in that stage I larvae swam faster (~0.28 $cm \cdot s^{-1}$), more diffusively (~ 0.77 $cm^2 \cdot s^{-1}$), and responded more to light stimuli in the first 24 hours of stage I compared to older larvae. No major morphological

changes are expected within stage I (Lavalli & Factor 1995) and, not surprisingly, swim speed during the first few days post-hatch varied by less than 15%. Indeed, we observed no significant three-way interaction between light, temperature, and age for swim speed. However, we did observe considerably more variable swimming behaviour (vertical position, linearity, and diffusivity) among temperature and light treatments for 1 and 2 day old larvae, confirming general observations of a diminishing light response with age reported long ago (Hadley 1908). Collectively, our observations of swim speed (1.8-2.2 $cm \cdot s^{-1}$) are comparable to observations of early stage American (Ennis 1986) and European lobster (Schmalenbach & Buchholz 2010) larvae, among other strong swimming larvae of invertebrate species (Foxon 1934).

Temperature can strongly influence swimming ability of small organisms such as meroplankton through mediation of physiological (e.g. metabolism) and environmental conditions. In particular, increased viscosity of water at colder temperatures increases resistance to movement, leading to a general expectation that warmer temperatures are more conducive to faster swim speeds (Chia et al. 1984, Fuiman & Batty 1997). Indeed, this pattern has been reported in larval fish (Guan et al. 2008, Stanley et al. 2012), post-larval American lobster (Rooney & Cobb 1991), larval sea stars (Civelek et al. 2013), larval sand dollars (Podolsky & Emlet 1993), and brine shrimp (Larsen et al. 2008), among others. Data for stage IV post-larvae in ours and previous studies (Rooney & Cobb 1991) conform to this hypothesis, with faster swimming and more diffusive movement associated with warmer temperatures. However, the movement paths of stage I larvae suggest the opposite, given that we observed a negative relationship between temperature

and swim speed/ diffusivity. This observation appears counter intuitive given the increased viscosity of water at lower temperatures, but might indicate physiological stress in stage I larvae at warm temperatures. Timing of spawning has been linked to temperature (Aiken & Waddy 1986) and often begins when temperatures exceed 12 °C, indicative of early summer (Harding et al. 1982). Our observation of inverse temperature-swimming relationships between stages I and IV could correspond to the relative temperatures expected for the two stages, given water temperatures on average would likely increases in temperature expected in the period between hatching and molt to stage IV (~1-3 weeks depending on temperature). Larvae in our study were progeny from areas where larvae experience some of the coldest surface temperatures in the species range (Aiken & Waddy 1986) and, correspondingly, the warmer treatments may have represented sub-optimal (e.g. decreased dissolved O_2), potentially more stressful conditions leading to a negative swim speed-temperature relationship.

Lighting condition significantly influenced stage I and IV behaviour beyond mediating the effect of temperature. For stage I larvae we found fastest swim speeds and generally more diffusive movement at the lowest light level. Increased swimming activity in low light might be related to larvae seeking out brighter lighting that may favour improved feeding rates or potential indication of reduced risk to visual predators. Differences in vertical position in response to light mirror previous *in situ* observations of variable vertical position and potential diurnal behaviour (e.g. Harding et al. 1987). Recent observations of *Homarus gammarus* suggested greater susceptibility of early larval stages to nutrient limitation, relative to later stages (Schoo et al. 2014). Ontogenetic

shifts in tolerance to food limitation might necessitate increased vertical movement in response to light and variable nutritional resources. Vertical swim trials demonstrated that larvae occurred higher in the water column under intermediate lighting conditions, similar to relative increases in surface abundances observed in the field (e.g. Harding et al. 1982, DiBacco & Pringle 1992, Annis et al. 2007). Lobster larvae are negatively buoyant (Schmalenbach & Buchholz 2010), necessitating active swimming to regulate or maintain depth distribution. Increased swim speed and diffusivity in stage I larvae at low light likely represents a behavioural response to regulate depth distribution. Similarly, Batty (1987) noted faster swim speeds in early stage larval herring at lower light, likely related to maintaining position in the water column. Indeed, Batty (1987) found that most behaviour for this larval stage occurred in the vertical plane at low light, presumably because available light was below foraging optima and larvae subsequently altered vertical position.

Light affected stage IV larval behaviour much less than stage I. Neither swim speed, nor linearity, nor diffusivity differed significantly among light treatments at temperatures above 10 °C for stage IV larvae. Near-surface distributions of stage IV larvae have been reported in the field (Harding et al. 1982; Stanley unpublished data) and lab (Boudreau et al. 1992) studies under a variety of lighting conditions. Despite stronger swimming capacities in stage IV larvae relative to stage I, their distributions were confined more to the upper water column than stage I, potentially negating the need to alter swimming behaviour in response to light. Rooney and Cobb (1991) noted significant increases in swimming activity during the day compared to dusk or night, however our

experiments did not account for any endogenous rhythms. Comparisons of stage I and IV swimming behaviour with previous observations might suggest endogenous rather than environmental control of stage IV swimming behaviour in response to light stimuli, as reported for shore crab (*Carceanus maenus*) (Zeng & Naylor 1996a).

Swimming at intermediate stages was slower and less diffusive than earlier and later stages, contrary to previous observations (Ennis 1986, Schmalenbach & Buchholz 2010). In particular, swim speeds for Stage II and III, 0.92 and 0.67 $cm \cdot s^{-1}$, respectively, were less than half the swim speeds recorded for the previous stage and showed little to no response to environmental cues, other than a small increase in speed and diffusivity for stage II at low light. Although our observations lacked any directed rheotactic cue, limited rheotaxis is expected during the zoeal stages (Ennis 1986). Correspondingly, we observed significantly lower variance in vertical position in stage II and III larvae, and individuals were never observed more than 20 cm from the bottom under any lighting condition. Given that cultures of stage III later metamorphosed into normal, active stage IV post-larvae, we do not believe the observed behaviour reflected poor larval health.

The relative shift in intermediate stages, relative to stage I and IV, might explain vertical distributions of larvae in deeper strata in the field (Harding et al. 1987, Boudreau et al. 1991). Typically only studies that utilize high volume, integrated sampling (below the neuston layer) obtain sufficient catches of intermediate larval stages to draw ecological inference (e.g. Hudon et al. 1986, Harding et al. 2005). Reduced speed, diffusivity, linearity, and response to light stimuli might be indicative of a behaviour that places larvae deeper in the water column. In particular, reduced linearity of movement,

such as we observed in intermediate stages, has been linked to maintenance of vertical position in several invertebrate species (Hidu & Haskin 1978, Jekely et al. 2008, Civelek et al. 2013) potentially facilitating the relatively static deep distributions we observed. Collectively our observations paint a very different picture of larval behavioural capacity for stage II and III compared to previous reports (Ennis 1986) that infer strong swimming potential.

4.4.3 Vertical distribution

Differences in vertical position, within in the context of heterogeneous vertical flow, can influence the dispersal potential of a variety of meroplankton species and biophysical models frequently incorporate this biological attribute (Cowen et al. 2000, Metaxas & Saunders 2009, Treml et al. 2015) particularly for larval fish (e.g. Fiksen et al. 2007) and decapods (Queiroga & Blanton 2005, Phelps et al. 2015). Deeper distributions could help constrain larval dispersal relative to surface layers, through counter current flow (Sponaugle et al. 2002) or potentially extend larval duration through placement in cooler temperatures (Mackenzie 1988). In the past decade several studies modelling larval lobster transport assumed basic behavioural capacity of larval lobster and static zoeal positions at the surface (Katz et al. 1994) or at the approximate mixed layer depth (Incze et al. 2010), but without behaviours beyond fixed vertical position. Static surface distributions (Katz et al. 1994) are likely appropriate for stage IV larvae, however, our study suggests light response could potentially alter distributions beyond the upper most water layer. Our observations confirm a relatively fixed deeper distribution of intermediate larval stages, II and III, as utilized by Incze et al. (2010), but not the fixed

surface position employed earlier by Katz et al. (1994). While predicting the vertical positions most likely occupied by intermediate stages is beyond the scope of our study, previous experiments by Boudreau et al. (1991) noted a significant influence of thermoclines on larval lobster distributions, suggesting that sharp changes in water density might impart a lower boundary to vertical movement. The assignment of a static positon for stage I appear more tenuous, given observations of variable distribution with light.

4.4.4 Summary

Observations of larval *Homarus americanus* swimming revealed that interactions between origin, ontogeny, and environment significantly influenced swimming behaviour, in terms of position, capacity, and orientation. In particular we observed significant and predictable variation in swimming behaviour, questioning the validity of assigning singular behaviour rules across environmental conditions, ontogenies, and species' range. Collectively our results quantify important sources of variability in the behaviour of meroplankton with links to environment. Identifying sources of variability and directly quantifying behaviour provides the basis for the formulation of behavioural rules to be tested in the field and employed in biophysical models.

In recent years lobster populations and fisheries in Atlantic Canada and the United States have generally flourished (Steneck & Wahle 2013), despite high exploitation rates of new recruits approaching 90% (Zeng & Naylor 1996a). Effective management and sustainability of this lucrative fishery requires understanding the factors that facilitate current population levels. Lobster populations are generally considered non-migratory

because of the relatively sedentary nature of adult stages (Zeng & Naylor 1996b, den Heyer et al. 2009), suggesting that the pelagic larval phase contributes the majority of variation in dispersal and source-sink dynamics (Incze & Naimie 2000, Kenchington et al. 2009). Indeed, numerous biophysical models predict source-sink dynamics and suggest strong dispersal in American lobster (e.g. Katz et al. 1994, Incze et al. 2010). An improved understanding of variability and capacity in larval behaviour will enable a more realistic parameterization of biophysical models (Metaxas & Saunders 2009, Butler et al. 2011), thus increasing accuracy and improving overall understanding of larval transport and dispersal-recruitment dynamics.

4.6 Tables

Table 4.1 Results of linear mixed effect models detailing biogeographic variation in stage I behaviour as a function of temperature (T), natal origin (O) and the interaction between treatments (O x T). Mixed effects models control for variance associated with observational sequence (random) nested within treatment. Type III sums of squares are reported and used in significance testing.

Behavioural index	**Variable**	**DF**	**SS**	**F-value**	**p**
Vertical swimming ($cm \cdot s^{-1}$)	O	1	0.23	3.56	0.063
	T	1	0.10	0.02	0.888
	O:T	2	0.41	7.21	**0.009**
Horizontal swimming ($cm \cdot s^{-1}$)	O	1	0.35	3.15	0.09
	T	1	0.57	22.22	**<0.0001**
	O x T	2	0.63	13.50	**0.001**
Linearity (%)	O	1	0.10	6.88	**0.015**
	T	1	0.41	17.1	**0.0003**
	O x T	2	0.03	1.20	0.283
Diffusivity ($cm^2 \cdot s^{-1}$)	O	1	0.098	2.0810	0.15
	T	1	1.48	11.2502	**0.005**
	O x T	2	2.28	9.3942	**0.002**

Table 4.2 Results of linear mixed effect models detailing intra-ontogenetic variation in larval behaviour as a function of light (L), temperature (T), age (A) and the interaction between treatments (i.e. A x L) within stage I (0-2 days post hatch). Mixed effects models control for variance associated with larval group and video sequence as random factor nested within treatment. Type III sums of squares are reported and used in significance testing.

Behavioural index	**Variable**	**df**	**SS**	**F-value**	**p**
Centre of mass depth (cm)	A	2	0.51	17.73	**<0.0001**
	L	2	0.06	2.79	0.07
	A x L	4	0.28	5.28	**0.001**
Swim Speed ($cm \cdot s^{-1}$)	A	2	0.430	3.99	**0.0218**
	L	2	3.84	40.62	**<0.0001**
	T	1	1.16	32.29	**<0.0001**
	A x L	4	0.45	2.44	*0.053*
	A x T	2	0.041	0.567	0.569
	L x T	2	0.990	11.44	**<0.0001**
	A x L x T	4	0.371	2.14	*0.082*
Linearity (%)	A	2	0.190	3.80	**0.026**
	L	2	0.425	1.90	0.156
	T	1	1.44	60.37	**<0.0001**
	A x L	4	0.012	0.27	0.897
	A x T	2	0.004	0.83	0.438
	L x T	2	0.420	8.57	**0.0004**
	A x L x T	4	0.436	4.08	**0.0045**
Diffusion ($cm^2 \cdot s^{-1}$)	A	2	5.92	7.88	**0.0007**
	L	2	4.20	12.04	**<0.0001**
	T	1	14.34	68.64	**<0.0001**
	A x L	4	0.26	0.71	0.585
	A x T	2	0.06	0.11	0.897
	L x T	2	5.34	18.51	**<0.0001**
	A x L x T	4	0.94	3.25	**0.016**

Table 4.3 Results of linear mixed effect models detailing intra-ontogenetic variation in larval behaviour as a function of light (L), temperature (T), ontogenetic stage (S) and the interaction between treatments (i.e. S x L). Mixed effects models control for variance associated with larval group and video sequence as random factor nested within treatment.

Behavioural index	**Variable**	**df**	**SS**	**F-value**	**p**
Centre of mass depth (cm)	S	3	1.06	37.86	**<0.0001**
	L	2	0.14	7.10	**0.0014**
	S x L	6	0.16	2.93	**0.012**
Swim Speed ($cm \cdot s^{-1}$)	S	3	57.267	400.4	**<0.0001**
	L	2	3.365	33.48	**<0.0001**
	T	1	0.005	0.34	0.55893
	S x L	6	1.898	7.32	**<0.0001**
	S x T	3	1.250	9.66	**<0.0001**
	L x T	2	0.564	4.56	**0.012**
	S x L x T	6	0.499	1.82	0.10
Linearity (%)	S	3	11.03	91.398	**<0.0001**
	L	2	0.750	9.741	**0.0001**
	T	1	0.169	4.298	**0.0403**
	S x L	6	0.112	0.391	0.884
	S x T	3	0.725	7.536	**0.0001**
	L x T	2	0.127	1.351	0.263
	S x L x T	6	0.297	1.398	0.222
Diffusion ($cm^2 \cdot s^{-1}$)	S	3	274.42	189.24	**<0.0001**
	L	2	4.85	4.916	**0.0089**
	T	1	0.12	0.029	0.865
	S x L	6	7.18	2.03	*0.067*
	S x T	3	14.22	11.63	**<0.0001**
	L x T	2	2.83	2.68	*0.073*
	S x L x T	6	7.82	2.80	**0.014**

4.7 Figures

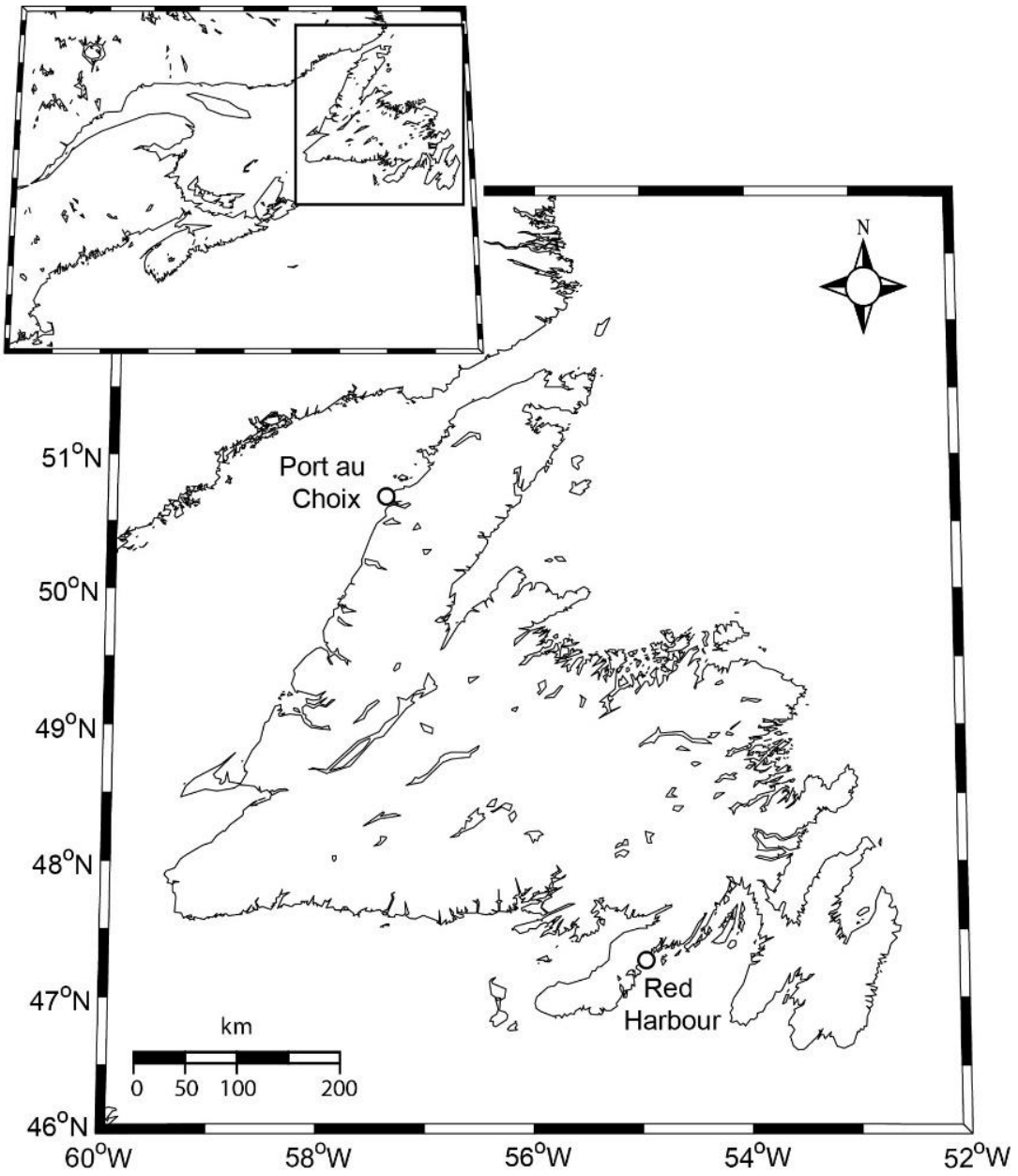

Figure 4.1 Map detailing locations of ovigerous female collections. Inset shows the Island of Newfoundland with respect to the American lobster range, eastern Canada and the northeastern United States. Port au Choix represents the cold-origin and Red Harbour the warm-origin.

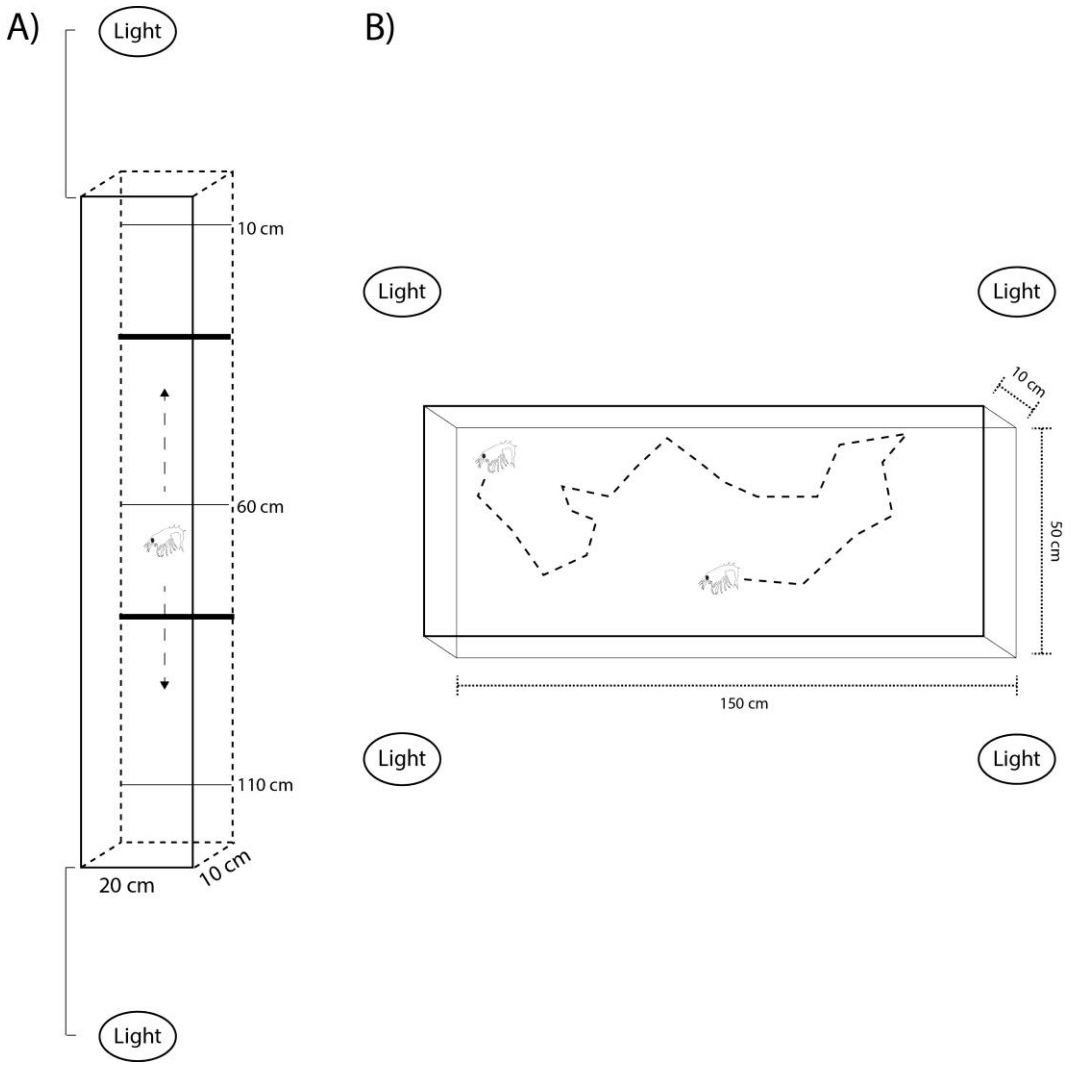

Figure 4.2 a) Schematic of vertical distribution and taxis observational chamber. Dark horizontal lines represent the start and end point of 50 cm swimming window in which vertical swim speed was measured. b) Schematic of the horizontal swimming arena. Dashed line represents example movement path recorded by camera, including larval track and orientation of movement.

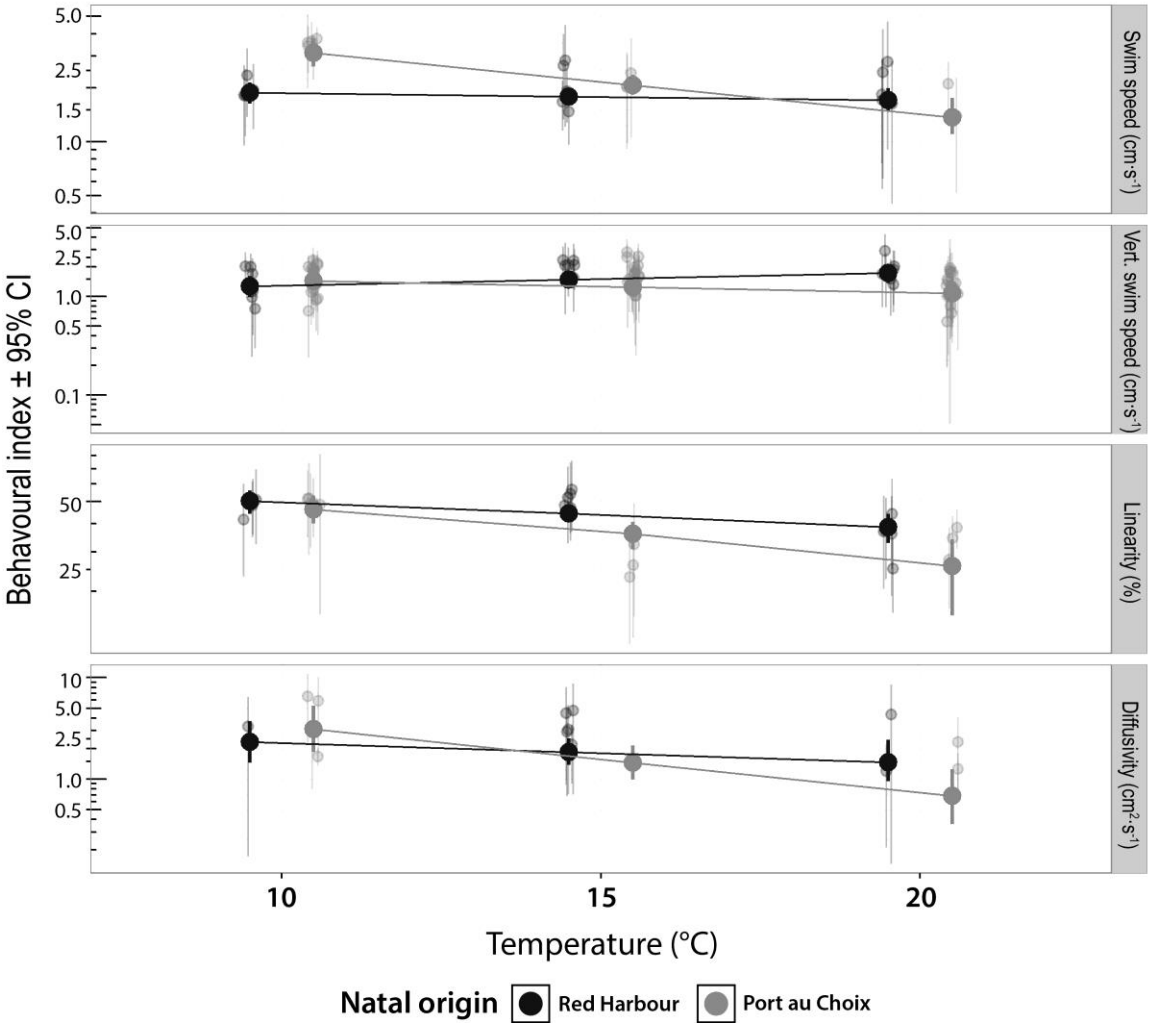

Figure 4.3 Biogeographic variation in stage I behaviour as a function of temperature and natal origin. Bootstrapped parametric means calculated from 1000 resamples (± 95% CI) are drawn from linear mixed effects models where video or observation sequence (vertical swim speed) is treated as a random factor. Observation sequence means are included as smaller points (±sd). Data on y-axis for diffusivity is log10 scaled, resulting in asymmetry in some error bars. Port au Choix represents the cold-origin and Red Harbour the warm-origin. Three temperature treatments were employed, offsets among points were introduced to better differentiate natal origins.

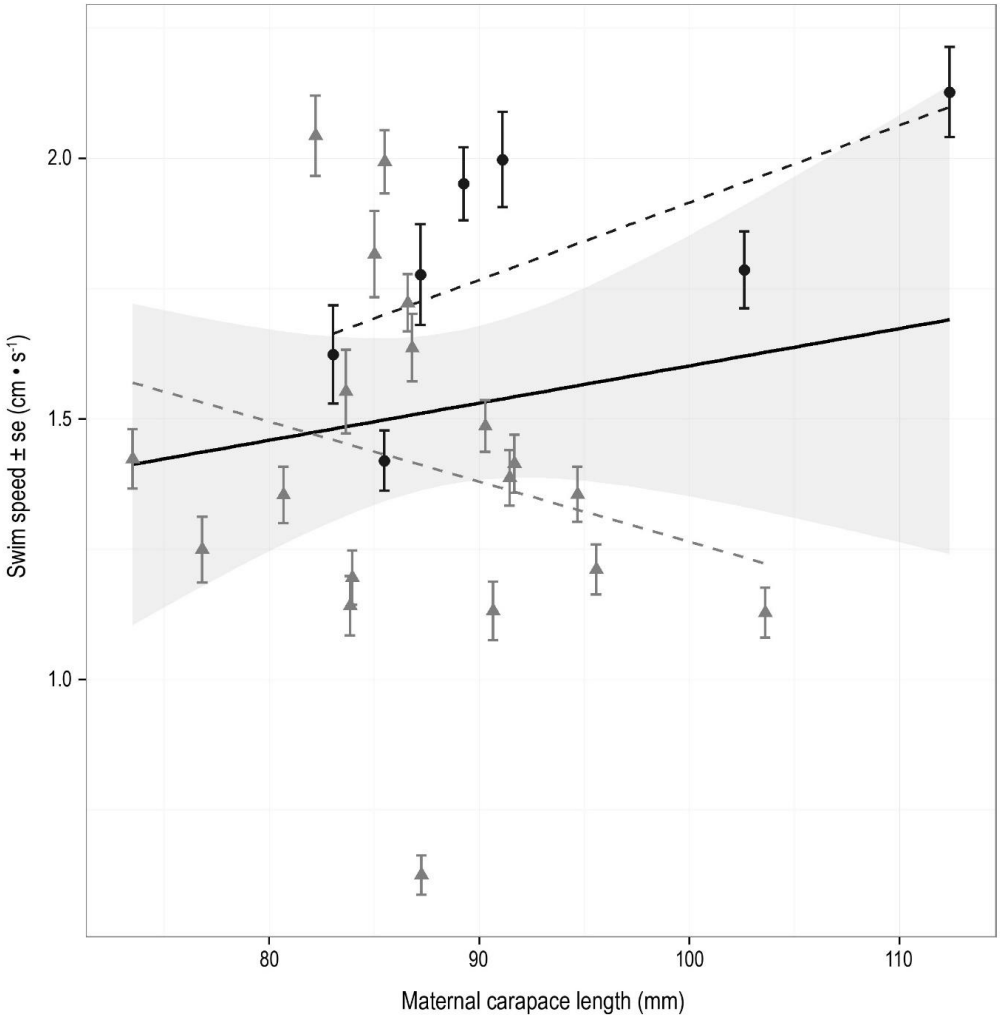

Figure 4.4 Maternal variation in newly emerged larval stage I swim speed. Grey triangles represent averages from Port au Choix (cold-origin) and black circles averages from Red Harbour (warm-origin). The black line represents the within group regression slope and the shaded grey region corresponds to the standard error of the regression. Dashed lines correspond to linear relationships partitioned to each region.

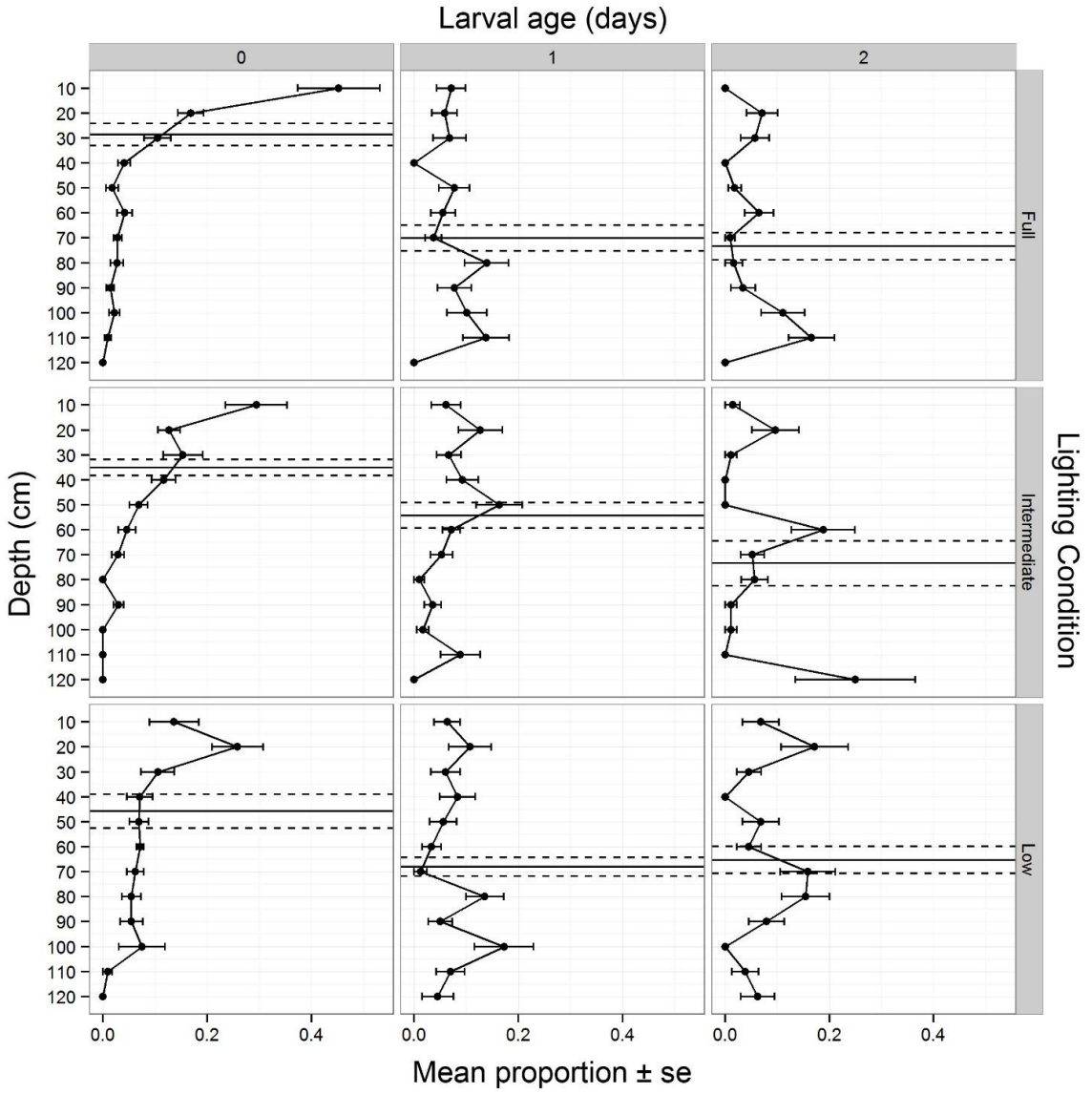

Figure 4.5 Larval stage I lobster vertical distribution as a function of age post-hatch and lighting condition. Horizontal solid lines represent the average of centre of mass for a given treatment and dashed lines represent one standard error.

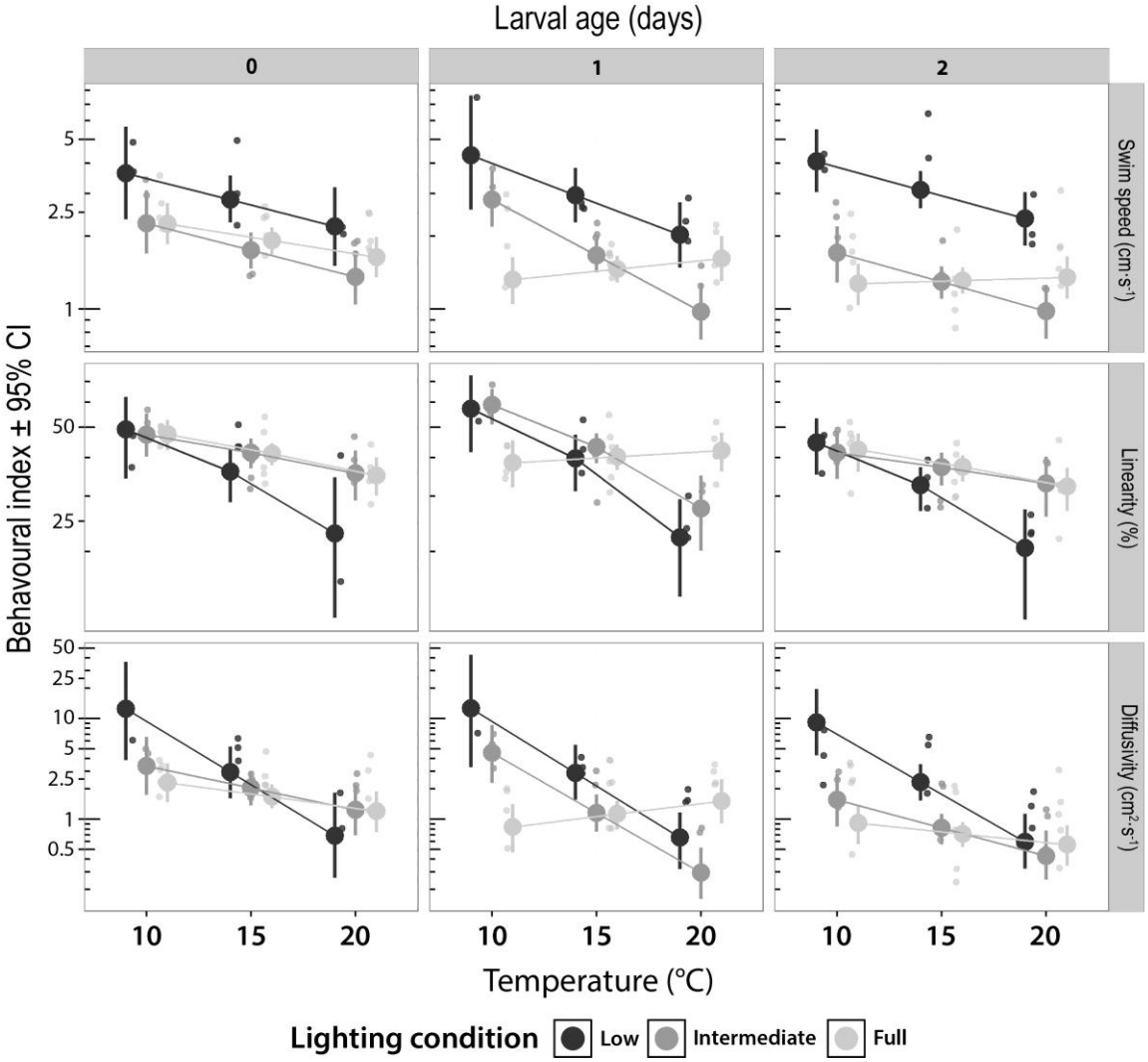

Figure 4.6 Intra-ontogenetic variation in behaviour of stage I larvae, as a function of temperature and lighting condition. Bootstrapped parametric means (± 95% CI) are drawn from linear mixed effects models where video observation sequence is treated as a random factor. Smaller points represent the average for each observational sequence within a given treatment. Data on y-axis is $\log_{10}$ scaled, resulting in asymmetry in some error bars. Three temperature treatments were employed, offsets among points were introduced to better differentiate lighting treatments.

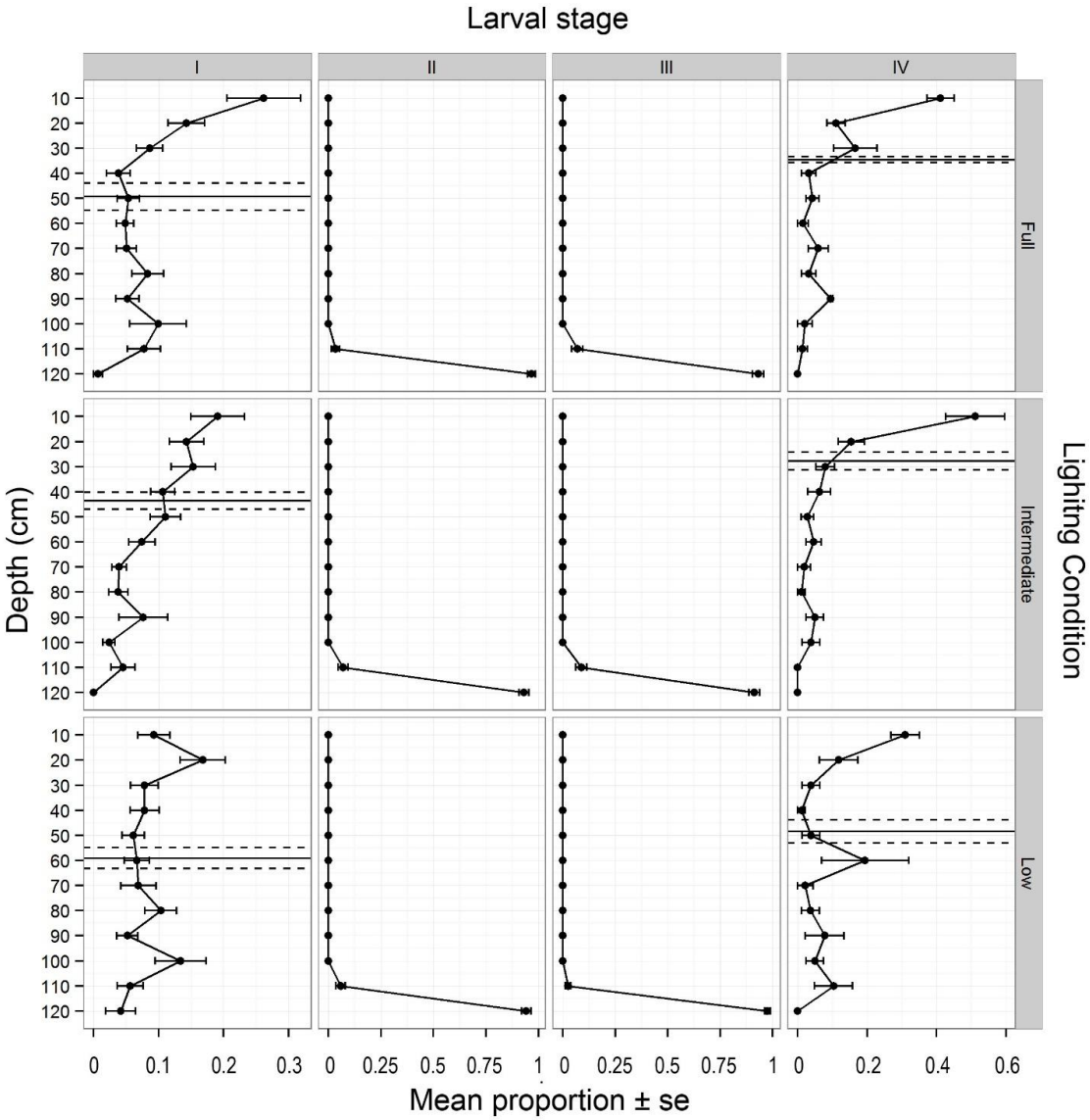

Figure 4.7 Larval lobster vertical distribution as a function of ontogeny and lighting condition. Horizontal solid lines represent the average of centre of mass for a given treatment and dashed lines represent one standard error.

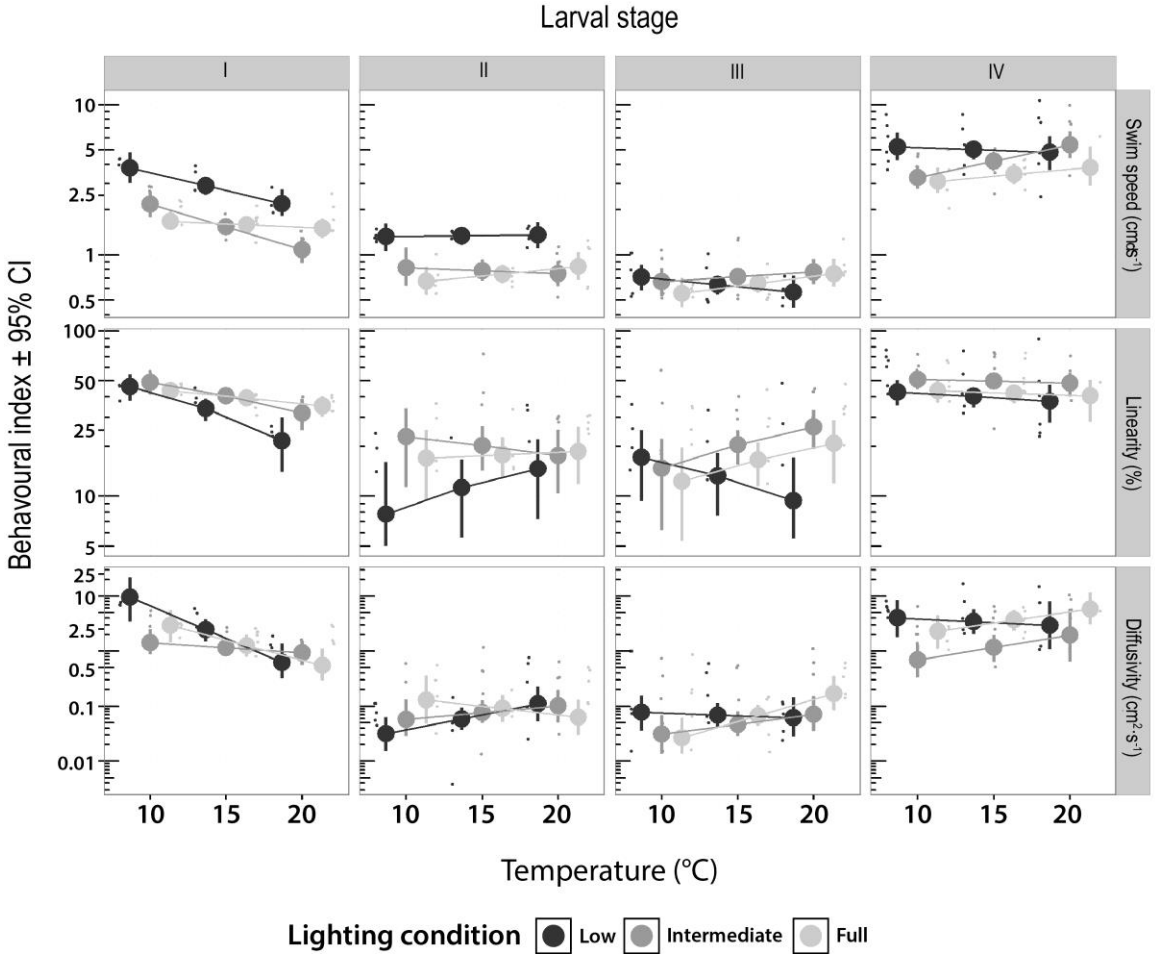

Figure 4.8 Inter-ontogenetic variation in behaviour as a function of temperature and lighting condition. Bootstrapped parametric means (± 95% CI) are drawn from linear mixed effects models where video observation sequence is treated as a random factor. Smaller points represent the average for each observational sequence within a given treatment. Data on y-axis is $\log_{10}$ scaled, resulting in asymmetry in some error bars. Three temperature treatments were employed, offsets among points were introduced to better differentiate lighting treatments.

Chapter 5 - Simulated dispersal of American lobster (*Homarus americanus*) larvae: a sensitivity analysis for physical and biological variables

5.1 Abstract

Using a semi-Langrangian biophysical model, we evaluated the relative influence of timing and location of spawning, vertical movement of larvae, and varying levels of horizontal diffusivity on potential dispersal of larval American lobster (*Homarus americanus*). We tested the sensitivity of predicted dispersal distance, pelagic duration, local retention, and settlement location to model physical and biological variables. Our evaluation compared traditional passive representations of larval behaviour to active vertical movements characteristics observed in a parallel behavioral study. Simulated larvae released in St. George's Bay, NS, Canada were tracked from hatching to settlement, over the summer larval period (July-September, 2008:2011). Overall fine-scale (<25 km) variation in spawning location influenced the spatial dynamics of settlement most, and significantly influenced predicted settlement location. Release month influenced dispersive duration most; duration was also the most sensitive to model parameters. Vertical movement significantly affected dispersal, although this effect varied considerably in magnitude and direction as shown by strong interactions with other model variables. Horizontal diffusivity had the weakest effect overall, with small but ecologically significant effects on retention and dispersal distance. Our results demonstrate how exposure to vertically and horizontally heterogeneous temperature and circulation, through ontogeny, influences the duration and extent of dispersal. Though

variable, and sometimes context dependant, connectivity to other lobster fishing management zones from St. George's Bay varied significantly depending on passive versus active vertical movement rules, resulting in differences in dispersal distance (10-100 km) and connectivity. Collectively these results identify the relevant scale of dispersal sensitivity and thus help prioritize physical and biological parameters to incorporate into dispersal models, improving simulations of larval transport in American lobster and similar meroplanktonic species.

5.2 Introduction

The extent of dispersal and survival to successful reproduction determines the geographic scale of natural populations (Cowen et al. 2006, Pineda et al. 2010). Together these processes dictate ecological connectivity and the evolutionary persistence of populations and species (Hilborn et al. 2003, Hastings & Botsford 2006). Indeed, the dynamics of dispersal directly influences the spatial structure and persistence of spatially distributed populations (Largier 2003). Accordingly, the successful management of aquatic resources requires identification of population links, or paths of recruitment, and their relative contributions to population persistence (DiBacco et al. 2006, Botsford et al. 2009, Grant et al. 2010). Such knowledge can facilitate stock identification (e.g. Cadrin 1995), marine spatial management (e.g. Neat et al. 2014), marine reserve design (e.g. Almany et al. 2009, Berglund et al. 2012), and the protection of marine biodiversity (Ruzzante et al. 2006). However, a multitude of physical and biological processes potentially influence distributions of marine populations with dispersive larval and adult stages, and complicate evaluations of connectivity among habitats and geographic regions

(Pineda et al. 2010). Future successful management of marine ecosystems will require development of methodologies to effectively evaluate and predict marine dispersal and connectivity.

Marine ecologists struggle to evaluate larval dispersal in the field accurately (Cowen et al. 2006), limited by the general inability to track relatively small larvae, or cohorts of larvae that disperse over long distances in dynamic physical environments. Recent genetic (e.g. Planes et al. 2009, Harrison et al. 2012) and otolith geochemistry approaches (e.g. Bradbury et al. 2011a, Stanley et al. 2015b, Chapter 4) offer powerful tools to evaluate dispersal dynamics in the field, but lack prognostic ability to model dispersal reliably. Biophysical models offer a tool for the exploration of the relevant factors which influence dispersal. Indeed, practitioners now commonly use biophysical models to evaluate dispersal and the factors that influence its outcome (Miller 2007).

The dispersal of marine larvae is a product of the duration in the water column and the physical environment transporting the larvae. The capacity of biophysical models to describe larval dispersal depends on the representation of both the passive and active components. Many biophysical simulations fall short in accurately describing the active components of dispersal (Metaxas & Saunders 2009), particularly the vertical position in the water column. Invertebrate larvae have been commonly treated as primarily passive, often with fixed vertical positions for the duration (e.g. Incze et al. 2010) or part (e.g. Harding et al. 2005) of their larval period with little or no consideration of larval behaviour or ontogenetic variance in behaviour. Often the lack of inclusion of behaviour

is based is pragmatic based on a limited ability to numerically model behaviour or the vertical structure of the water column.

Limited incorporation of behaviour into biophysical models likely relates to inadequate information on swimming abilities, coupled with a general assertion of weak swimming capacity relative to the horizontal velocities imparted by circulation. Despite limited larval swimming ability in some species, swimming abilities of larvae of many others (e.g. fish and some decapod crustaceans) meet or exceed vertical current velocities (Metaxas & Saunders 2009). Distributions of marine meroplankton often vary with depth (reviewed by Cohen & Forward 2009, Lloyd et al. 2012b), resulting from both physical forcing and active response to environmental stimuli (Sulkin 1984). Vertical swimming that places larvae in specific flow conditions (e.g. selective tidal stream transport; Miller & Morgan 2013, Moksnes et al. 2014) or at different positions in the water column as a function of time of day (Cowen et al. 2006), and-or ontogeny (Butler et al. 2011), can strongly influence horizontal transport. Vertical swimming by meroplankton can therefore influence horizontal transport by exposing larvae to heterogeneous physical flow environments (Queiroga & Blanton 2005, Fiksen et al. 2007, Leis 2007, Cowen & Sponaugle 2009).

Incorporation of vertical movement into biophysical models can potentially influence dispersal outcomes (Butler et al. 2011, Phelps et al. 2015) and biological "active" inputs thus influence model outputs. However, successful incorporation of vertical swimming behaviour requires matching the resolution in the associated physical models to the scales over which vertical movement exerts significant influence.

Regardless of the simulation mechanisms for larval behaviour (e.g. diel migration vs. ontogenetic development), the vertical advective resolution of a biophysical model must accurately describe the dynamic nature of the water column (Daigle et al. 2015).

Most studies linking vertical swimming and dispersal report variable position and exposure to heterogeneous flow conditions as determining the key evaluating factors (Queiroga & Blanton 2005, Fiksen et al. 2007). However, vertical gradients in circulation often corresponds to heterogeneity in water temperature which, in turn, relates directly to pelagic larval duration (PLD) of many meroplanktonic species, both as a function of larval duration or for a given ontogenetic/developmental stage (Mackenzie 1988, Quinn et al. 2013). Therefore integrating realistic PLDs into a model that includes equally realistic temperature fields better reflects duration of larval exposure to different flow conditions, an aspect many biophysical models ignore (Treml et al. 2015).

We chose American lobster, (*Homarus americanus*, hereafter lobster), as a model species to study the influence of behaviour and other model parameters on larval dispersal. American lobster are among the most commercially valuable fished species in North America, representing over 35% of the total landed value of the Atlantic Canadian fishery (DFO 2015). Patterns of genetic homogeneity using microsatellite markers (Kenchington et al. 2009) and evidence of relatively limited adult movement (den Heyer et al. 2009), suggest considerable mixing and larval connectivity among lobster stocks, highlighting the importance of a thorough investigation of mechanisms that influence dispersal. The four pelagic larval stages of American lobster vary in their swimming capacity and response to stimuli (Ennis 1986, Chapter 4). Planktonic swim speeds of

zoeal stages reach 1-3 $cm \cdot s^{-1}$ (Chapter 4) and post-larval stages can swim at ~20 $cm \cdot s^{-1}$ (Cobb et al. 1989). The relatively strong swimming capacity of larval lobster, coupled with heterogeneity in vertical position in the water column (Ennis 1975a, Harding et al. 1987, Chapter 4), suggest a strong potential active contribution to dispersal akin to that reported for larval fish (e.g. Cowen et al. 2006, Leis 2007). Chapter 4 provided the data on swimming ability and vertical position necessary to develop rules on vertical movement to incorporate in a biophysical model.

Early evaluation of dispersal in larval lobster assumed larvae dwell primarily at the surface and therefore likely follow surface currents and/or prevailing wind patterns (e.g. Templeman 1937, Templeman & Tibbo 1945, Scarratt 1964). Associations of larvae with features such as Langmuir circulation (Harding et al. 1982) or gyres (Lund & Stewart 1970), as well as concordance between larval distributions and wind patterns (Templeman 1937, Templeman & Tibbo 1945, Rogers et al. 1968, Harding et al. 1982, Hudon et al. 1986, Hudon 1994) support the assertion of passive dispersal. However, other studies report horizontal distributions of stages that contradict passive predictions (Squires 1970, Caddy 1979) and that larval lobster are not restricted to the upper neuston layer (Ennis 1975a, Harding et al. 1987), suggesting that vertical movement could influence dispersal (e.g. Pezzack et al. 1992, Incze & Naimie 2000, Chasse & Miller 2010). Furthermore, clear patterns of variation in larval morphometrics (Rogers et al. 1968, Harding et al. 1993), larval behaviour (Chapter 4), and genetics (Benestan et al. 2015) indirectly suggest a much more complex mechanism for realized dispersal than passive circulation alone.

Biophysical simulations offer a potential tool to evaluate the influence of passive and active components of larval lobster dispersal. Past simulations of lobster dispersal employed a spectrum of behavioural and diffusive model parameters. Studies to date have modelled behaviour as passive, with static vertical positions (Incze & Naimie 2000, Xue et al. 2008, Incze et al. 2010), or active with simplified representations of behaviour, limited to specific ontogenetic stages (shoreward swimming for stage IV: Katz et al. 1994, diel movement for stage I: Harding et al. 2005). Similarly previous larval dispersal models used a spectrum of diffusive terms, despite evidence that dispersal can play a relatively minor (Xue et al. 2008), or major role in dispersal (Pepin & Helbig 1997) relative to advection. To date, no study has explicitly examined how realistic physical and biological variables can influence dispersal of larval lobster.

Evaluation of dispersal models requires understanding the consequences of larval behaviour and movement diffusivity. Our study has two objectives. First we evaluate how different vertical movements could influence dispersal, and second we determine how the predicted influence of behaviour could vary as a function of space, time, and diffusivity. To accomplish these objectives we vary simulated larval lobster behaviour based on empirical observation (Chapter 4) and published reports. We then simulate dispersal using three unique levels of horizontal diffusivity to evaluate potential interaction of diffusion with behaviour in influencing dispersal. Finally we vary the origin and timing of spawning to evaluate how the subsequent patterns of dispersal vary both spatially and temporally. The influence of each factor, and interaction among factors, is gauged relative to the simulated magnitude, duration and extent of dispersal.

We simulate dispersal using semi-Langrangian particle tracking, tracking particles based on specific circulation and diffusive parameters within the biophysical model. The Lagrangian perspective offers advantages over Eulerian methods because it does not smooth particle positions over grid cells, thus resulting in higher resolution that can incorporate behaviours.

5.3 Methods

5.3.1 Study Site

We based our simulation on data collected in St. George's Bay, Nova Scotia, Canada, a coastal embayment approximately 45 km^2 open on the north to the Northumberland Strait and the Gulf of St. Lawrence (Figure 5.1). The relatively homogeneous bathymetry of the bay averages 20 m with a maximum depth of ~40 m at the mouth. With little freshwater runoff, tides and wind primarily force currents in the bay (Daigle et al. 2014b). Tidal currents in the bay are weak mixed diurnal to semidiurnal with a tidal range < 2 m (CHS 2015). Early observations of circulation suggested consistent clockwise circulation in the bay (Petrie & Drinkwater 1978), however, more recent data suggests variable circulation that lacks consistent features (Lesperance et al. 2011a, b). Flushing time within the bay is approximately one month (Petrie & Drinkwater 1978); Daigle et al. (2014a,b) and Daigle et al. (2015) provide a more detailed summary of physical characteristics of St. George's Bay.

5.3.2 Field sampling

We utilized field sampling of St. George's Bay (SGB) to help derive some of the physical and biological features of the area for our biophysical models. We sampled

larval lobster abundance and associated physical variables at 16 stations in SGB (Figure 5.1c) during the summer months (July – September) of 2009-2010 to evaluate timing and location of larval presence in SGB (n= 6 and 3 for 2009 and 2010 respectively). We deployed a 1 x 2 m 500 µm neuston net from a commercial lobster boat to sample the surface water (~upper 90 cm), hauling the net at ~ 1 $m \cdot s^{-1}$ for 30 minutes in a spiral, non-overlapping pattern around the station coordinate. Volumes of each net tow were quantified using two flowmeters, the highest of which was considered the sample volume. Given known decreases in surface abundance for all stages in full sunlight (Ennis 1973, Harding et al. 1987) we sampled only during the early morning (06:00 – 10:00) and late afternoon (15:00-19:00). All samples were preserved in 95% ethanol and later identified to larval instar stage (n=4) according to Ennis (1995).

We profiled temperature and salinity of the water column using a Seabird 25 Conductivity-Temperature-Depth (CTD) profiler (Figure 5.2), and estimated thermocline and pycnocline depth as the average sequential 0.25 m depth interval where maximum change occurred. Prior to calculating thermocline and pycnocline depth, we smoothed temperature data using a sliding 1-m filter.

5.3.3 Biological variables

We selected biological parameters in the model to represent the vertical movement and swimming capacities observed in Chapter 4). The swimming speeds for each larval stage were calculated from swimming coordinates of larvae swimming at 15 °C under fully lit conditions. Speeds were calculated using the average distance traveled between successive 1 minute (1800 frames at 30 fps) time lags among 50 randomly

chosen larvae for each stage; 0.18 ±0.03, 0.09 ±0.04, 0.08 ±0.3 and 0.34 ±0.14 $cm \cdot s^{-1}$ (mean ± sd) for stages I-IV respectively. From these measurements we developed a normal distribution of potential swimming velocities for each larval stage. Previous studies report larval swimming capacities based on maximum swim speeds, often calculated over short time intervals (<10 seconds) (Chia et al. 1984). Laboratory observations of lobster (Chapter 4) and other invertebrates (e.g. Daigle & Metaxas 2012) suggest that larvae rarely sustain maximum swimming capacities for longer than 10 seconds (e.g. Ennis 1986), and swim speeds calculated over short time intervals likely overestimate larval swimming capacity (Daigle et al. 2015). Our swimming velocities, calculated as a product of average displacement after 1 minute, offer a more conservative and realistic estimate of average larval behavioural capacity in the field. To reduce the complexity in the particle tracking algorithm, we utilized swim speed distributions assembled for each larval stage which were not temperature-dependent, as observed in Chapter 4.

Vertical release positions were designed to reflect potential scenarios of larval vertical movement in the field, based on historical and current views of larval lobster behaviour. We chose a passive 'null' model where larvae maintain fixed surface positions through ontogeny and 4 behavioural models where larvae either occupy contrasting depth positions or actively undergo diel migrations that vary with ontogeny (Table 5.1). Behavioral rules spanned the spectrum of potential lobster behaviours in order to highlight how active behaviour might change expected transport relative to passive surface distribution, as well as hypotheses linking horizontal position to surface

circulation patterns (e.g. Templeman 1937, Scarratt 1964, Hudon 1994). In addition, these behaviours provide a simple model to explore how ontogenetic variation in vertical position (stages I – III; A2 and A3) could influence dispersal outcomes. For all behaviours we fixed the position of stage IV at the surface because past studies clearly document this distribution (e.g. Annis 2005).

Pelagic larval duration in the model was temperature dependant based on published developmental equations for larvae (Mackenzie 1988) and modified for post-larvae (Incze et al. 1997) (Table 5.2). Cumulative development accrued at 1-hour time intervals based on temperature measured at the depth of a simulated larva at that time step (Table 5.2). Once a simulated larva completed an ontogentic stage they began the next stage, assumed the behaviours and developmental rates assigned to that developmental stage. We utilized developmental models estimated by Mackenzie (1988) because they derived duration under environmental ranges similar to our study system and because this approach allowed comparison with previous biophysical models (Chasse & Miller 2010, Incze et al. 2010). Because Quinn et al. (2013) and Annis et al. (2007) derived their models for temperature ranges different than expected for our study, we chose not to use them.

The model treated the surface of the water column and land as solid boundaries, and we assigned simulated larvae that reached a new position at a solid boundary to the previous time step (1 hr). For simulated larvae between the limits prescribed by its ontogeny and behavioural model (Table 5.1), and not constrained by an active movement path (i.e. diel movement), the model set direction of vertical movement randomly using a

random number generator with a 50% probability of ascending or descending during the time step. If assigned a behavioural preference (i.e. diel movement), the model directed simulated larval movement until they reached the prescribed depth layer.

5.3.4 Physical variables

Simulations of dispersal tested three different levels of horizontal diffusivity (0, 5, and 25 $m^2 \cdot s^{-1}$) fixed across the model domain and tracking period (similar to Harding et al. 2005; 25 $m^2 \cdot s^{-1}$). We incorporated diffusivity as additional random horizontal movements, accumulated at each model time step according to Smagorinsky (1963). Particle diffusion was based on the premise that the average squared rate of diffusion of a simulated particle will increase at a rate of 2K, where K is the specified diffusivity level (Taylor 1922). From this premise we extrapolate the change in position for an individual particle to be $R\sqrt{2K\Delta T}$, where R is a random number between -1 and 1, with a mean of zero and standard deviation of 1, and T is the time step of the model. We modelled our upper diffusivity boundary, 25 $m^2 \cdot s^{-1}$, based on empirical and modelled estimates derived in the eastern Northumberland Strait (Hrycik et al. 2013), adjacent to our larval seeding area in SGB (Figure 5.1).

5.3.5 Hydrodynamic model and particle tracking

We employed a biophysical model derived from previous simulations in the area (Chasse & Miller 2010, Daigle et al. 2015). Three-dimensional ocean fields were obtained from a NEMO-OPA 3D hydrodynamic model of the Gulf of St. Lawrence, Scotian Shelf, and Gulf of Maine (GSL-SS-GOM) at a higher resolution than described in Brickman and Drozdowski (2012). We based the modelling system on the ocean code

OPA version 9.0 (Madac 2008), with a spatial resolution of 1/24° and 46 levels of variable thickness vertical strata, averaged over the top 6 m and then increasing in resolution with depth. Temperature and salinity fields were free to change with time, constrained only by freshwater runoff and surface forcing. The model incorporated tidal forcing using surface elevation and open boundaries. Freshwater entered the model domain (Figure 5.1a) through precipitation and the main rivers of the GSL-SS-GOM system. The model also incorporated annual barotropic transport cycles originating at the Belle-Isle Strait as well as baroclinic transport calculated from monthly temperature and salinity fields of the NEMO-OPA model. The model employed internal and external mode splitting, with time steps of 240 s and 4 s for internal (baroclinic), and external (barotropic) modes. We investigated circulation and transport for the simulation period (2008-2011) using a hindcast, forcing the ocean with the Canadian Meteorological Center (CMC) GEM atmospheric model. Prior to simulations, the model was initialized for two months (November-December 2007) from temperature and salinity climatologies calculated for November averaged over all available data (1981-2010).

Currents were averaged over 3-hour intervals from the NEMO-OPA hydrodynamic model to calculate larval trajectories. The disk space required to store the model output across the entire domain required averaging currents, which nonetheless provided sufficient temporal resolution to resolve the major effects of tides and local circulation. We regained temporal resolution when calculating simulated dispersal trajectories using linear interpolations of circulation through time, based on the model tracking time step of 1 h. We tracked simulated larvae using a tracking algorithm based

on the Runge-Kutta method including a predictor-corrector scheme, and compiled the model using previously published FORTRAN code (Chasse & Miller 2010, Daigle et al. 2015); these previous studies who also simulated dispersal events in the southern Gulf of St. Lawrence.

For every combination of behaviour and diffusivity, we released fifty larvae from 4 release sites within SGB (Figure 5.1c), at weekly intervals between July and September in 2008 through 2011. Each particle was tracked for 90 days, although larvae reached settlement age and location prior to the end of tracking.

We utilized the endpoints of simulated larval dispersal events, at the end of stage IV (according to Incze et al. 1997; Table 5.2), to calculate response variables of dispersal distance, dispersal duration, and potential settlement location. We consider connectivity in this study as the link between the origin and the endpoint of a dispersal event. We calculated dispersal distance using least-cost path analysis between the start and endpoints of each dispersing particle, treating land as a barrier to the dispersal path. Connectivity matrices were calculated for different treatments using the different release locations (n=4) as start points and the zone (Figure 5.1c) and Lobster Fishing Area (LFA) of settlement (Figure 1b) as endpoints. We calculated dispersal duration as the number of days between larval release and the end of the final pelagic larval stage (IV) according to (Mackenzie 1988). Here we consider the completion of stage IV as an endpoint for the pelagic larval period and the beginning of the settlement process (Wahle & Incze 1997). Finally, we coarsely divided simulated dispersal events into those retaining individuals (within 50 km of start position) versus those that did not. This approach provided a

relative index of local recruitment at the approximate scale of SGB. We calculated all response variables in R (R Development Core Team 2015).

5.3.6 ADCP model

Although hydrodynamic models provide a compelling mechanism to model dispersal over large horizontal scales (Figure 5.1a), they offer relatively limited vertical resolution relative to current profilers such as ADCPs (Acoustic- Doppler Current Profiler) which, in turn, offer limited horizontal resolution. To bridge the divide between vertical and horizontal resolution, we calculated dispersal trajectories using vertical flow fields estimated for each of the ADCP moorings deployed in 2009 and 2010 (Figure 1c). For ADCP dispersal simulation we seeded 1000 virtual larvae at the beginning of ADCP deployment in 2009 and 2010 (July 11th and 21st respectively). Given the length of time needed to reach settlement stage (~ 3-6 weeks), we chose these release dates to ensure that development for all particles overlapped with available vertical flow data from ADCP deployments. We utilized temperature profile data from CTD casts during larval surveys in 2009 and 2010, averaging the data from surface (0-5 m: 19.2 ± 1.4 °C), intermediate (5-13 m: 17.1 ± 2.6 °C) and deep (13-15 m: 14.2 ± 3.3 °C) layers respectively, representing positions occupied by larvae within vertical movement limits. ADCP flow data was averaged at 30 min time steps for 1 meter intervals, and larval behaviours and development followed parameters outlined in Tables 5.1 and 5.2 respectively. We constrained larval movement to one direction only at the beginning of daily diel migrations (descent at 6:00 and ascent at 18:00) until larvae reached the depth layer (surface, intermediate, or depth) assigned by the behaviour (Table 5.1). As in the

biophysical model, larval swimming velocities where drawn at random from a distribution of potential swimming velocities derived for each stage measured in the lab (Chapter 4). Given the limited horizontal scope of the ADCP moorings (one point in space), we chose to exclude horizontal diffusivity in dispersal simulations.

We utilized the cumulative dispersal distance, relative to the start point, as a response variable for ADCP simulations. These simulations provided insight into how simulated behaviour interacted with high resolution vertical circulation to influence dispersal. Given the limited horizontal representation, we present output from these simulations only to complement the hydrodynamic model and highlight how exposure to vertically heterogeneous circulation interacts with vertical position to influence dispersal.

5.3.7 Statistical analysis

We explored relationships between larval collections in the field and features of the water column using general linear models (GLM). Specifically, we evaluated the relationship between water column structure (thermocline and pycnocline depth) and the concentration of each larval stage. Given the patchy spatial and temporal distribution of larvae in SGB (Harding et al. 1982 and Supplementary Figure 5.1) we chose to evaluate relationships between larval concentration and water column features using only data from samples that captured at least one larva of a given stage (83, 30, 21 and 36% of samples for stages I-IV respectively).

The primary objective of our biophysical simulations was to evaluate how behaviour, diffusivity, and spatial-temporal aspects of spawning might influence dispersal outcomes, in order to develop broadly applicable hypotheses regarding their relative

effects when modelling dispersal. To model how these factors influenced dispersal we utilized a varying intercept mixed effect model (MM) that treated release date and year as a random grouping variable. We chose MMs because they provide a means to account for variation statistically that cannot be generalized to the fixed effects (variation among and within release days and years). We then evaluated significant differences among treatments using specified contrasts and pairwise comparisons.

We defined retention as simulated larvae that reached settlement age, but dispersed no further than 50 km from the release point, selecting this distance to represent the approximate scale of SGB and the LFAs (26a and 26b) in which larvae were released. We estimated the influence of release location, month, behaviour, and diffusivity on retention using a generalized mixed model fitted with a binomial (retained or not retained) response.

Given the large sample size of simulated dispersal particles (n=143100), evaluations of significant effects using distributional tests are highly prone to Type II error, and we complemented significance tests with measures of sample size effect ('effect size' sensu Cohen 1988). We used partial Omega squared ($\widehat{\omega}^2$) scores to partition the relative contribution of each model term to the total proportion of variance explained (r^2) by the model (Cohen 1988). $\widehat{\omega}^2$ values were calculated using the penalized weighted residual sum squares from mixed effects models according to (Bates et al. 2015). Cohen's $\hat{f}$ was used to evaluate the omnibus effect size of each model term. Here we considered Cohen's $\hat{f}$ values of 0.1, 0.25, and 0.4 to represent small, medium, and large size effects respectively (Cohen 1988, Lakens 2013). We used Cohen's *d* effect size estimates to

compare mixed model estimates of dispersal distance for simulated larvae with variable vertical positon (D, A1:A3; Table 5.1) to larvae restricted to the surface (NS). Cohen's *d* was calculated for all possible model terms, and associated interactions, which included behaviour. From this analysis we looked at the distribution of Cohen's *d* values to interpret differences in dispersal distance estimated from the mixed model relative to the variability in modeled estimates. Cohen's *d* was calculated as:

Equation 1: $$d = \frac{m_b - m_n}{SD_{pooled}}$$

where m_b and m_n represent the adjusted mean response from the model for a given behaviour and null control, respectively. SD_{pooled} represents the pooled standard deviation among behaviours compared, partitioned for each model term and interaction. Adjusted means were estimated from each model fit using the *effects* package in R (R Development Core Team 2015). We considered Cohen's *d* value estimates of 0.2, 0.5 and 0.8 to represent small, medium, and large size effects sizes, representing differences greater than 20, 50 and 80 % of the standard deviation respectively (Cohen 1988). For both Cohen's $\hat{f}$ and *d* we used the "small" size effect noted by Cohen (1988) as the minimum threshold for an *ecologically significant* effect and/or difference.

Behaviour can influence dispersal by altering larval exposure to different circulation and temperatures within a vertically heterogeneous water column. Thus behaviour can potentially influence the duration and realized distance of dispersal. To partition the influence of flow from temperature variance, we ran the ADCP model fixing temperature at the average mixed layer value (~17.5 °C). For each mooring location and

release time, we compared the average dispersal distance among fixed and variable temperature models as a percentage. This percentage represents a relative index of the amount of dispersal attributable to developmental differences versus those attributable to circulation, taking advantage of horizontally discrete, high vertical resolution representations of circulation.

All data presented as boxplots follow a common format. Solid lines denote medians (50^{th} percentile), each boxes span the 25^{th} to 75^{th} percentile, vertical lines (whiskers) span the 5^{th} and 95^{th} quantiles and points represent outliers.

5.4 Results

5.4.1 Field sampling

Water temperature varied significantly among model depth layers (MM: df_{num} =2, df_{den} =202, MS = 281.11, F-value=125.1, $p<0.001$, random intercept =CTD cast) with decreasing temperature with depth; 20.1 (± 1.3), 18.2 (±2.1), and 15.4 (±3.1) °C for surface, intermediate and deep, depth layers respectively. Thermocline depth varied between 5 – 18 m, averaging 13.9 m (± 6.7) and 12.2 m (± 4.22) for 2009 and 2010 respectively (mean ± sd). Permutational Pearson correlations (n= 100 permutations; Legendre 2005) indicated a strong correlation between pycnocline and thermocline depth (pycnocline ~ thermocline: R = 0.85, p=0.01, 100 permutations).

Neuston surveys of St. George's Bay documented the presence of first stage larvae (I) in all months sampled, July – September, with concentration declining through the sampling period (Figure 5.3). Larval concentrations in surface waters were an order of magnitude higher than those previously reported in nearby Northumberland Strait

(Scarratt 1964) and SGB itself (Harding et al. 1982) using similar surface sampling gear corrected for swept area. Although we found higher concentrations, the relative proportions of each stage approximated those previously reported. Stage I larvae occurred throughout SGB, although often with highest concentrations on the western side of SGB near the locations of the ADCP moorings and within LFA 26a (Supplementary Figure 5.1). Concentrations of intermediate (II-III) and post-larval (IV) stages fluctuated between 0-15 larvae per 1500 m^3 (Figure 5.2), with no obvious trend other than highest concentrations of stage IV larvae in August.

Larval concentrations in the neuston layer showed evidence of a declining relationship significantly with increasing thermocline depth and varied among stages (Figure 5.3; Table 5.3; *parameter estimates available in* Supplementary Table 5.1). Stages II-III larval concentrations, in particular, related most strongly and negatively with thermocline depth, followed by a much more variable thermocline depth relationship for stage I larval concentrations. The shallower slope between Stage IV larval concentrations and thermocline depth suggests a weaker link than preceding stages (Figure 5.3). Models with pycnocline depth as the covariate produced nearly identical results.

5.4.2 Circulation model

Circulation in St. George's Bay varies strongly both horizontally and vertically, largely driven by variability in wind patterns, although tidal circulation does also play a role (see Daigle et al. 2014a for more details). Circulation features such as upwelling and vertical shear occur in the area and were captured by both the circulation model and

ADCP moorings. Overall circulation was weaker at depth than at the surface, and generally fit previous observations (Petrie & Drinkwater 1978).

Comparison of modelled circulation patterns to the ADCP data at each of 5 mooring locations within SGB showed overall similarity among model estimates and observed circulation patterns temporally, similar to comparisons reported by Chasse and Miller (2010), although the model often under-predicted the average current magnitude (Supplementary Table 5.2; Figure 5.4). Higher variability in average current observations from the ADCP moorings relative to the model suggests some underlying complexity in circulation not captured by the large-scale model (Figure 4). Differences between predicted and observed currents decreased with depth on average, with estimated mean error ~ half at depth (mean error ± sd: - 1.6 ± 0.2 $cm \cdot s^{-1}$) than at the surface (mean error ± sd: -3.4 ± 1.2 $cm \cdot s^{-1}$) (Supplementary Table 5.2; Daigle et al. 2015). Although we limited our empirical circulation observations to within SGB, comparison of these data with those reported by Chasse and Miller (2010) suggests that the model provides an appropriate template to obtain first order dispersion patterns originating in SGB and to evaluate the influence of model variables on dispersal.

5.4.3 Influence of model parameters

We estimated significant influence and effect size for each modelled variable on the distance, retention, and duration of simulated larval dispersal (Table 5.4). Overall, location affected dispersal distance and retention most strongly explaining approximately 50% of the explained variance in the model ($\widehat{\omega}^2$= 0.5 for both), whereas month of release influenced pelagic larval duration most ($\widehat{\omega}^2$= 0.73; Figure 5.5). Behaviour significantly

influenced ($p<0.0001$, Cohen's $\hat{f} > 0.1$) total distance dispersed and pelagic larval duration, both across all treatments and when partitioned among treatments (interaction), however, the effect on proportion retained was small. Diffusivity had the weakest overall effect on dispersal distance and pelagic larval duration (Cohen's $\hat{f} \sim 0.1$ and $\widehat{\omega}^2 = 0.03$), although it significantly influenced the likelihood of simulated retention (Cohen's $\hat{f} = 0.29$ and $\widehat{\omega}^2 = 0.1$) (Figure 5.5). Dispersal duration was more sensitive to model variables than proportion retained and dispersal distance, with the majority of Cohen's $\hat{f}$ estimates significantly greater than 0.25.

Higher dispersal and lowest retention rates characterized larvae released from the mouth of SGB, and NE and NW sites (Figure 5.1) pooled across treatments, relative to sites at the head of the bay, SE, and SW (Figures 5.6 and 5.7). Significantly higher dispersal distances from sites near the mouth of SGB persisted across months and behaviours, despite significant interactions (Figure 5.6; Table 5.4). We gave simulated particles vertical positions reflecting those reported in Chapter 4 (A3; Table 5.1) and, when released from the northern sites, we observed significantly larger kernels (variability in dispersal distance outcomes) than those particles characterized by passive surface distributions (NS). The opposite was true for southern release sites (Figure 5.6). Overall larvae restricted to the surface produced a much more broadly distributed dispersal kernel than those occupying deeper water strata (NS vs A3; Figure 5.7).

Larvae released in September experienced the longest pelagic duration and correspondingly the shortest dispersal distance, relative to releases in July and August

(Figures 5.7 and 5.8). Interestingly, despite a significantly shorter median dispersal duration in August (Table 5.4; Figure 5.8), dispersal distance exceeded larvae released in August, suggesting strong seasonal variation in circulation in the model domain among modelled years (2008:2011). Although average retention decreased from July through September, distributions of realized retention varied significantly between 0 and 100% and likely reflected variation among release locations (Figure 5.8) and some temporal variation within months and among years (controlled as random intercepts in mixed effects models).

Variation in horizontal diffusivity also significantly influenced dispersal metrics in the model. Dispersal distance increased and, correspondingly, the proportion of larvae retained decreased, with increasing diffusivity (Figure 5.9). Duration of dispersal generally decreased with increasing diffusivity, although variation among diffusivities was smaller than observed for dispersal distance and proportion retained (Figure 5.5). Interaction of diffusivity with behaviour was relatively less pronounced than for location and month of release (Figures 5.6- 5.8). Generally the magnitude change among diffusivities was higher than the effect of changing behaviours within a given diffusivity.

Behaviour significantly influenced larval lobster dispersal, with Cohen's $\hat{f}$ estimates exceeding 0.1 for the majority of model parameters and associated interactions (Table 5.4 and Figure 5.5). Relative to passive surface distributions, larvae assigned vertical positions and behaviours (A3) tended to exhibit shorter dispersal distances and increased probability of local retention (Figures 5.6-5.9). However, overall pelagic larval duration increased when larvae were given variable vertical positons relative to surface

distributions, as expected based on declining temperature with depth and an inverse relationship between temperature and development (Mackenzie 1988, Quinn et al. 2013). Interestingly, the influence of behaviour was inverted between July and August/September. Higher dispersal distance characterized larvae exhibiting variable vertical position in July, in contrast to larvae that exclusively occupied the surface layer, whereas the opposite was true for larvae released later in the season. Examination of dispersal distance in the context of release location and year (2008:2011) revealed a more variable but similar pattern (Figure 5.10). Higher dispersal characterized larvae that move vertically compared to those confined to the near surface across years, except in July 2008 and September of 2009, confirming annual variation in circulation previously reported for the southern Gulf of St. Lawrence (Chasse & Miller 2010). This variability contributed to the fit of each mixed model, resolving less than half of the variance inherent to the simulated dispersal (r^2; Table 5.4). However, active and passive scenarios yielded significant effects on dispersal, irrespective of whether positive or negative (76.2 km, 52.6 ± 5.7 km; median and mean ± se) (Figure 5.10).

Cohen's *d* effect size estimates also demonstrated the influence of behaviour, based on parametric estimates from mixed models, comparing vertically structured (D, A1:A3) and surface restricted (NS) behaviours (Figure 5.11) partitioned among interactions in the model. We found that behaviour produced ecologically significant effects (Cohen's d > 0.2) in most instances, especially for higher order interaction terms. Cohen's $\hat{f}$ also showed behaviour had the largest influence on the dispersal duration, however, interaction with other model parameters produced this influence given that

behaviour alone had a small, likely non-ecologically significant influence. Similarly, behaviour significantly influenced dispersal distance and proportion retained with the effect increasing, on average, with higher order model terms. Averaged across all partitioned comparisons, and pooled among active behaviours, the effect of imposing a variable or fixed vertical distribution was 0.3, 3.8, and 10.1 (sd: 0.28, 3.6, and 6.9) for the proportion retained, dispersal distance, and duration respectively. Average effects were highest for the laboratory derived behaviour (A3) compared to other vertical distributions (Figure 5.11). Not surprisingly larvae migrating dielly were more similar (smaller average effect) to larvae confined to the surface, because these individuals experienced similar circulation and thermal conditions during ontogeny.

Connectivity matrices add a spatial context to the dispersal summaries provided by analyses of dispersal distance, duration and retention. Larvae from the mouth of SGB generally dispersed the farthest, reaching settlement age in LFAs beyond adjacent management zones (LFAs 25 – 27; Figure 5.1b), whereas larvae originating at the head of SGB were more likely to remain within and around their origin (Figure 5.12), as shown in analysis of retention (Figures 5.8-5.10). Larvae released from the NE location dispersed farther and to more LFAs (Figures 5.6 and 5.12). As the larval season progressed into September, the likelihood of larvae reaching settlement age in more distant locations increased and likelihood of larval retention decreased in the area immediately surrounding SGB. Behaviour interacted with both release month and location to augment overall connectivity. Larvae confined to the surface (NS) were more likely to settle in LFAs to the west of their release location (LFAs 22, 24 and 25) than larvae with some

vertical movement. This pattern was particularly striking in August of 2011 (Figure 5.12) when larvae dispersing at the surface did not reach settlement age in LFAs east of SGB. Larvae dispersing at depth (D) were also transported primarily west of SGB, whereas larvae with laboratory-derived behaviours reached settlement age in LFAs on the northeast coast of Nova Scotia and near the Bay of Fundy (LFAs 30-33). The addition of horizontal diffusivity to this trend did not significantly augment this pattern but increased variability in settlement locations (Supplementary Figures 5.2 and 5.3).

Ontogenetic variation in behaviour among zoeal stages (I-III) also significantly influenced dispersal. Net dispersal for behavioural models with fixed larval behaviours (A1) differed significantly from those estimated for models with variable behaviour (averaged absolute values: 33.87 ± 5.4 and 40.47 ± 5.37 km, n=148; for A2 and A3 respectively). Correspondingly the estimated dispersal durations were greater for those larvae exhibiting vertical movement compared to those at the surface (4.1 ±0.52, 9.75±1.05 days; n=148; for A2 and A3 respectively). Both dispersal distance and duration yielded Cohen's *d* effect sizes exceeding 0.2 in the majority of pairwise comparisons, suggesting relatively strong discrimination and effect size for model estimates among vertical and surface behaviours (Cohen 1988). The magnitude and direction of this difference, positive or negative, varied with location and time of sampling as suggested by other analyses (e.g. Figures 5.8 and 5.11).

5.3.4 ADCP results

Behaviour significantly influenced dispersal distance in simulations using ADCP data and, like the biophysical simulations, the influence of behaviour varied in both

magnitude and extent depending on the release location in SGB (MS=11.8, 9.91, 15.42; df=4, 4, 16 and $p<0.0001$; for behaviour, location and their interaction; respectively), similar to that observed in the biophysical model. Active behaviour constrained dispersal distance for simulated larvae originating at the head of SGB (M3 and M4), compared to those originating closer to the mouth (M1, M2, and M5) where dispersal was extended, relative to larvae constrained to the surface (Figures 5.13 and 5.14). Behaviour in some cases also changed the direction of dispersal (e.g. south westerly movement for the depth and active model 3 versus north easterly dispersal for diel and surface models in Mooring 5). Models incorporating the location of the mooring and-or the interaction between mooring and behaviour produced significantly higher AIC scores and therefore performed better than models based on active behaviour alone. Overall ADCP models that incorporated vertical structure resulted in significantly different dispersal outcomes compared to those with larvae constrained to dispersal at the surface (Figure 5.13).

Variation in vertical swimming among ontogenetic stages (I-III) also influenced dispersal outcomes. Simulations that permitted vertical migration for all zoeal stages resulted in reduced dispersal compared to scenarios with variable migration behaviour. Differences among uniform and variable vertical movement varied in both magnitude and direction (Figure 5.14) among mooring locations, averaging 12.3 (±4.5) and 34.9 (±13.6) km in absolute difference between uniform behaviour (A1) and variable behaviour (A2 and A3, respectively).

In order to evaluate the relative influence of placement in vertical heterogeneous circulation versus placement in vertically heterogeneous temperature, and thus rate of

development (passive physiologically controlled), we ran the models with a fixed temperature at all depths. The average difference (pooled among moorings) for variable temperature and temperature fixed ADCP models was 39, 66, 69 and 61 % (± se, 7.0, 8.6, 7.8 and 8.5) for D, A1, A2, and A3, respectively (Table 5.1). This result suggests that variable circulation was responsible for the majority of dispersal when larvae exhibit some vertical heterogeneity. When larvae remained within a deeper depth layer, the delayed development time associated with exposure to colder temperatures increased dispersal distance. Indeed, dispersal duration differed from surface to depth scenarios by ~ 2 weeks if larvae were held at that depth for the entire larval period (mean ± sd calculated among all CTD casts; 22.5±0.61, 23.7±0.79, 27.3±2.25, 36.2 ±5.23 days; for depth layers 2.5, 7.5, 12.5, and 17.5,m respectively). Fixing larvae at 5-m depth intervals highlights the implication of horizontal and vertical variability, with greater dispersal distances associated with deeper depth strata but varying among mooring locations (Figure 5.14). Simulations using mooring data from the head of the bay often dispersed larvae in a direction opposite and over less distance relative to those closer to the mouth of the bay. Depth strata below the upper 5 m show some coherence as previously reported (Lesperance et al. 2011a, b), noting less structured surface circulation primarily driven by wind events.

5.5 Discussion

This is the first study to show how different potential realizations of larval behaviour interact with physical processes and spawning characteristics (e.g. timing and release location) to influence predicted settlement locations of larval American lobster. In

particular, we demonstrate that small-scale variation in hydrodynamic conditions (>20 km) among release locations can result in significant variation in how behaviour influences dispersal and connectivity, consistent with findings from other systems (e.g. Shanks 2009, Phelps et al. 2015), albeit at a much smaller scale. Differences in dispersal outcomes observed among behaviours ranged 10s - 100s kms, exceeding the spatial extent of many management areas for lobsters in eastern Canada.

Effective application of management and conservation strategies for exploited and-or threatened species requires a comprehensive understanding of the factors that influence the spatial dynamics and connectivity among populations of interest (Bradbury et al. 2008b, Snelgrove et al. 2008). Particularly for meroplanktonic species, the pelagic dispersal phase during the early life history strongly influences connectivity (Fiksen et al. 2007, Cowen & Sponaugle 2009, Stanley et al. 2013). The geographic scale of dispersal for larval meroplankton represents a combination of active and passive processes (Sponaugle et al. 2002), where the interaction between behaviour and the physical environment determines displacement. Despite increasing evidence that behaviour mediates dispersal, many studies fall short of incorporating relevant biological attributes into dispersal models (Metaxas & Saunders 2009), and thus potentially miss small-scale patterns of connectivity (Cowen et al. 2006). Indeed, understanding connectivity originating from the pelagic dispersing stages of meroplankton in strongly advective environments requires a balanced biological and physical approach to modelling, particularly where the objective is to evaluate the relative influence of individual model parameters on dispersal (Treml et al. 2015).

5.5.1 Behaviour

Observations of vertical distributions of larvae in the field showed declining average concentrations of each of the zoeal stages with thermocline depth. This trend was particularly strong for stages II and III which were occurred at nearly the same concentrations as stage IV. Given that we limited our sampling to the surface layer, the relationship with the water column structure provides some evidence of the vertical positioning, and presumably the behavioural range of larvae. We observed a nonsignificant relationship between thermocline depth and stage IV concentration, confirming previous reports that stage IV larvae are confined to the uppermost surface layers above the thermocline (Annis 2005). The concentrations of stages II and III declined significantly with thermocline depth, suggesting that these stages occupy deeper water layers, just as we observed in the lab (Chapter 4) and was reported in previous field studies (Harding et al. 2005). These observations coupled with a nearly equivalent, if not smaller, concentrations of intermediate compared to post-larval stage lobster suggest that stages II and III occupy deeper water layers not sampled, agreeing with the active behavioural models we used in our dispersal simulations.

The addition of vertical swimming behaviours significantly affected dispersal of larval lobster, but this effect was more variable and context specific than other factors. The weak oceanographic features of our study system (e.g. lack of consistent estuarine flow; Lesperance et al. 2011a, b), relatively limited PLD compared to other studies (e.g. Butler et al. 2011), or limitations imposed on the ability of these behaviours to result in differences given the limited vertical resolution of our physical model likely explain the

small effect size of behaviour, relative to the location and month of release. The physical model employed in our simulations increased in resolution with depth, meaning that the upper mixed zone (0-15 m), where we simulated larval vertical movement, only included 2-4 unique layers, and may have been unable to resolve important vertical features of the water column including thin-layer processes (Daigle et al. 2015). However, the ADCP derived progressive vector model provides an important contrast in this respect. The ADCP model incorporated considerably higher vertical resolution (~13 unique layers) where larval dispersal changed through ontogeny and included behaviour the thin-layer processes our larger oceanographic model potentially missed. Similar to our model output and a previous shorter-term progressive vector analysis (Lloyd et al. 2012a), ADCP simulations revealed significant interactions between the vertical movement of larvae and release location on realized dispersal distance. In all cases, vertical movement for some (A3) or all (A2 and A3) of the larval period produced dispersal outcomes that differed significantly from predictions based on passive vertical distributions. Not surprisingly the increased vertical variability in flow captured by the ADCP yielded greater differences in dispersal distances. However, both models produced similar patterns for given release locations and behaviour, and both models demonstrated a greater influence of vertical movement near the opening of SGB.

Variation in swimming behaviours among stages, as observed in previous studies (Ennis 1975a, 1986, Chapter 4), also influenced dispersal outcomes significantly. The ADCP and biophysical model both demonstrated that variation in swimming behaviour (A2 and A3) yielded different dispersal outcomes than those derived from uniform larval

behaviours (A1). This difference was particularly pronounced in the biophysical model where the variation in behaviour changed the dispersal outcome by ~ 34 and 41 km and 4 and 10 days for larvae with progressively changing diel capacities (A2) and larvae that reflected changing swimming performance with ontogeny in the laboratory (A3). These differences persist even when averaged across all release locations, months, and years, all of which represented significant sources of variability in our study.

The changes in vertical position linked to variation in circulation and duration that we documented in this study demonstrate the importance of considering ontogenetic variation in behaviour (Leis 2007, Corell et al. 2012). Indeed, simulations of larval reef fish dispersal that incorporated both passive and active phases demonstrated large effects of circulation and variation in vertical movement on larval duration and thus dispersal outcomes (James et al. 2002, Cowen et al. 2006). Similarly, surveys in the northeastern Atlantic found dynamic patterns in larval fish distributions consistent with changes in larval ontogeny, swimming capacity, fluid dynamics, and presumably active mediation of vertical and horizontal position (Stanley et al. 2012). Collectively, the results of these studies and ours highlight the need to incorporate ontogenetic variation in larval behaviour when simulating active contributions to larval transport.

Variability in dispersal itself varied as a function of behavioural rules. Smooth, relatively unimodal dispersal kernels characterized larvae that occupied deeper water layers (i.e. A1 and A3) relative to those generated from other behaviours. Similarly, Sundelof and Jonsson (2012) reported more Gaussian distributed dispersal kernels for simulated larvae that spent a higher percentage of their time in deeper water strata than

those near surface, which tended to produce multi-modal dispersal kernels. The behaviors chosen in simulations will therefore impact precision in matching model predictions to settlement indices (e.g. Incze et al. 2010) or genetic patterns (e.g. Kenchington et al. 2009, Benestan et al. 2015). Multi-model dispersal kernels, as expected of surface restricted simulated particles, would be expected to create spatially patchy settlement patterns along the coast relative to simulations that place larvae in deeper water strata (Sundelof & Jonsson 2012). This partitioning of variance among predictions, coupled with changes in the magnitude and direction of larval transport, further highlight the need to incorporate realistic behaviours.

Numerous studies have demonstrated the influence of vertical movement on dispersal of pelagic larvae, especially in systems with consistent vertical heterogeneity in flow (e.g. Fiksen et al. 2007, North et al. 2008). Timing of vertical migrations appears particularly important in systems strongly influenced by tidal forcing. For example, simulations evaluating the relative influence of vertical movement, like our study, demonstrated that incorporation of behaviour significantly influenced larval transport, but this difference depended on synchronizing movement relative to tides and the ability of larvae to migrate between vertical positons at speeds fast enough to be meaningful within a tidal cycle (Sundelof & Jonsson 2012). Our simulation supports this dependency, with relatively fast swimmers that can span depth layers within the span of a tidal cycle. However, our model triggered behaviors in response to light (diel movement) and therefore produced migrations asynchronous with tidal cycles. Available information on the response of larval lobster to tidal cycles remains scant, particularly for zoeal lobster.

Whereas stage IV lobster apparently exhibit some rheotactic response (Ennis 1986; Stanley unpublished data), there is no consensus on the ontogenetic progression of this trait, and therefore no meaningful way to parameterize the model. However, the synchronicity of stage-dependant movement relative to tidal cycles may not be particularly important in our study system. Using slower moving invertebrate larvae and the same physical model, Daigle et al. (2015) demonstrated that simulated larvae swimming with random, diel, or tidally-linked patterns produced similar connectivity patterns, suggesting that vertical migrating per se was more important than the specifics of that movement. If larval lobster exhibit tidally oriented behaviour, our model potentially underestimates the influence of swimming behaviour (Savina et al. 2010, Sundelof & Jonsson 2012), though our results and those of Daigle et al. (2015) suggest modest variability associated with timing relative to the behavioural rules we assigned in our model.

The swimming ability of larval lobsters varies as a function of temperature and natal origin (Chapter 4). However, to simplify computational resources we utilized a simplified temperature-independent behavioural model. This simplified model may smooth some of the variability in behaviour expected of larvae. Variation in larval swimming velocities, particularly for stage I, varied from 10-40%, depending on differences in temperature, natal origin, or the interaction between the two. Swimming velocity interacts with vertical placement of larvae which, in turn, modifies larval exposure to different temperature and circulation fields. Differences among active behavioural models (A1-A4) highlight this point, showing that variability in swimming

capacity among stages influenced the dispersal outcomes. However, we believe the trade-off between computational limitations and realistic behaviour is likely minimal. Observations of temperature dependence in the lab demonstrated that early and late stages (I and IV) exhibited the strongest temperature dependence, likely in part because they swam the fastest. In our biophysical model stage IV larvae moved within the upper mixed layer and therefore their swimming velocities would have little to no interaction with vertical structure in circulation. This simplification would likely alter dispersal predictions only in the first larval stage. Swimming capacity in stage I larvae in our experiments (Chapter 4) was inversely related with temperature. Assuming this observation translates to larval lobster from the southern Gulf of St. Lawrence, our model may underestimate the rate of diel vertical movement for stage I larvae, particularly later in the season when the upper mixed layer begins to cool (R.R.E. Stanley unpublished data). Given that the predicted duration of this ontogenetic stage comprises less than 20% of the PLD of a larva, this biological detail would likely contribute little variation relative to the other factors explicitly tested in our study. Overall, increasing the range of possible swimming velocities for simulated larvae would likely increase variability in our dispersal estimates but add little to estimated differences among behavioural sub-models.

We chose variation in vertical position to parameterize models and test sensitivity of simulated dispersal to different larval behaviour, based on literature and laboratory observations. Our study simulation represents the first study to scale up empirical observations of swimming behaviour, particularly of zoeal stages, into dispersal simulations. Although our parameterization offered a reasonable approximation given

limited field data (Metaxas & Saunders 2009, Miller & Morgan 2013), we nonetheless simplified field conditions. First, we fixed themocline depth at 15 m throughout the domain, which clear it oversimplifies spatial and seasonal variability in water column structure (Lesperance et al. 2011a, b; Stanley unpublished data) and empirical data on larval concentrations and water column structure. We chose a fixed depth to simplify the behavioural sub-model and maintain consistency when comparing outcomes among contrasting swimming behaviours. Our fixed behavioural limits potentially extended larval durations and reduced variability in dispersal outcomes relative to larvae limited to shallower depth layers. Dispersal outcomes correlated poorly with wind patterns in our study area (see Daigle et al. 2015), suggesting our simplification of placing larvae at deeper depths unlikely biased the magnitude of dispersal (unlike Xue et al. 2008, Incze et al. 2010), particularly given that our model resolves a course vertical gradient between 0 and 10 m. Our model also ignored parameters which defined selectivity at settlement (e.g. affinity to certain substrate types; Butman 1987, Wahle & Steneck 1992, Lillis & Snelgrove 2010), and nor did we permit late stage lobster to actively delay settlement to seek optimal habitat (sensu Katz et al. 1994). Whereas past studies demonstrate suitability of habitat at settlement as an important aspect of connectivity in larval lobster (Chasse & Miller 2010), and habitat preference has been relatively well documented (e.g. Barshaw & Rich 1997), we again chose to simplify the model to focus on pelagic dispersal rather than settlement. Similarly we ignored mortality, which varies spatially and temporally and would better suit models geared to field data. The inclusion of variable mortality,

particularly linked to depth distribution, would likely further accentuate the importance of behaviour (Chasse & Miller 2010).

5.5.2 Location and timing

The influence of vertical movement on dispersal varied spatially and temporally. We observed that overall location had the most significant effect on dispersal outcome among tested factors, despite spatial differences in release location of less than 20 km. This influence was particularly strong for release locations near the opening of SGB which were more likely to deliver larvae to distant LFAs than those near the head. Variation in circulation likely drives some among-sites differences (Chasse & Miller 2010), because difference in dispersal distance generally exceeded between-site distances. Not surprisingly, the influence of behaviour varied with release location, mirroring variation in circulation. The biophysical and ADCP model both demonstrated that vertically migrating larvae were exposed to more variable currents and, tended to differ more in transport among sites than larvae constrained to the surface. Other studies report variation in behavioural influence with location (e.g. Savina et al. 2010, Phelps et al. 2015). For example, vertical migration may extend or reduce dispersal of larval Norwegian lobster (*Nephrops norvegicus*), depending on release location, relative to surface drifters in the Irish Sea (Phelps et al. 2015).

We initially varied release locations within SGB to replicate model results rather than test an explicit model factor. However, a parallel modelling study noted significant influence of location (Daigle et al. 2015) and we therefore considered it within our statistical analysis. This inclusion proved important, because pooling among locations

greatly reduced our capacity to partition effects of other factors as their effect on dispersal, which varied in magnitude and direction among locations. Although we demonstrate variation over relatively small distances, these differences highlight the need to evaluate spatial variability in hydrodynamics when deriving estimates of population connectivity, particularly when incorporating larval behaviour.

Hindcast models by Chasse and Miller (2010) suggested that SGB sourced larvae come primarily from external areas, with only about 37% of larvae retained within the bay and the remaining larvae largely settling in the adjacent Northumberland Strait. Similarly, our analysis documents ~44% median local retention from SGB. However, our analysis also documents fine-scale partitioning among the endpoints of dispersal and the release location within SGB. We demonstrate that larvae originating at the northwesterly sites reach settlement age in the Northumberland Strait (LFAs 26a, 25) whereas larvae released from northeastern locations predominantly move towards Cape Breton (LFAs 26b, 27). This fine-scale partitioning of dispersal outcomes, and their interactions with behaviour, are important in linking spawning sources to sink locations, particularly when considering connectivity among management units.

Previous studies link timing of spawning in American lobster to temperature (Aiken & Waddy 1986), with spawning triggered when temperatures rise above 12 °C, indicative of early summer (Harding et al. 1982). Newly hatched lobster larvae emerge from June through September (Scarratt 1964; this study), and timing of spawning strongly influences dispersal potential of marine species (Mitarai et al. 2008, Siegel et al. 2008). We found that dispersal distances generally scaled negatively with ambient temperature,

where the shortest dispersal distances occurred in August, the warmest month. Transport of larvae restricted to the surface, a differed most from those integrated throughout the water column during the warmest month, reflecting water column temperatures. Not surprisingly dispersal duration also followed this trend. We found that more pronounced variability in dispersal among months for larvae with simulated dispersal limited to the surface, confirming Sundelof and Jonsson (2012) who postulated lower seasonal effects for larvae that occupy deeper layers during dispersal, given increased seasonal variability at the surface. As in our study, Treml et al. (2015) modelled a suite of biological and environmental factors that might influence dispersal and found that PLD was among the major drivers of dispersal distance. Modelling in the Gulf of Maine showed that incorporation of realistic PLDs instead of fixed time periods significantly changed predicted dispersal distance and direction (Xue et al. 2008). As in our study, these authors attributed extended PLDs to placement of larvae in deeper layers by exposing larvae to different currents that could influence dispersal outcomes (Incze et al. 2010). Studies evaluating connectivity, both genetic and demographic, among source and sink locations must consider the temporal distribution of spawning, particularly for larvae that move vertically.

5.5.3 Diffusivity

We chose three levels of diffusivity as benchmarks to reflect biophysical simulations either as fixed values or stochastically generated. While models with no diffusivity provide a null model to tease apart the relative role of each of the other model parameters, such models simplify the diffusion implicit to the system. However, we found

a relatively small effect of adding horizontal diffusivity compared to all other factors. Variable diffusion proved important only for probability of retention though the effect was not significant. Higher levels of diffusivity reduced the likelihood of local retention and, correspondingly, reduced the likelihood particles moved shorter distances. Diffusion had little effect on the duration of dispersal relative to other factors, and did not functionally alter the influence of behaviour on any of the response variables. Collectively our results show that the relative effects of release month, release location and their interactions with behaviour are robust to varying levels of diffusivity.

5.5.4 Connectivity

Our simulations reveal dispersal scales, across all behaviours and release sites (median = 90.9 ± 97.8 km), that far exceed the movement ranges expected of lobster adults over the same time period (~ 12.3 km; den Heyer et al. 2009). Larval transport therefore likely explains relatively complex spatial structure recently demonstrated through genetic analysis of lobster populations (Benestan et al. 2015), given that most exchange of individuals likely occurs at this stage (Kenchington et al. 2009). Lobsters are almost continuously distributed across their range lobsters where suitable habitat permits, but nonetheless retain some spatial structure (Kenchington et al. 2009). Biophysical dispersal models developed for the southern Gulf of St. Lawrence demonstrated some spatial structure in recruitment but with “leaky” discontinuities that defined structure (Chasse & Miller 2010) and an overall pattern in genetic connectivity fitting isolation by distance (Kenchington et al. 2009, Benestan et al. 2015). Our observation of temporal (year to year and month to month) and spatial (<20 km) influences on connectivity agree

with a leaky spatial structure, as does reduced mixing after settlement characteristic of decapod metapopulations as posed by Fogarty and Botsford (2006). Although this description varies from all tenets of a 'classic' metapopulation (e.g. Levins 1969), where populations undergo extinctions and then subsequent recolonizations from connected sub-populations (Smedbol & Wroblewski 2002, Kritzer & Sale 2004), it nonetheless provides a useful framework to model source-sink dynamics of a managed area. Importantly this framework highlights the significance of the larval phase, and the need to develop more robust biological parameters when modelling larval dispersal. Understanding patterns and variability in settlement is particularly important where the scales of management and dispersal do not align, resulting in a disconnect between conservation efforts and essential recruitment (e.g. Reiss et al. 2009, Kerr et al. 2014), as observed in our study and others (e.g. Chasse & Miller 2010). Successful application of metapopulation tools to Atlantic Canadian lobster will require accurate modelling of larval dispersal that can account for sources of variation.

5.5.5 Summary

Previous models of dispersal of American lobster enhance understanding of connectivity and settlement processes of this economically valuable species. Our study represents the first investigation to utilize a 3-dimensional coupled hydrodynamic, individual-based model designed specifically to evaluate the sensitivity of dispersal predictions to variable behavioural, spatial, temporal, and diffusive inputs for American lobster. In particular, our study is the first to integrate and focus on empirically derived ontogenetic behaviours.

Our results emphasize the need to parameterize larval behaviour in Lagrangian simulation studies. Exposure to variable circulation, both horizontal and vertical, intrinsically defines dispersal potential, but changes in pelagic larval duration associated with changes in temperature at depth also play a role. The integration of realistic estimates of larval duration with behaviour contributed to significant behavioural effects, even in a study system with variable oceanography. We highlight variable influence of larval behaviour as a function of timing of spawning and particularly with geographic location, suggesting that vertical migration effects on larval dispersal may be difficult to generalize. Nonetheless, our results demonstrate how this often overlooked and seemingly ephemeral biological attribute can measurably influence larval dispersal and connectivity. Our work contributes to the growing body of literature documenting how variability in vertical position associated with relatively small-scale larval behaviours can influence dispersal at much larger scales (e.g Miller & Morgan 2013, Moksnes et al. 2014, Phelps et al. 2015).

A robust methodology to link spawning sources and areas of settlement represents an important step in evaluating management strategies and forecasting future status of lobster populations. To date, settlement indices provide the best mechanisms to link settlement of post-larvae with landings in a given assessment area (Wahle et al. 2004, Burdett-Coutts 2011). Linkages between egg production and landings, however, remain few (notable exceptions; Xue et al. 2008, Chasse & Miller 2010, Incze et al. 2010). Dynamics of larval exchange can profoundly influence how populations respond to disturbance, thus defining their resilience to fishing pressure (Fogarty 1998), as well as

providing a basis to link conservation actions to realized benefits (e.g. recruitment; Chasse & Miller 2010). This study provides an important step towards an improved understanding of the factors that regulate larval transport, and can thus guide the development of more robust and precise models of larval dispersal in American lobster and similar meroplanktonic species.

5.6 Tables

Table 5.1 Behavioural models applied to biophysical simulations of larval behaviour. Note that we fixed diel migrations so that larvae descend at 06:00 and ascend at 18:00.

Model	Code	Ontogenetic stage(s)	Vertical constraints
Null surface model	NS	I-IV	0-2m
Depth model	D	I-III	10-15m
		IV	0-2m
Active model 1	A1	I-III	Diel migrations to 15 m
		IV	0-2m
Active model 2	A2	I	Diel migrations to 9 m
		II	Diel migrations to 12 m
		III	Diel migrations to 15 m
		IV	0-2m
Active model 3	A3	I	Diel migrations to 15 m
		II – III	10-15 m
		IV	0-2m

Table 5.2 Temperature-dependant rate of larval (I-III) and post-larval (IV) development in American lobster according to Mackenzie (1988) and Incze et al. (1997), respectively. D = duration in days, T = temperature (°C).

Ontogenetic stage(s)	**Equation**
I	$D = 851 \bullet (T - 0.84)^{-1.91}$
II	$D = 200 \bullet (T - 4.88)^{-1.47}$
III	$D = 252 \bullet (T - 5.30)^{-1.45}$
IV	$D = 703.5 \bullet (T)^{-1.26}$

Table 5.3 Results of general linear model evaluating larval density as a function of thermocline depth (TD), ontogenetic stage (S), and the interaction (TD*S).

Parameter	Df	F-value	p-value
TD	1	9.47	0.0025
S	3	12.80	<0.0001
TDxS	3	3.58	0.0154

Table 5.4 Results of mixed effects models examining the effects of vertical swimming behaviour (B), release location (L), diffusivity (D), and month of release (M) on dispersal distance, dispersal duration, and retention to within 40 km of the release location. Asterisks indicate significance, * $p < 0.001$ and ** $p < 0.0001$. F-values reported as $*10^2$ notation. Coefficient of determination values (r^2) are partitioned among model terms according to partial omega squared ($\widehat{\omega}^2$) estimates. Cohen's $\hat{f}$ values represent estimates of effect size.

		Dispersal distance $r^2 = 0.28$			Duration $r^2 = 0.40$			Retention $r^2 = 0.39$		
Term	**df**	**F**	$\hat{f}$	$\widehat{\omega}^2$	**F**	$\hat{f}$	$\widehat{\omega}^2$	**F**	$\hat{f}$	$\widehat{\omega}^2$
B	4	1.98**	0.15	0.02	3.17**	0.177	0.02	0.08*	0.20	0.02
L	3	73.2**	0.69	0.5	3.72**	0.165	0.023	2.28**	0.84	0.50
D	2	5.6**	0.13	0.03	10.7**	0.156	0.03	0.60**	0.29	0.10
M	2	66.0**	0.43	0.3	289**	0.899	0.728	1.00**	0.38	0.17
BL	12	0.39**	0.2	0.01	0.62**	0.231	0.011	0.013	0.04	0.00
BD	8	0.24**	0.1	0.005	1.33**	0.229	0.016	0.01	0.01	0.00
LD	6	1.33**	0.19	0.02	0.65**	0.134	0.008	0.14**	0.42	0.07
BM	8	0.09*	0.06	0.002	1.22**	0.219	0.015	0.01	0.00	0.00
LM	6	4.68**	0.35	0.07	2.89**	0.263	0.029	0.15**	0.42	0.07
DM	4	0.50**	0.08	0.005	6.79**	0.272	0.046	0.009	0.00	0.00
BLD	24	0.06**	0.14	0.003	0.20**	0.247	0.001	0.003	0.00	0.02
BLM	24	0.17**	0.25	0.01	0.35**	0.358	0.013	0.009	0.00	0.00
BDM	16	0.17**	0.17	0.006	1.22**	0.465	0.034	0.004	0.00	0.01
LDM	12	0.28**	0.17	0.008	0.38**	0.191	0.008	0.008	0.00	0.00
BLDM	48	0.04**	0.24	0.004	0.17**	0.512	0.014	0.002	0.00	0.04

5.7 Figures

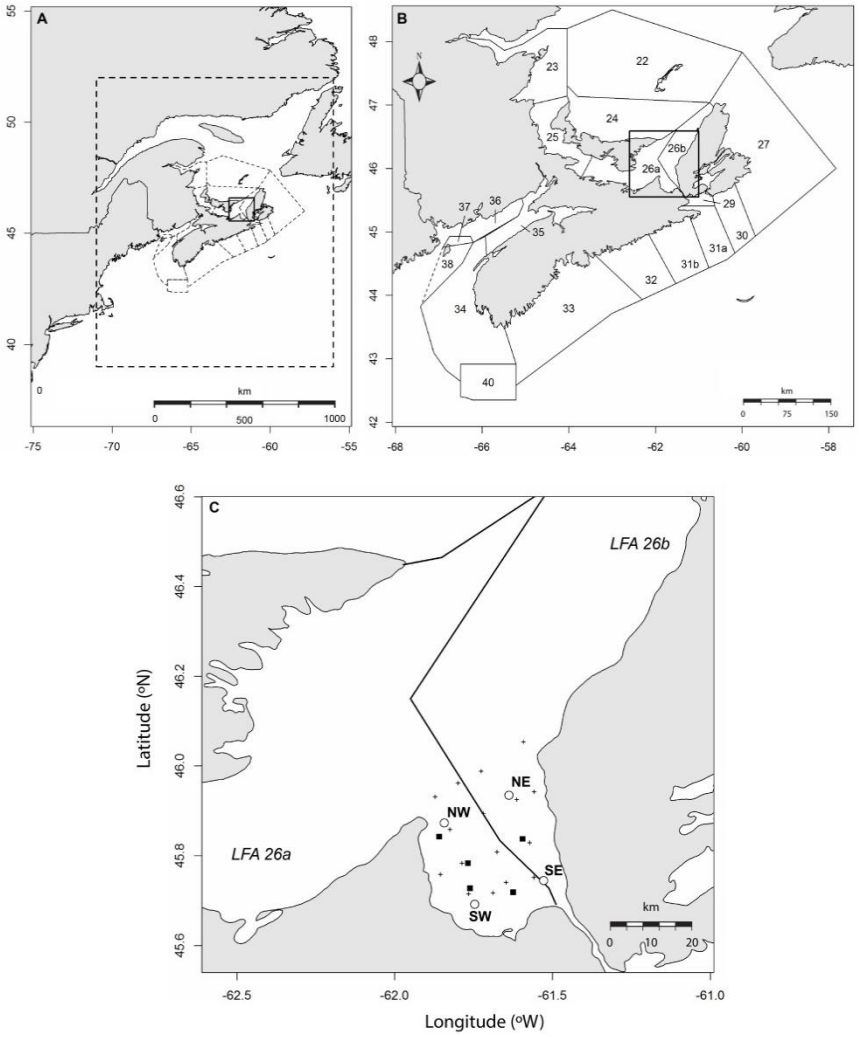

Figure 5.1 Maps depicting the: A) model domain (dotted line) B) Lobster fishing areas in which we simulated larval release and C) simulated larval release sites (○), sample locations (+), and the four corners of St. George's Bay (SE, SW, NE, NW). Mooring locations (▪) denote moorings M1 – M5 counter clockwise from the northwestern most mooring location. Note panel C denotes a zoomed version of panels A and B.

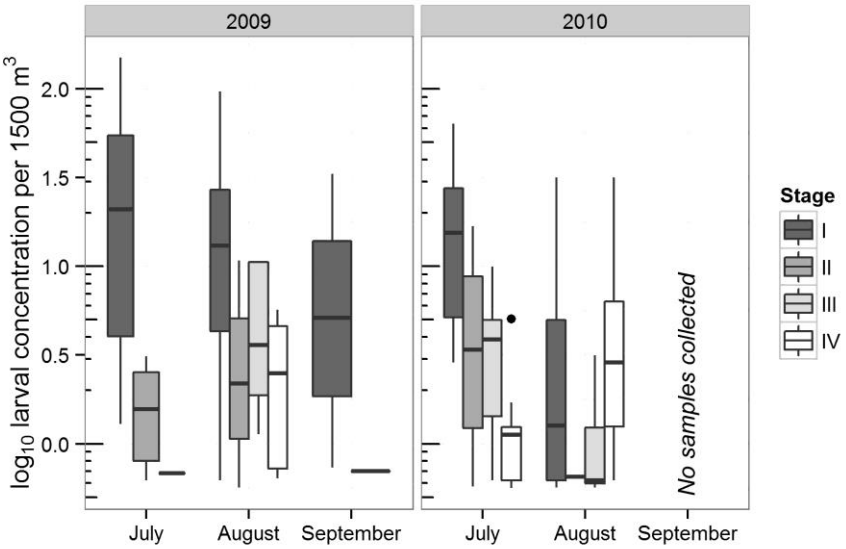

Figure 5.2 Distribution of catch of different larval stages (I-IV) pooled among neuston surveys in St. George's Bay, 2009-2010. Larvae were not sampled in September of 2010. Refer to statistics section for description of boxplot visualization.

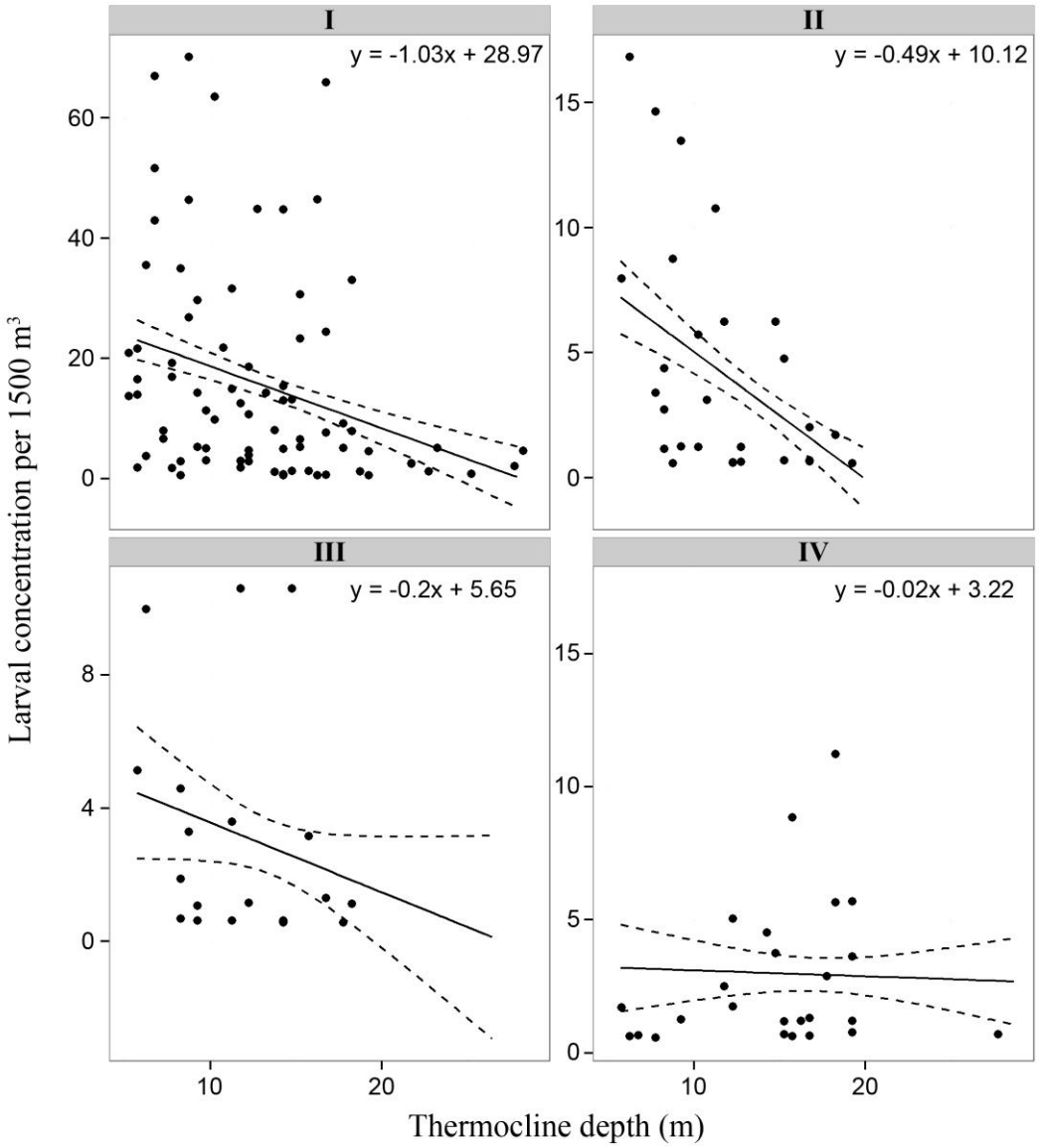

Figure 5.3 Relationship between larval concentration and thermocline depth for each of four larval stages (I-IV). Lines represent fits from GLS model (± se) showing the general relationship with depth. Data pooled among sample years (2009-2010) and standardized to a sample size of 1500 m^3. Model parameters included in each panel and in Supplementary table 5.1. Note scales of panel are set relative to the stage specific data.

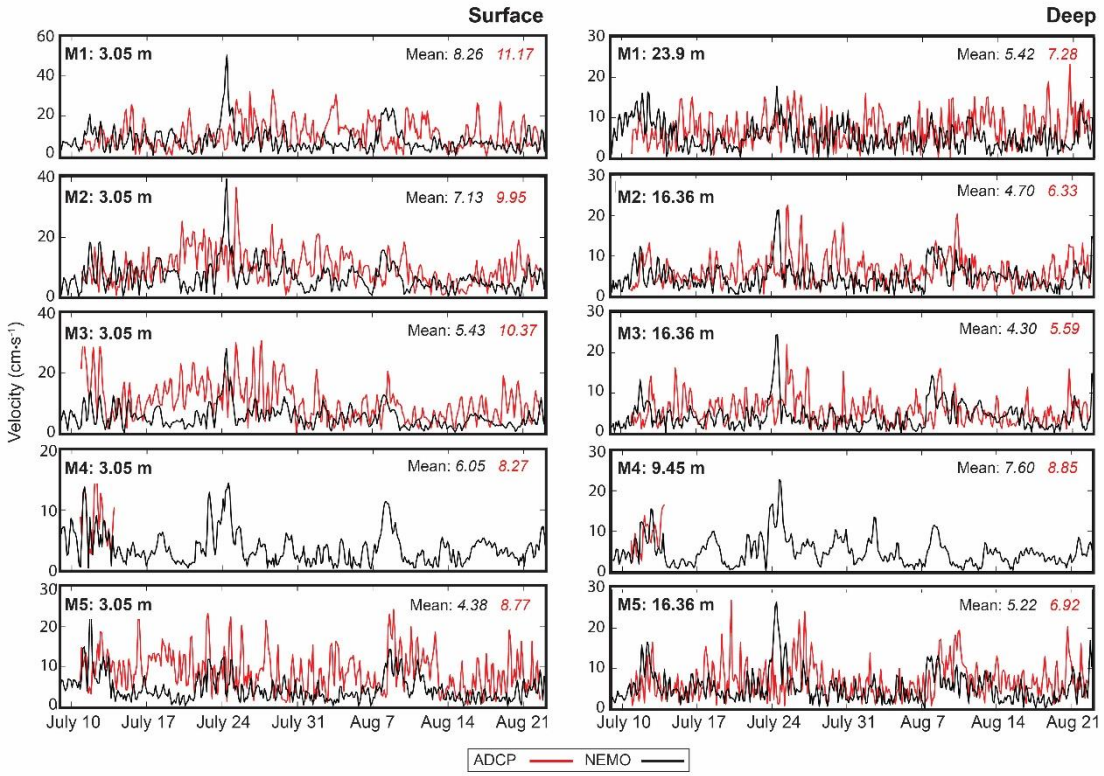

Figure 5.4 Time-series of ADCP measured current amplitude (red) and NEMO modelled current amplitude (black) for the surface and deep depth layers of each mooring. Depth of comparison reported for each mooring and means reported in $cm \cdot s^{-1}$. Data presented is from 2009 and originally reported in Daigle et al. (2015).

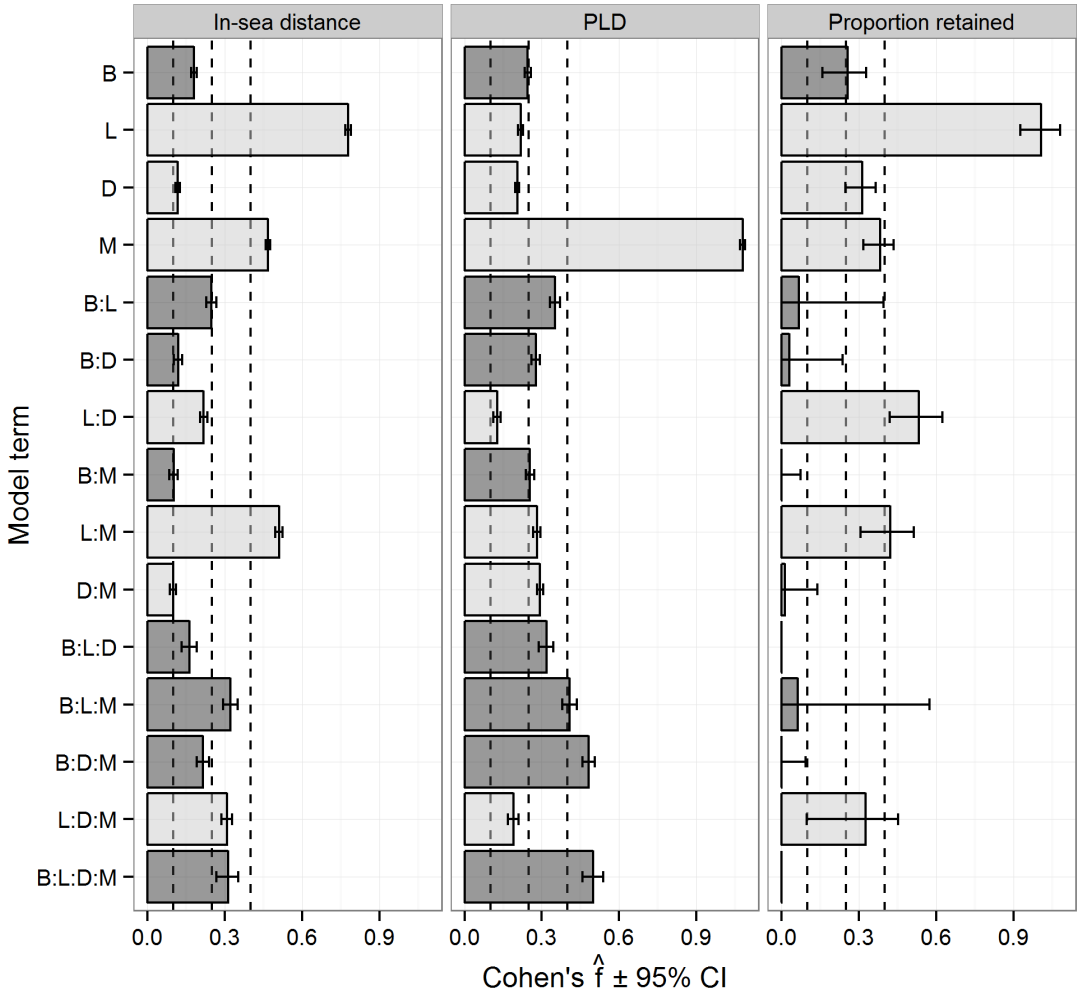

Figure 5.5 Effect sizes for each term in linear mixed effects models examining the influence of behaviour (B), location (L), diffusion (D), and month of release (M) on dispersal distance, distance from shore, and proportion of larvae retained (within 40 km of starting point). Dashed lines represent small, medium and large effects (Cohen's $\hat{f}$ = 0.1,0.25, and 0.35 respectively), (Cohen 2013), bars denote means, and horizontal lines denote 95% CI. Shaded bars represent terms that include behaviour. Colons (:) denote interactions.

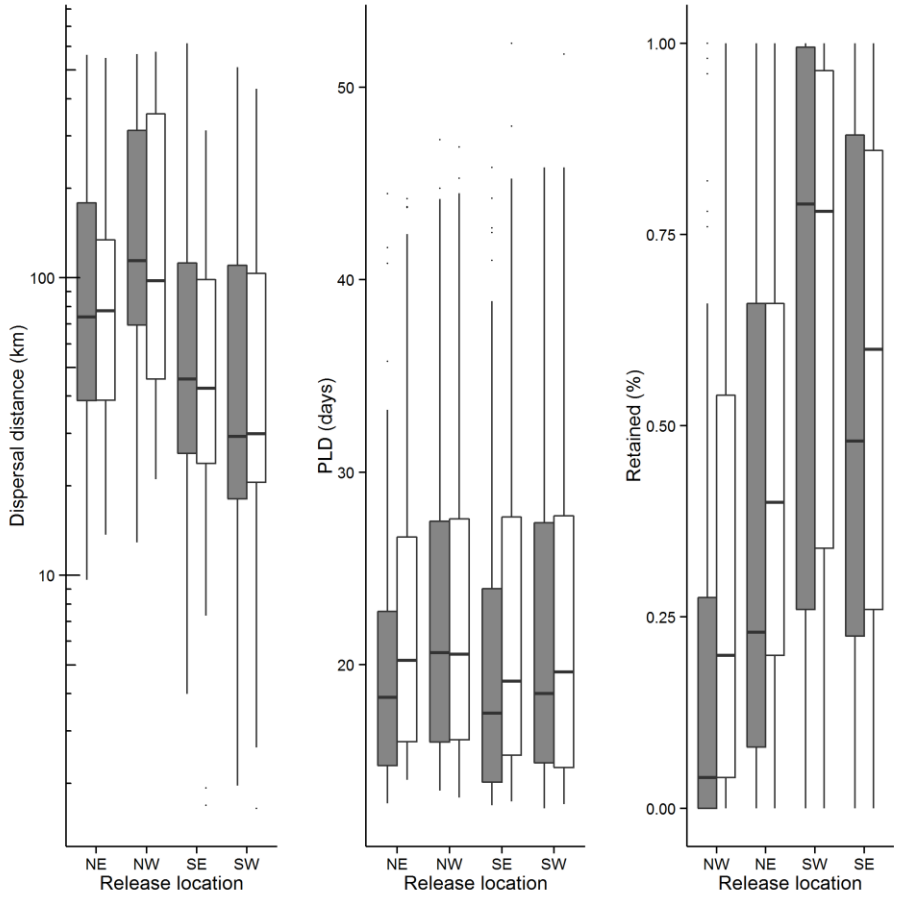

Figure 5.6 Summary of model simulations of larval dispersal as a function of release location. Data incorporates passive (grey) and laboratory derived (white) behavioural rules (NS and A3 respectively; Table 5.1), all release dates, and no diffusivity. Note that dispersal distance is plotted on a $\log_{10}$ scale. Refer to statistics methods for description of boxplot visualization.

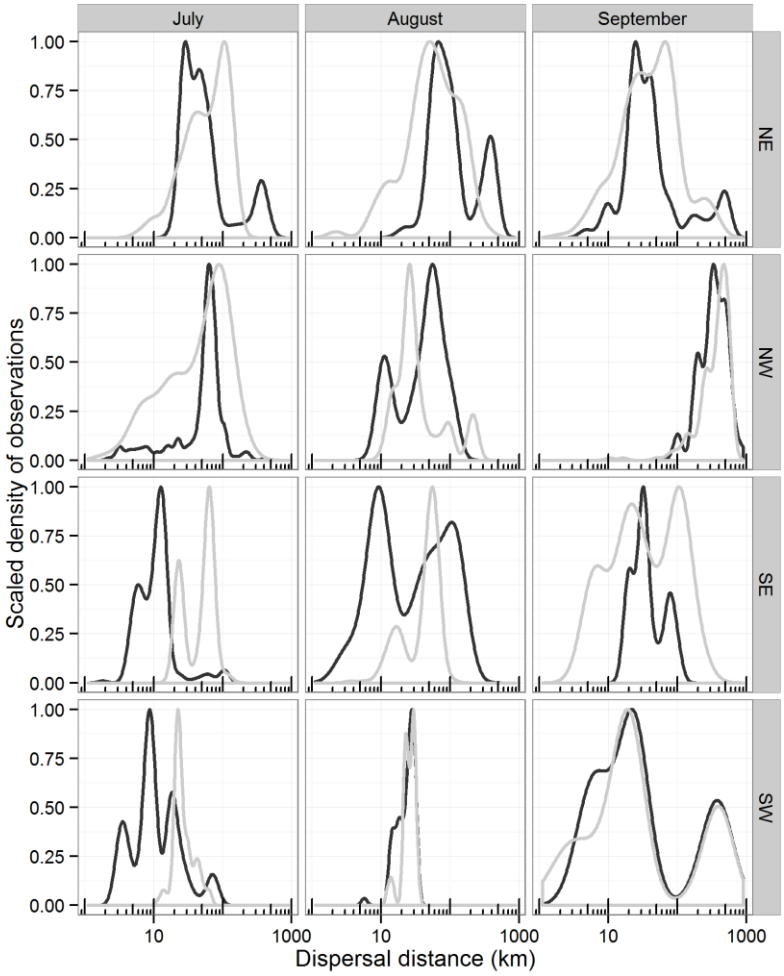

Figure 5.7 Dispersal kernels partitioned to the month and site of origin among passive surface (NS; black line) and active vertical (A3; grey line) behaviours (Table 5.1). Kernels were derived by pooling the day and year of simulated release with no diffusivity.

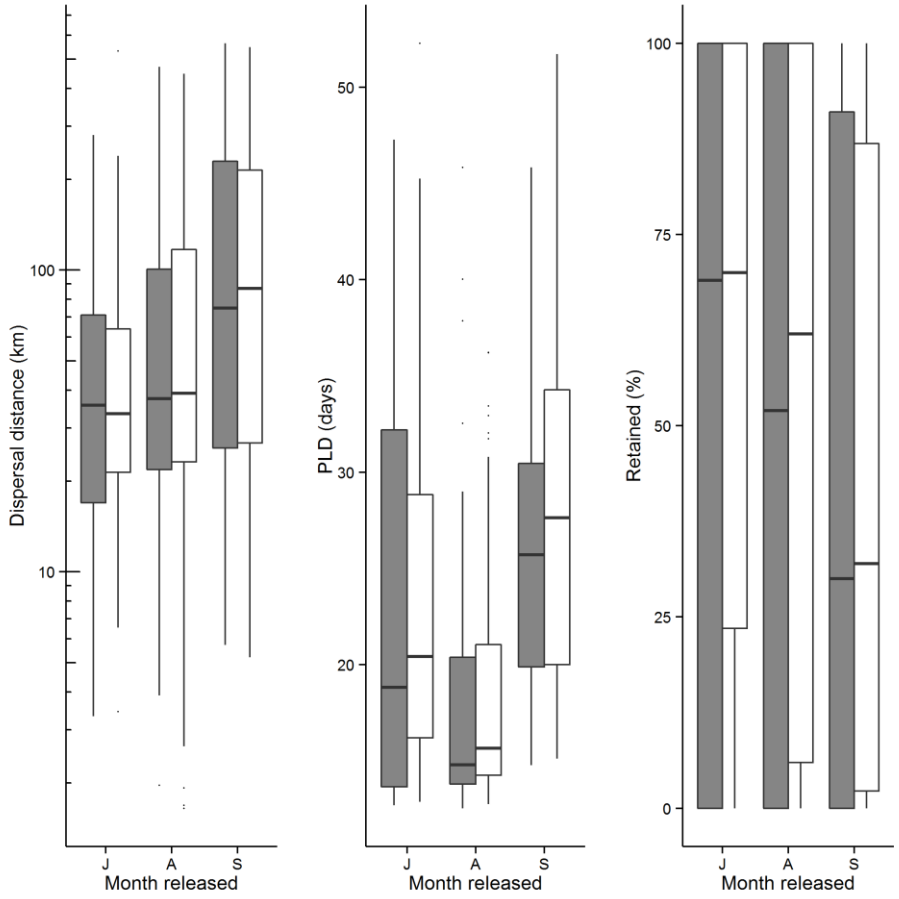

Figure 5.8 Summary of model simulations of larval dispersal as a function of the month of release; July (J), August (A) and September (S). Data includes passive surface (grey) and laboratory derived (white) behavioural rules (NS and A3 respectively; Table 5.1), all release days and years associated with each month and no diffusivity. Note that dispersal distance is plotted on a $\log_{10}$ scale. Refer to statistics methods for description of boxplot visualization.

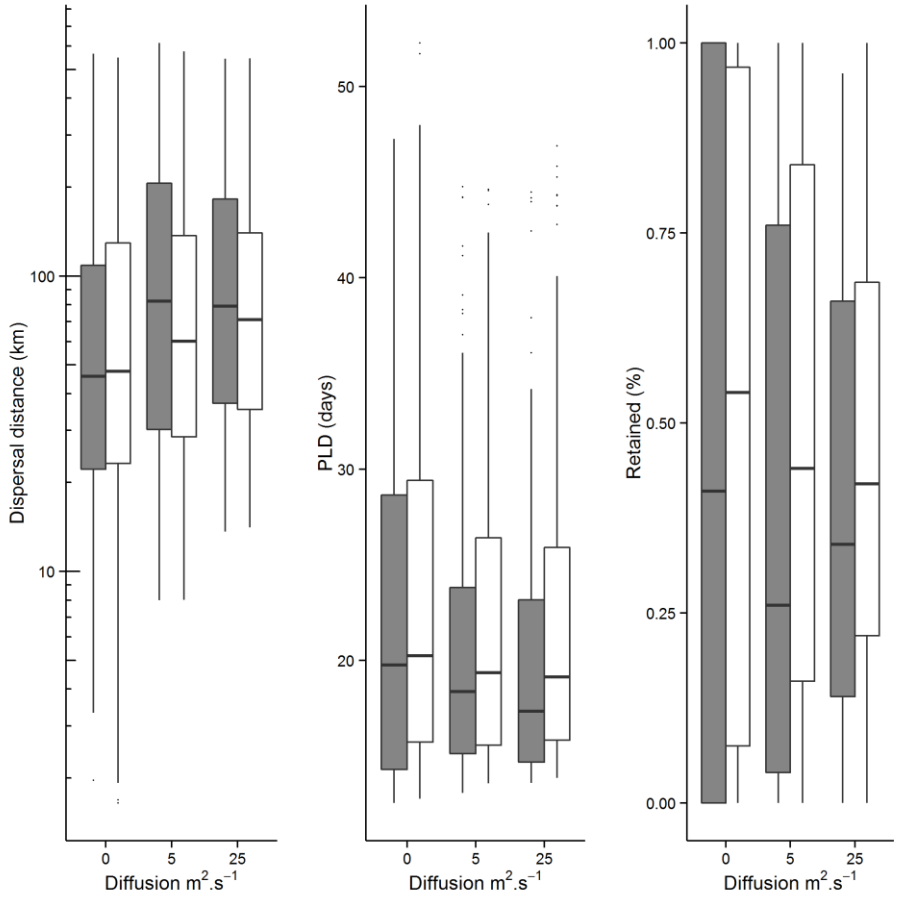

Figure 5.9 Summary of simulated larval dispersal as a function of model diffusivity. Data include passive surface (grey) and laboratory derived (white) behavioural rules (NS and A3 respectively; Table 5.1) and all release dates. Note that dispersal distance is plotted on a $\log_{10}$ scale. Refer to statistics methods for description of boxplot visualization.

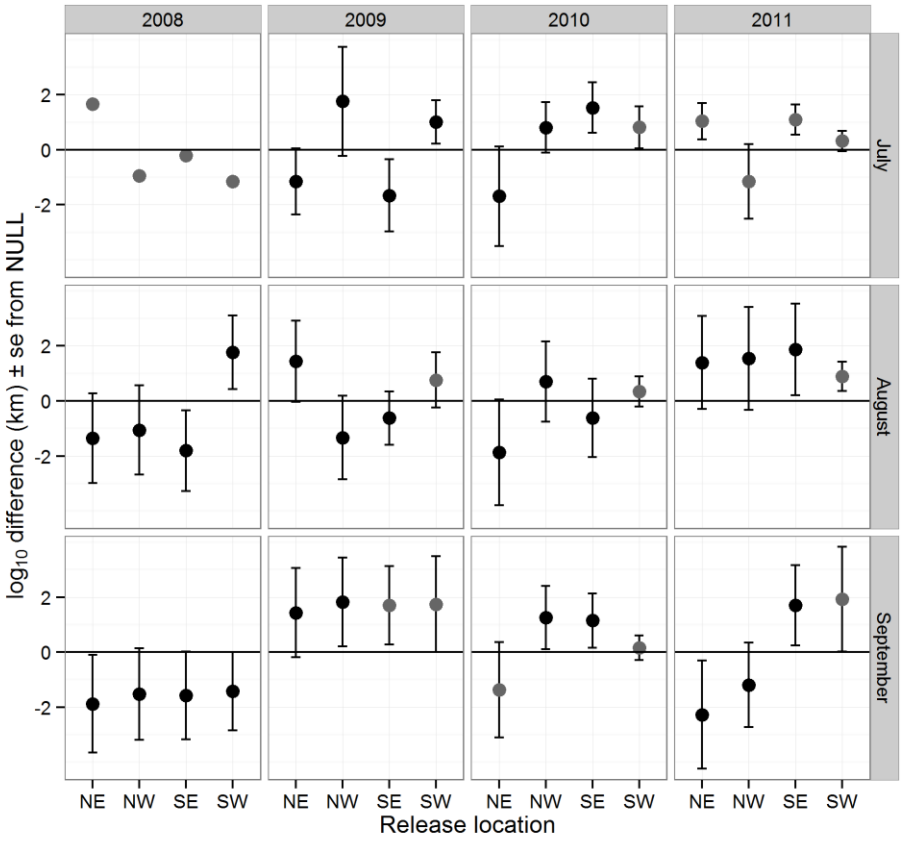

Figure 5.10 Difference between mean dispersal distance estimated for passive surface (NS) and active vertical (A3) movement (Table 5.1) with no horizontal diffusivity. Positive values denote realized dispersal further for larvae exhibiting vertical behaviour. Grey data points represent differences not significantly different than zero (NS = A3). Data aggregated by year month and release location and pooled among sampling days. Note differences are presented on a log scale and multiplied by -1 for instances where dispersal was farther for particles with no active behaviour. Circles denote means and bars denote 1 SE.

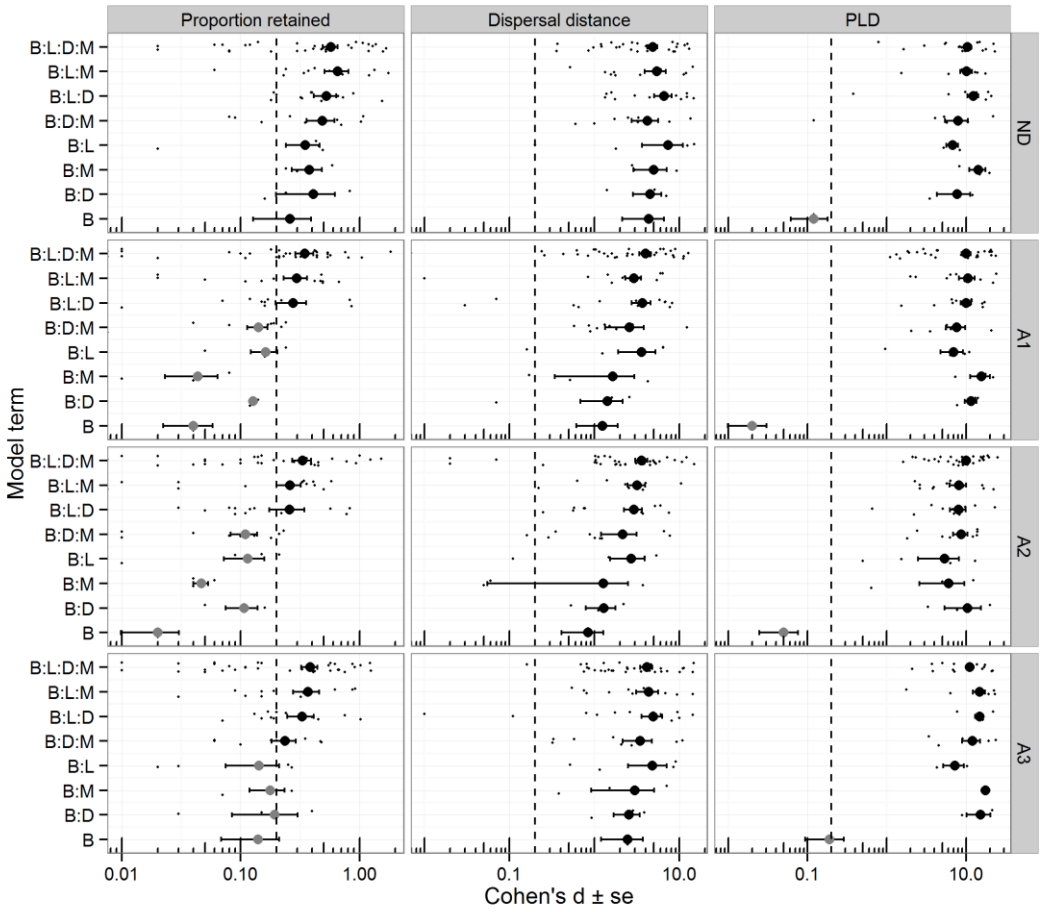

Figure 5.11 Effect sizes (Cohen's *d*) for each larval behaviour partitioned among model term variables relative to the static surface distributions, examining the influence of behaviour (B), location (L), diffusion (D) and the month of release (M) on the dispersal distance, distance from shore and pelagic larval. Dashed lines represent the threshold for a significant effect size (0.2), small points represent estimates of Cohen's *d*, and solid points represent the mean effect size for a given model term and behaviour. Mean effect sizes less than 0.2 are shown in grey. Note x axis is presented on individual $\log_{10}$ scales for each facet. Circles denote means, lines denote 95% CI and points denote individual data values.

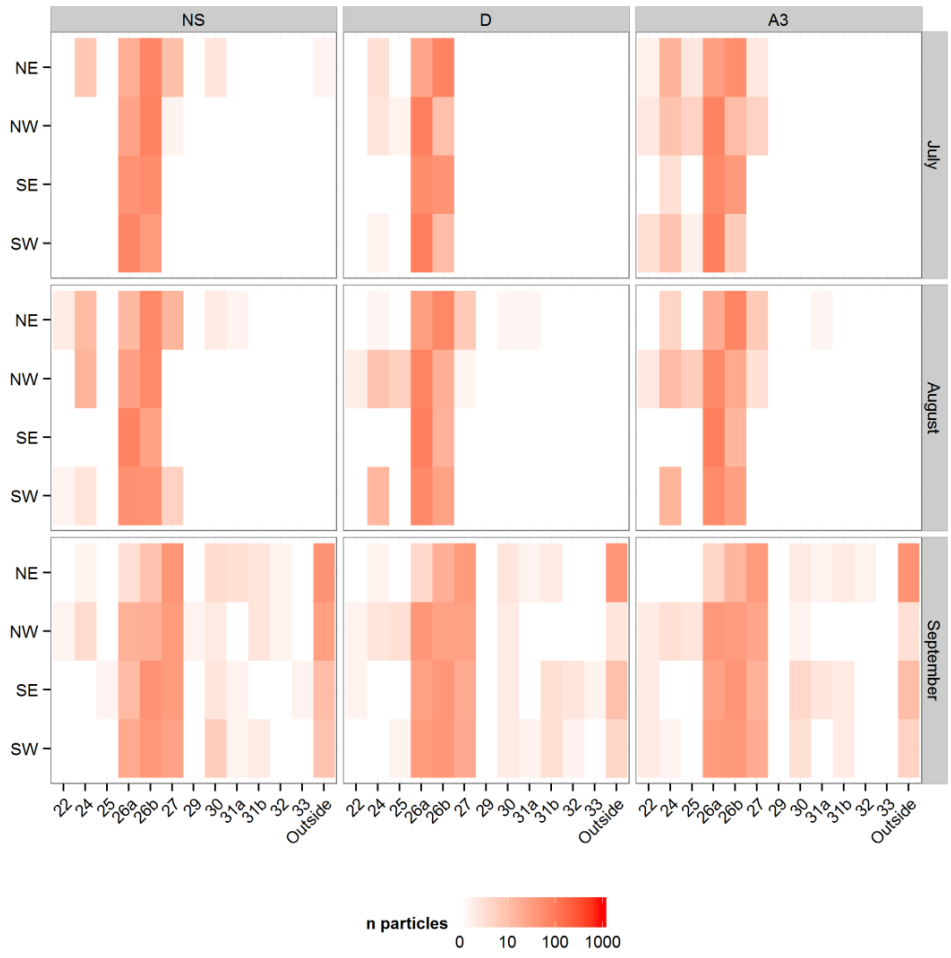

Figure 5.12 Connectivity matrices summarising the dispersal of larvae exhibiting surface passive (NS), depth passive (D), and laboratory derived vertical structure (A3) behaviours (Table 5.1). Release locations (y axis) represent start points and the corresponding LFAs (x axis) define where larvae achieve settlement age (See Figure 5.1b). Fill colours correspond to the number of larvae settling in each LFA of a possible 1000 simulations for each release location. Each matrix represents the dispersal outcome for larvae dispersing with no horizontal diffusivity and aggregated to 2011. Note that the gradient fill of the plot corresponds to a $\log_{10}$ scale.

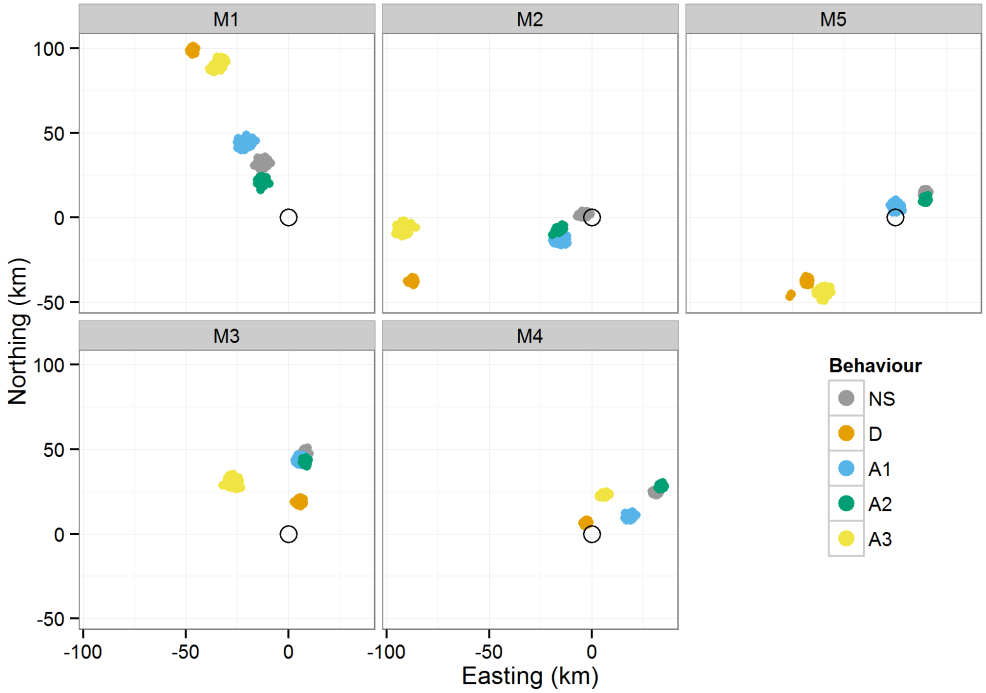

Figure 5.13 Endpoints of simulated dispersal events based on ADCP data. Points are rastered on a Cartesian plane relative to the release location at each mooring point denoted by '○'. Behavioural rules correspond to Table 1 and mooring locations are located on Figure 5.1c.

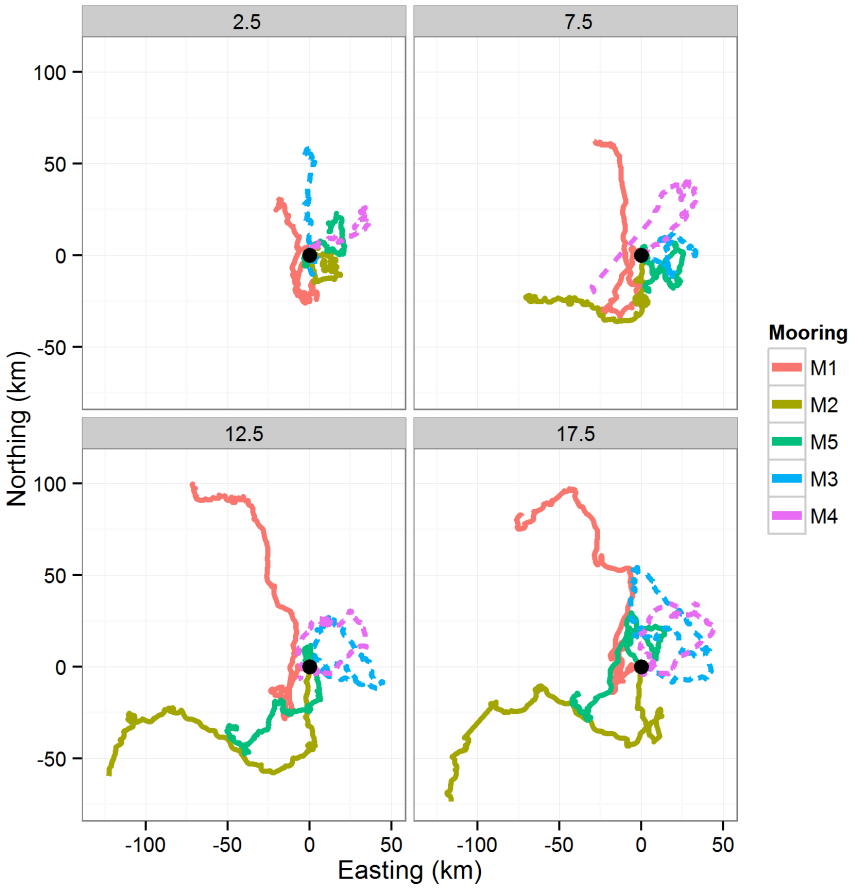

Figure 5.14 Simulations of dispersal at set depth strata (panels denoting depth in m) with variable temperature according to CTD casts. Note dashed lines correspond to mooring locations at the head of St. George's Bay and the solid lines correspond to those closer to the mouth. All simulations are rastered on a Cartesian plane at a common starting point of 0,0.

Chapter 6 –Conclusions and future directions

6.1 General conclusions

Connectivity of marine populations and the degree to which they are open or closed has important implications for the ecology and management of commercially harvested species (Cowen et al. 2000). In contrast to success in use of artificial tagging techniques for measuring spatial dynamics of adult life histories, size constraints and elevated mortalities limit the application of these techniques to earlier life history stages. Indeed, difficultly in reliably quantifying connectivity during the early life history remains a significant hurdle in evaluating spatial-temporal dynamics of marine systems (Thorrold et al. 2002). Sustainable management of marine species requires a broad focus that incorporates the spatial-temporal structure of the exploited population(s) though the complete life history. An incomplete evaluation of connectivity brings into question the capacity of management practices to support sustainable exploitation (Begg et al. 1999, Kerr et al. 2014). This limitation applies particularly to meroplanktonic of species in which larval dispersal and recruitment largely control spatial dynamics (Botsford et al. 1994), but population assessment is based on landings of adults.

Advances in the application of biophysical simulations and geochemical tagging have significantly improved the ability of marine ecologists to model spatial dynamics of larval dispersal and subsequent recruitment to the benthos. These tools represent an important step to resolving the key demographic linkages that define spatial structure in marine populations. Combined with genetics, these approaches represents a powerful

alternative to artificial tagging when identifying putative source-sink dynamics of marine species (Thorrold et al. 2002).

The precision and utility of any approach scales with the lowest resolution parameter and accuracy of any assumptions. Utilizing connectivity tools without an understanding of the complexity of the system they describe can potentially lead to unrealistic representations of spatial-temporal dynamics, and thus misinform management (Reiss et al. 2009). This principle formed the core of my doctoral research. Specifically I utilized laboratory experiments to calibrate the design and guide the interpretation of both otolith fingerprinting and biophysical models. Through field surveys and simulations I tested the sensitivity of each approach to laboratory calibrations and variation in the implied assumptions.

6.1.1 Otolith chemistry

In Chapter 2, I demonstrated that composition of juvenile cod otoliths responded predictably to variation in salinity and temperature fish would experience in situ. This chapter provides a comprehensive assessment of temperature and salinity effects on a substantial suite of both elemental and isotopic constituents in juvenile Atlantic cod otoliths. To date, this study evaluates the largest suite of otolith constituents in Atlantic cod as a function of environmental variability. Temperature and salinity both significantly affected all elements and isotopes measured except magnesium, which was apparently incorporated into otoliths independent of salinity. Importantly I demonstrated significant interactive effects between temperature and salinity on otolith geochemistry for the majority of constituents measured. Significant interactions suggest caution in using

otolith chemistry to reconstruct the history of a single environmental variable (e.g. temperature; Hoie et al. 2004), unless using a constituent primarily influenced by only one factor (e.g. magnesium as a function of temperature).

Otolith chemistry presents an important opportunity to explore spatial and environmental histories of fish, providing insight into historical spatial variation and a potential link between adult and juvenile habitat. However, the applicability of otolith chemistry to provide retrospective information is predicated on an explicit understanding of how temperature and salinity jointly influence otolith composition (Elsdon and Gillanders 2002). Chapter 2 provides the necessary information to guide the use of retrospective analysis of otolith chemistry in Atlantic cod, and to date is the ***only*** study to explore the influence of temperature and salinity on multiple otolith constituents in tandem. Collectively the results of Chapter 2 highlight areas of opportunity and limitation when using otolith geochemistry to reconstruct environmental histories and discriminating natal habitats in Atlantic cod.

Chapter 3 builds on Chapter 2 with a series of field surveys of juvenile cod from five large embayments in eastern Newfoundland. Surveys were designed to be synoptic, targeting all available eelgrass habitats using published records of juvenile abundance in nearshore Newfoundland (Methven et al. 1998). From this baseline, I determined the scale (10s to 100s km) at which otolith chemistry effectively differentiates natal habitats of juvenile cod. Discrimination success was generally low at the scale of sample site, but varied among sites (26-58% for sites with more than 15 fish sampled). Discrimination improved when sites were clustered at larger spatial scales, particularly at the largest scale

where ~78% of samples assigned successfully to the correct coast of origin, partitioned as north and south of the Avalon Peninsula. These results demonstrated that otolith chemistry varied sufficiently to discriminate at a spatial scale much smaller than previously reported. Using different subsets and combinations of capture locations, I demonstrated how variation within the scale of an embayment can mediate successful discrimination. Assignment success decreased when more sites were added to the assignment analysis. Using a predictive model derived from laboratory experiments in Chapter 2 and environmental measurements from the field I was able to develop a otolith Null composition for each sample site. Using residuals from the observed composition and that predicted by the model I was able to demonstrate the presence of a residual signal in otolith chemistry, likely reflecting the underlying complexity of the system (water chemistry, diet, etc.), that was diluted by environmental similarities among assignment scales (e.g. site or bay).

Results from Chapter 3 provide the first fine-scale (<100 km) baseline for juvenile Atlantic cod otolith chemistry in the Northwest Atlantic, and likely represents one of the highest spatial resolution datasets available for the species. Using this data, I demonstrate that otolith chemistry varies sufficiently to differentiate natal coasts and, in some cases, bay of origin at scales much finer than previously documented. This fine-scale variation could provide important insight into the composition of adult groupings (e.g. diversity of natal habitats among grouped individuals), highlighting the complexity at which adult spatial patterns arise and potentially providing direct links to specific natal environments. Importantly my work demonstrates that environmental variability within a spatial

grouping can significantly reduce the power of discrimination analyses, a consideration often not addressed by assignment studies. I suggest that while otolith chemistry can provide novel information, inferences drawn from assignment-based analyses must consider the scale of environmental variation and the resolution of sampling when collecting baseline data. .

Previous studies demonstrate sufficient variation in otolith chemistry to discriminate among adult sub-population units in northwestern Atlantic cod at large (>100 km) spatial scales (e.g. Campana et al. 1994, D'Avignon & Rose 2013). At these large scales, modest environmental variation within spatial groupings relative to other sources of variation likely drives discriminatory power (e.g. genetic, diet, and water chemistry differences). However, such large-scale comparisons do not address a pressing need to link adult spawning groups to juvenile and subsequent adult life histories. Some 44% of all known Atlantic cod spawning groups reside in small inshore groupings (Robichaud & Rose 2004) and larval dispersal originating from inshore spawning likely occurs at smaller scale than previous studies of otolith chemistry indicated (Bradbury et al. 2000, Stanley et al. 2013). Collectively, Chapters 2 and 3 provide insight into the application of otolith chemistry in evaluating the spatial dynamics of juvenile cod, and the scales at which researchers or managers could use retrospective otolith analysis to delineate spatial patterns of juvenile cod. Chapter 3 also highlights issues with under-sampling when measuring assignment power and demonstrates how increased sampling coverage can decrease assignment success. These results provide new baseline information on necessary considerations for successful application of otolith

fingerprinting when evaluating connectivity among nursery areas and exploited adult cod stocks.

6.1.2 Biophysical modelling

Chapter 4 focused on measuring swimming capacity and behaviour in larval American lobster in response to temperature and light cues. Although numerous previous studies documented larval behaviour (Templeman 1936, Ennis 1975a, 1986, Rooney & Cobb 1991) in American lobster, no previous study had directly quantified lobster larval swimming, nor had any study measured ontogenetic, biogeographic, or maternal origin as a significant source of variation. My experiments demonstrated that larval lobster swimming does not progressively improve, as previous studies suggested for American (Ennis 1986) and European (Schmalenbach & Buchholz 2010) lobster. I found a notable reduction in capacity of intermediate larval stages in both horizontal and vertical swimming trails. My results show that larval lobster swimming capability exceeds many other invertebrates (Chia et al. 1984), achieving swimming velocities of $cm \cdot s^{-1}$ at all stages. Although my observations of decreased swimming capacity in intermediate larval developmental stages contradict previous indirect laboratory observations based on position in flow (Ennis 1986, Schmalenbach and Buchholz 2010), they better match observations from field surveys that report lower densities of intermediate stages than might be expected given abundances of other larval stages (Scarratt 1964, Harding et al. 1987, Harding et al. 2005; Chapter 5).

Chapter 4 demonstrated biogeographic variation in swimming capacity of larval lobster, the first documentation of such variation for this species. Through a common

garden experiment, I demonstrated that swimming performance under different temperatures reflected environmental conditions of the natal site. In both vertical and horizontal swimming trials, larvae performed better in temperatures similar to those typical of their natal habitat, drawing analogies to cogradient variation (Marcil et al. 2006). This variation in swimming traits was pronounced (25-43% difference), despite relatively close proximity of the ports of origin (<800 km) that were previously considered a single genetic grouping (Kenchington et al. 2009). Recent genetic surveys suggest significantly more genetic structure in Atlantic Canada (Benestan et al. 2015), than previously reported (Kenchington et al. 2009). The observed variation in swimming traits appears to support these new findings. Given that the larval dispersal stage likely contributes most to connectivity in American lobster (Kenchington et al. 2009), the behavioural capacity of larval lobster could play a significant role in the dispersal-recruitment process (Pezzack et al. 1992, Incze et al. 2010) and thus any realized genetic structure.

Collectively, Chapter 4 provides the most comprehensive assessment of the movement ecology of the pelagic larval stages of American lobster to date. Importantly, Chapter 4 is the only study to provide direct measurements of larval lobster swimming capacity through individual-based tracking algorithms. Given that flow rate will vary depending on vertical position in the flume (Lillis and Snelgrove 2010), and that larvae tend to swim to the bottom in flow (R. Stanley *unpublished data*), observations from flume-based studies without three dimensional tracking (e.g. Ennis 1986, Schmalenbach and Buchholz 2010) are prone to inaccurate estimations of the flow conditions

experienced by the larvae. This error would be particularly pronounced for the intermediate stages, which seek deeper distributions and therefore likely experience boundary conditions and slower flow rates (Lillis and Snelgrove 2010). In this respect, data and methods outlined in Chapter 4 mark an important improvement to our understanding and ability to resolve larval American lobster behaviour.

In Chapter 5 I derived behavioural sub-models based on behavioural observations in Chapter 2, which I incorporated into a 3-dimensional hydrographic model. I then used this combined biophysical model to evaluate how observed larval behaviour could influence dispersal outcomes relative to passive representations of behaviour used in previous dispersal simulations (Katz et al. 1994, Chasse & Miller 2010, Incze et al. 2010). I found that the addition of vertical movement significantly influenced pelagic larval duration, dispersal distance, and settlement location[3]. This influence varied, however, resulting in different outcomes depending on the time (year and month) and location of larval release. In particular I demonstrated that release locations ~20 km apart resulted in significantly divergent dispersal outcomes. Like Chapter 3, this result highlights the importance of resolving the fine-scale variability in a system when drawing conclusions on connectivity.

Larval dispersal is a product of the interaction between larval behaviour and the oceanographic conditions experienced by larvae (Metaxas and Saunders 2009). For American lobster, the lack of data has limited the integration of behaviour into

[3] Location where larvae reached settlement age.

biophysical models and correspondingly has potentially limited accuracy (e.g. Pezzack 1992, Incze et al. 2010). Chapter 5 provides the first integration of larval behaviour for each pelagic larval stage into a dispersal simulation of American lobster (*sensu* Butler et al. 2008). In this chapter, I demonstrate that behaviour has a clearly influences dispersal and pelagic larval duration (10-100 km and 4-10 days, relative to passive behaviour respectively), and that this influence itself, reflecting spatial-temporal variation in circulation. In addition, I provide a framework for the development and integration of behaviour into biophysical models based on *in-situ* observations in the lab (Chapter 4). Collectively, Chapter 5 provides an important resource when interpreting results of dispersal simulations and developing behavioural parameters, highlighting the potential tradeoffs among computational resources, accuracy, and biological detail.

American lobster represent an extremely lucrative fishery for Atlantic Canada, and despite reports of record landings of lobster through the majority of its range over the past decade (Steneck & Wahle 2013), the spatial and temporal dynamics of the species remain relatively poorly understood. Indeed, previous dispersal simulations documented significant overlap in dispersal and recruitment among management zones in the Southern Gulf of St. Lawrence (Chasse & Miller 2010), suggesting a decoupling between egg conservation efforts and realized recruitment. Mismatches between the scales of management response and biological processes threaten sustainability in the face of environmental change and high exploitation rates (Begg et al. 1999, Reiss et al. 2009) observed across the lobster's range (Fogarty & Idoine 1986). Collectivity Chapters 4 and 5 provide the first comprehensive assessment of the movement ecology of larval American lobsters and its potential mediating influence on lobster dispersal and

connectivity. These chapters provide the baseline to incorporate behaviour in biophysical dispersal models of larval lobster. Inclusion of behaviour is particularly important for evaluating dispersal-recruitment processes at small scales as well as defining the influence of variable mortality (sensu Chasse & Miller 2010), noting the relationship between behaviour and larval duration.

6.1.3 Summary

In conclusion, my thesis bridges the divide between laboratory based experiments and field applications of connectivity tools. By improving aspects of design or challenging assumptions each chapter provides a template for future use of otolith chemistry and biophysical modelling, and also punctuates the need to calibrate and understand the assumptions of applying each strategy to dynamic field conditions (Figure 6.1). Both Atlantic cod and American lobster are important species both culturally and economically to Atlantic Canada. The ability of management frameworks to adapt to continued changes in community composition (e.g. Thompson et al. *submitted*) and environment (e.g. Pershing et al. 2015) expected in the northeastern Atlantic will require a comprehensive understanding of connectivity, and the underlying factors that influence its persistence. With these tools and this information managers can make more informed decisions about how best to apply spatially explicit management such as management units or protected areas, in addition to optimizing use of source-sink dynamics for other management decisions.

6.2 Future directions

Recent studies document increased abundances of Atlantic cod on the Scotian shelf (Swain & Mohn 2012) and the northeastern Newfoundland shelf (DFO 2013b). This

recovery parallels a concomitant rebound in the groundfish community to a composition similar to that documented before the cessation of the groudfish fisheries in the early 1990s (Thompson et al. under review). Out migration or larval recruitment from inshore spawning groups will likely contribute to any recovery of offshore Atlantic cod (Snelgrove et al. 2008, Stanley et al. 2013). Therefore, the ability to partition natal origins at the scales demonstrated in our analysis could potentially help in identifying sensitive nursery habitats and spawning groups that contributed to the continued recovery of the groudfish stocks. Results from Chapters 2 and 3 provide baseline information on variability in otolith chemistry as well as a framework for sampling strategies at scales less than 100 km.

Fisheries and Oceans Canada has identified marine protected areas as a priority conservation strategy for Canadian oceans (Canada 2005). For MPAs, effective design requires knowledge of spatial distribution of production (Ludford et al. 2012, Stanley et al. 2015a), however, establishing putative sources and sinks requires understanding underlying variability in the system first (Chen et al. 2014). This issue was particularly important and complex when evaluating the conservation benefit of the lobster MPA in Eastport, NL (Janes 2009, DFO 2014a). The Eastport MPA was designed as a tool to supplement local stocks by protecting an area known to support spawning females. Though monitoring to date has documented complex demographic trajectories within the MPAs relative to outside areas (Rowe 2002, Janes 2009), no operational model exists to predict where larvae from this surplus production from the MPA (Ennis et al. 2003) will settle and eventually recruit to the fishery. This gap in information limits the ability of

any monitoring program to discern whether the MPA meets its management objectives to maintain a viable population of American lobster within the Eastport Lobster Management Area (EPLMA). Biophysical models offer the best tool available to answer this question (Stanley et al. 2015a). Given the relatively small assessment scale of the EPLMA (~ 400 km^2) any biophysical model should incorporate larval behaviour, as demonstrated in Chapter 5. Given the relatively colder water temperatures that characterize the northeastern coast of Newfoundland (Colbourne et al. 1998), longer durations within each ontogenetic stage might well increase the influence of behaviour (Mackenzie 1988). The approaches outlined in Chapters 4 and 5 could inform a biophysical model for the Eastport MPA, potentially answering many of the dispersal questions posed in recent evaluations (DFO 2014a, Stanley et al. 2015a).

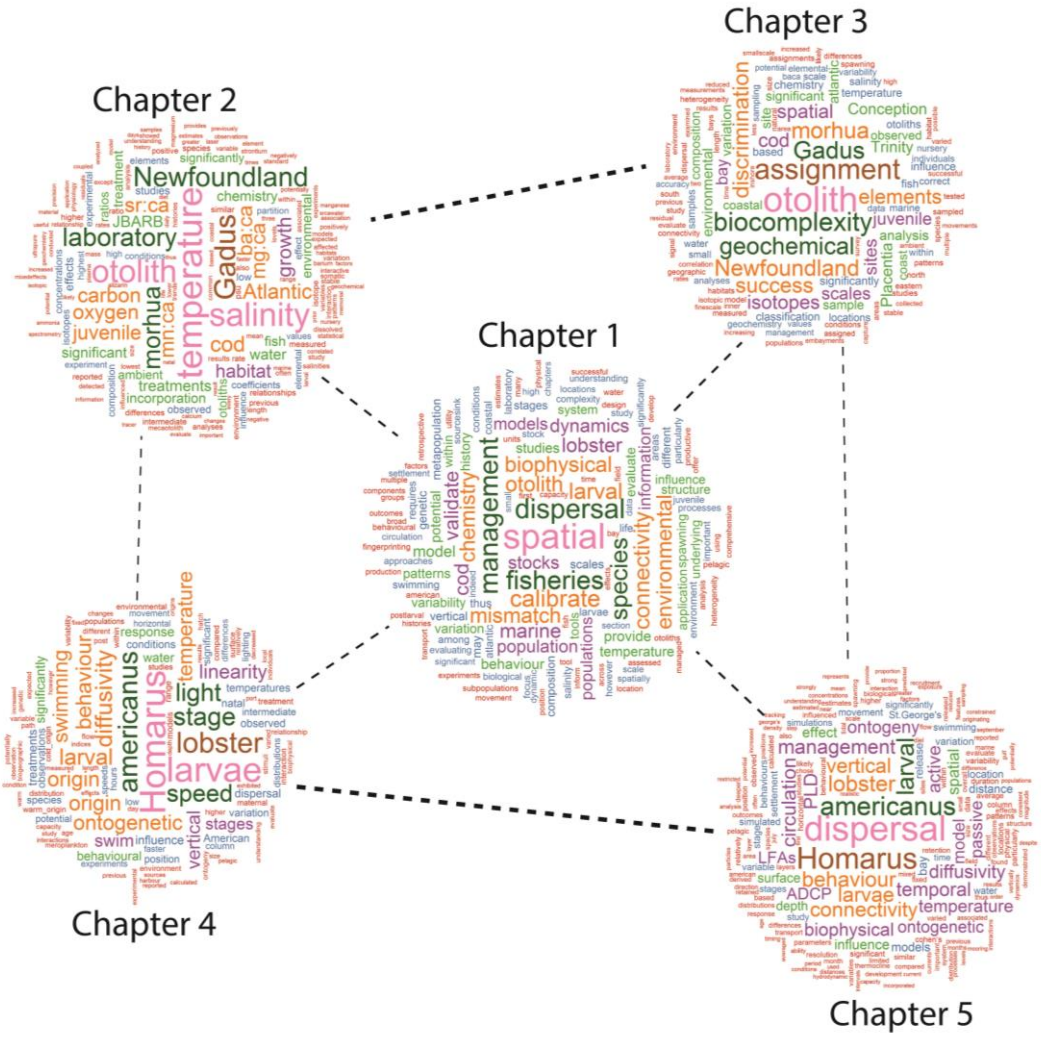

Figure 6.1 Word cloud diagram highlighting the connectivity among thesis chapters. Word size and colour correspond to the relative frequency of each word within the individual chapter's text.

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Appendices

Supplementary Table 2.1 Correlations between somatic (S) and otolith (O) growth and elemental partition coefficients and isotope values in juvenile *Gadus morhua* grown under experimental conditions. Pearson correlation coefficients presented with *Benjamini-Hochberg* adjusted α controlling for multiple comparisons * α <0.05, ** α <0.01. Isotopes in ‰ relative to VPDB. Sample size of each treatment and element is denoted by n.

	Temp	Low			Med			High			Pooled
	Sal	Low	Med	High	Low	Med	High	Low	Med	High	
D_{Mg}	**S**	0.15	0.03	-0.01	0.29	0.36	-0.27	0.1	0.05	-0.17	**0.21***
	O	0.17	-0.2	0.6	0.36	0.27	0.26	0.22	-0.17	-0.22	**0.19***
	n	*37*	*21*	*28*	*31*	*33*	*27*	*29*	*38*	*25*	*269*
D_{Mn}	**S**	0.26	-0.52	-0.19	0.41	0.42	0.12	0.06	0.14	0.02	**0.33***
	O	0.41	-0.17	0.28	0.45	**0.66***	**0.55***	0.36	0.16	0.05	**0.34***
	n	*39*	*21*	*25*	*28*	*31*	*29*	*31*	*39*	*24*	*267*
D_{Sr}	**S**	-0.32	0.87	-0.39	-0.3	-0.4	-0.51	-0.14	-0.01	-0.47	**-0.56***
	O	-0.42	-0.25	0.01	-0.48	**-0.74***	**-0.42**	**-0.56***	-0.28	-0.21	**-0.57***
	n	*37*	*22*	*24*	*27*	*32*	*29*	*29*	*38*	*27*	*265*
D_{Ba}	**S**	-0.05	-0.63	0.21	0.17	0.05	0.33	0.03	-0.04	-0.1	0.03
	O	0.08	0.15	0.17	0.22	0.2	0.33	0.36	0.32	0.39	0.13
	n	*33*	*23*	*28*	*27*	*32*	*29*	*31*	*39*	*27*	*269*
$\delta^{13}C$	**S**	0.19	-0.03	-0.42	0.08	-0.04	0.01	0.26	0.7	0.22	**0.45***
	O	0.31	0.07	-0.6	-0.07	-0.35	0.22	0.26	-0.5	0.01	**0.37***
	n	8	15	16	13	15	15	11	10	14	116
$\delta^{18}O$	**S**	-0.27	0.31	-0.24	0.38	-0.33	-0.06	-0.69	0.35	0.27	**-0.41***
	O	0.11	0.11	0	0.06	0.1	0.29	**-0.86***	0.11	0.08	**-0.34***
	n	*14*	*14*	*16*	*11*	*13*	*15*	*9*	*10*	*14*	*116*

Supplementary Table 3.1 Summary of environmental data for each sample site. Temperature data queried from SST telemetry using a 25 km radius of available data for two months prior to collection for each site. Salinity data was collected at 1m depth at the time of sampling with ~3 replicates for each site. Bay of sampling coded as Placentia (PB), St. Mary's (SMB), Conception (CB), Trinity (TB) and Bonavista (BB). Data presented as mean ± (1 standard deviation) for temperature and salinity.

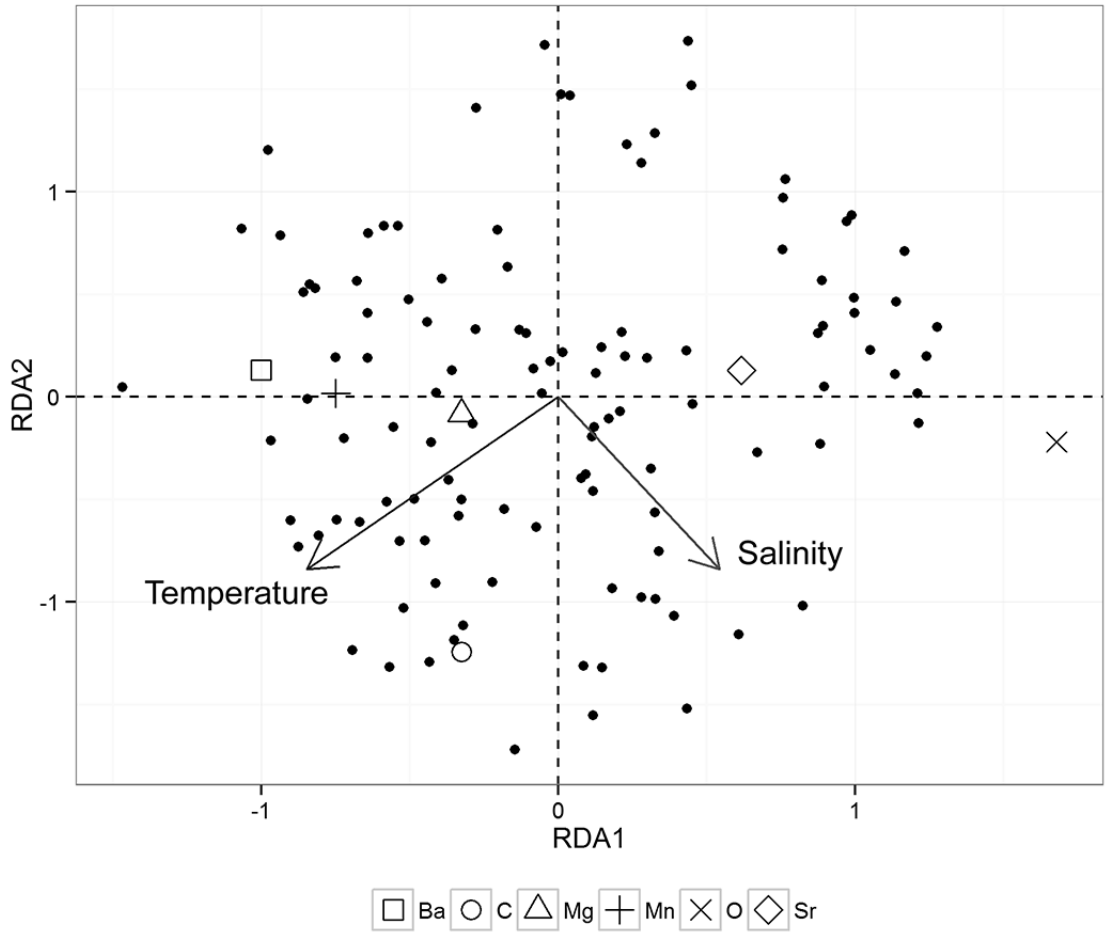
ID	Month	Day	Bay	Site	Code	Temp (°C)	Sal (PSU)
1	Oct	25	PB	Great Brule	GB	12.2 (±0.18)	30.2 (±0.93)
2	Oct	25	PB	St. Bernard's Cove	BC	12.6 (±0.18)	30.6 (±0.95)
3	Oct	18	PB	Woody Island	WE	13.2 (±0.29)	30.2 (±0.68)
4	Oct	18	PB	Spanish Room	SR	12.5 (±0.09)	31.3 (±0.98)
5	Oct	30	PB	Harbour Buffett	HB	12.1 (±0.25)	30 (±0.49)
6	Oct	10	SMB	Colinet Island	CI	13.5 (±0.09)	30.6 (±0.28)
7	Nov	8	CB	Harbour Grace	HG	10.1 (±0.12)	29.9 (±0.48)
8	Oct	24	CB	Holyrood	HR	11.3 (±0.09)	30 (±0.58)
10	Oct	4	TB	Little Mosquito Cove	LMC	11.2 (±0.26)	30.1 (±0.79)
11	Oct	1	TB	Smith Sound	SS	12.1 (±0.13)	30.5 (±0.39)
12	Oct	15	TB	Dildo	DL	11.5 (±0.18)	30.6 (±0.78)
13	Oct	2	TB	Tappers Cove	TC	12.0 (±0.19)	30 (±0.48)
14	Oct	3	BB	Cannings Cove	CC	13.3 (±0.29)	29.9 (±0.2)
15	Oct	3	BB	Bread Cove	BC	13.2 (±0.28)	29.9 (±0.89)
16	Oct	3	BB	Piper's Cove	PC	11.8 (±0.5)	29.5 (±0.54)
17	Oct	23	BB	Indian Bay	IB	10.0 (±0.26)	27.6 (±0.6)

Supplementary Table 5.1 Results of Generalized Least Squares (GLS) model evaluating larval density as a function of thermocline depth. Parameters are listed with associated standard error.

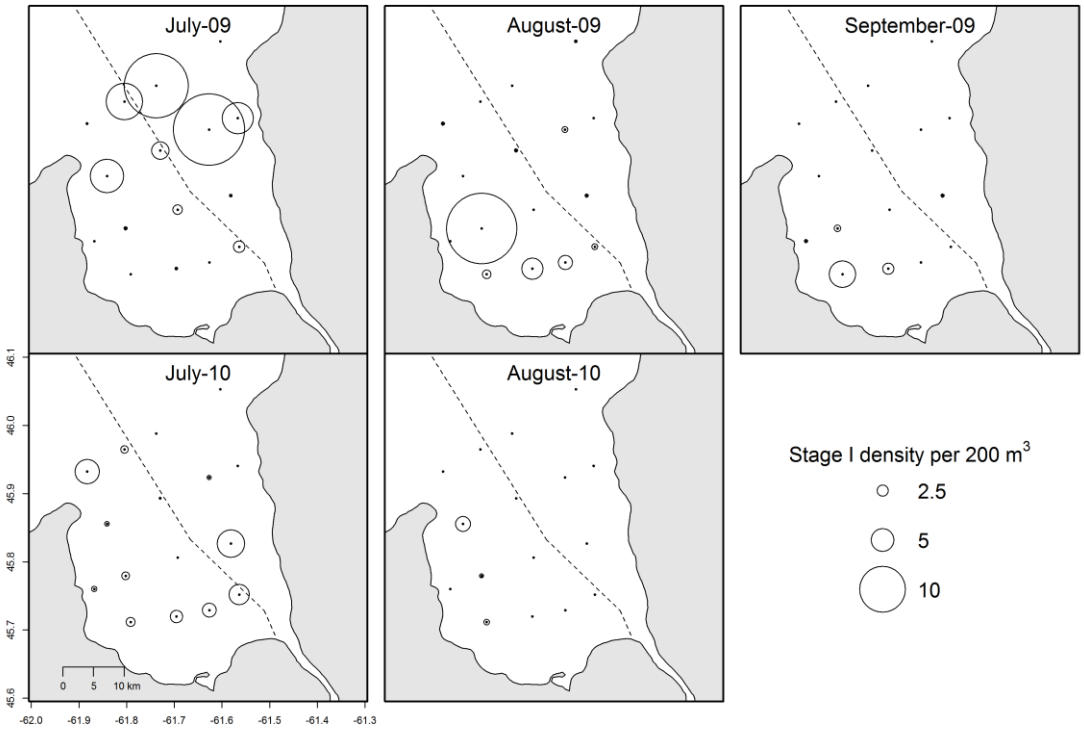
Stage	Slope	Intercept
I	-1.03 (± 0.34)	28.97 (± 5.03)
II	-0.49 (± 0.38)	10.12 (± 5.65)
III	-0.20 (± 0.41)	5.65 (± 5.97)
IV	-0.02 (± 0.36)	3.22 (± 5.55)

Supplementary Table 5.2 Average current amplitudes and associated standard deviations (SD) of the 3-hourly amplitudes for both simulated and observed currents, as well as the mean difference and associated SD between the circulation model and ADCP estimates at each mooring location (Figure 1c). All units are in cm· s^{-1}. The surface and deep layers span 0-12 and 12-24 respectively. Data from this table first reported in Daigle et al. (2015).

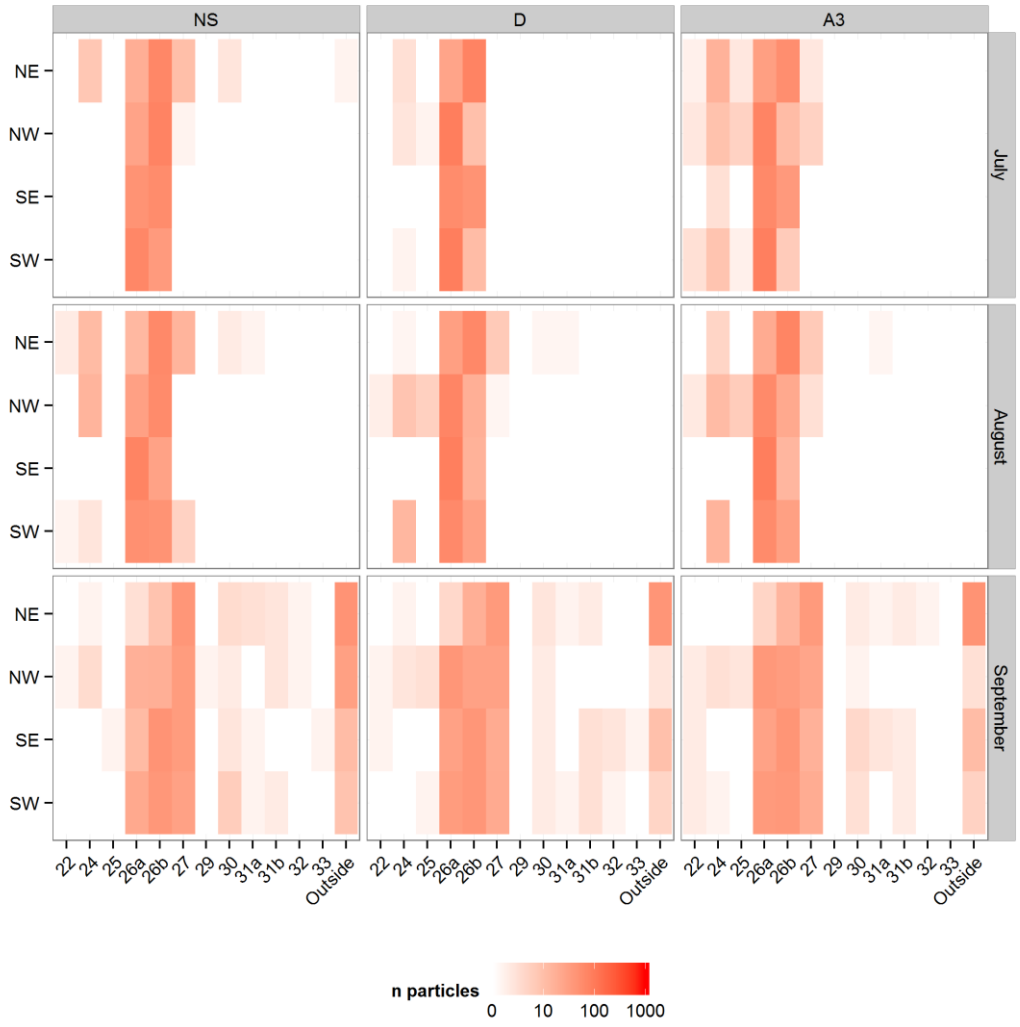
Layer	Station	Mean OPA	Mean ADCP	SD OPA	SD ADCP	Error	SD Error
Surface	M1	8.254	11.167	5.962	6.718	-2.913	8.403
Surface	M2	7.055	9.945	4.735	5.358	-2.89	5.911
Surface	M3	5.42	10.374	3.617	6.261	-4.954	6.253
Surface	M4	6.296	8.271	3.392	4.242	-1.975	3.316
Surface	M5	4.392	8.766	3.336	4.954	-4.374	5.221
Deep	M1	5.465	7.279	3.301	3.906	-1.814	5.15
Deep	M2	4.662	6.329	3.06	3.704	-1.667	4.089
Deep	M3	4.288	5.587	3.255	3.371	-1.3	3.884
Deep	M4	7.216	8.849	4.047	4.074	-1.633	3.364
Deep	M5	5.18	6.922	3.829	4.395	-1.742	5.343
All	M1	4.285	5.809	2.792	3.487	-1.524	4.323
All	M2	3.687	7.307	2.239	3.951	-3.62	4.746
All	M3	3.55	6.18	2.282	3.541	-2.631	4.374
All	M4	3.782	6.822	2.051	3.007	-3.04	3.507
All	M5	3.575	6.994	2.657	3.795	-3.418	4.206



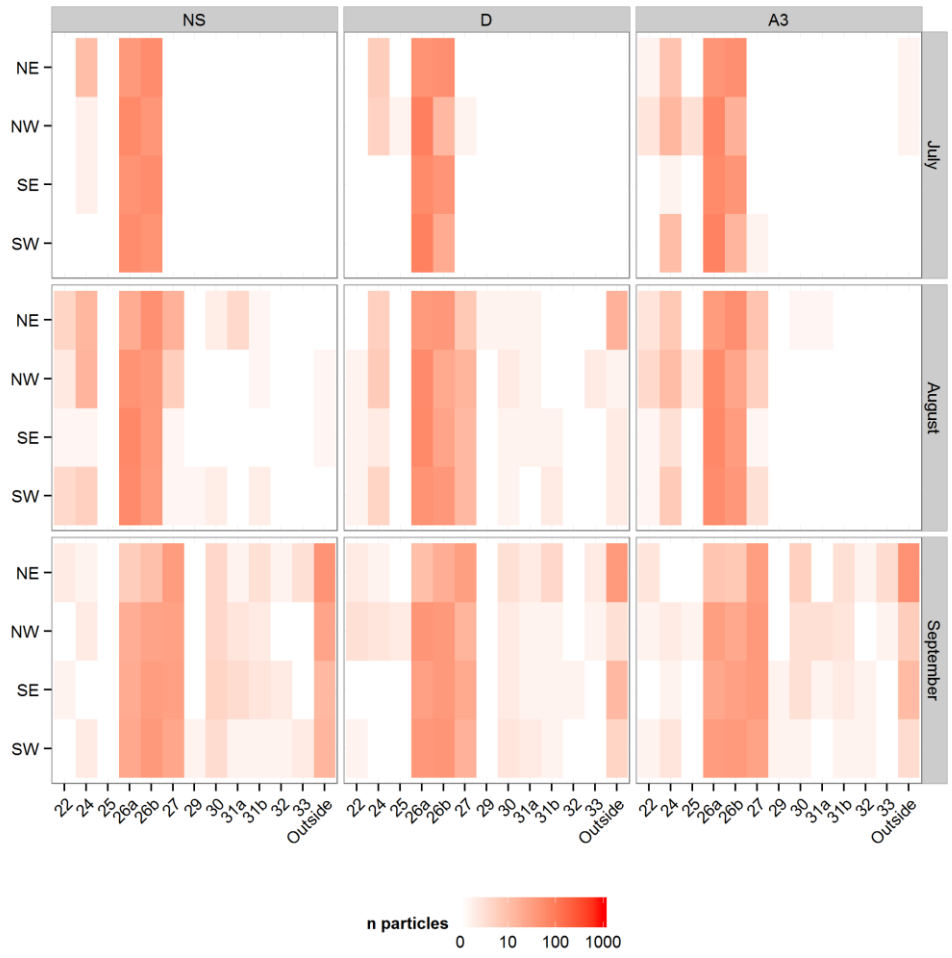
Supplementary Figure 3.1 Summary plot of the redundancy analysis (RDA) predicting otolith chemistry as a function of temperature and salinity. Elemental centroids are plotted for each otolith constituent. Arrows represent eigenvectors for each environmental variable. Data for model based on (Stanley et al. 2015, Chapter 2).



Supplementary Figure 5.1 Distribution of stage I larvae captured during neuston surveys of St. George's Bay 2009-2010.



Supplementary Figure 5.2 Connectivity matrices summarising the dispersal of larvae exhibiting surface passive (NS), depth passive (D) and laboratory derived vertical structure (A3) behaviours (Table 3). Release locations (y axis) are the start points and the corresponding LFAs (x axis) where larvae achieve settlement age (See Figure 1b). Each matrix represents the dispersal outcome for larvae dispersing with a horizontal diffusivity of 5 $m^2 \cdot s^{-1}$ and aggregated to 2011. Note that the gradient fill of the plot corresponds to a log10 scale.



Supplementary Figure 5.3 Connectivity matrices summarising the dispersal of larvae exhibiting surface passive (NS), depth passive (D) and laboratory derived vertical structure (A3) behaviours (Table 3). Release locations (y axis) are the start points and the corresponding LFAs (x axis) where larvae achieve settlement age (See Figure 1b). Each matrix represents the dispersal outcome for larvae dispersing with a horizontal diffusivity of 25 $m^2 \cdot s^{-1}$ and aggregated to 2011. Note that the gradient fill of the plot corresponds to a $\log_{10}$ scale.