The behaviors, habitat preferences, and ecology of distinct Atlantic cod phenotypes in the Gulf of Maine

by Christian W. Conroy

B.S. in Biology, Co-Major in Ecology, Emory University
M.S. in Marine, Estuarine, and Environmental Sciences, University of Maryland

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Jonathan H. Grabowski
Professor of Marine and Environmental Sciences
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Abstract of Dissertation

Diversity in spatial behaviors and associated divergence of phenotypes are common across fish taxa. Behavior and phenotype are shaped by geology, oceanography, climate, prey availability, predation, competition, and the cumulative effects of these factors on past generations. In stochastic natural systems, diversity may enhance stability, resilience, and even productivity of populations and population complexes. Among the behaviors of fish that vary between and within populations is migration. Migration is most often related to travel between spawning and feeding grounds and is integral to population productivity. However, members of the same species and even population may adopt vastly different migratory strategies, including long-distance migration and strict site residence. Across taxa, these strategies are often correlated with metabolism, growth, fecundity, and productivity. In the context of fisheries management, the spatial and physiological variance associated with different migratory strategies may lead to complicated or unexpected responses by managed stocks to disturbance. Understanding the processes that lead to the adoption and maintenance of migratory strategies, how these behaviors affect habitat use, and how behavior and habitat are related to phenotype, are necessary to the development of effective fisheries management policies and regulations.

Atlantic cod (*Gadus morhua*) adopt a range of spatial behaviors across the North Atlantic, with effects on productivity and growth. As in other fish taxa, resident cod have lower growth rates and fecundity than conspecific migrants. Many identified groups of cod residents are physically isolated from nearby migrants, offering little insight to many of the important questions surrounding these behavioral strategies. In the Gulf of Maine, sympatric resident and migrant cod have been identified from a number of locations.
This research focused on the interactions between behavior, habitat use, and phenotype of cod in the Gulf of Maine. A visually distinct cod phenotype found at several locations throughout the region features red pigmentation and a deeper body than the familiar form of cod. As is observed elsewhere in the species range, this red phenotype remains in relatively shallow, structured habitats and grows at lower rates compared to the familiar migrant olive phenotype. The connection between low growth, resident behavioral strategy, and body morphology (deep vs. fusiform) has been observed in a number of salmonids and may be common among fishes that occupy diverse environments. Red phenotypes were highly selective of certain habitats, and the spatial behaviors of all cod regardless of phenotype changed seasonally, possibly due to the influence of seasonal changes in environmental conditions like temperature. Phenotype-specific behaviors were consistent both inshore and offshore, generally following resident and migrant strategies. Offshore red phenotypes expressed strict site fidelity, but this may in part be due to habitat limitation, as nearshore red phenotypes adhered to shallow depths but moved up and down the coast.

Regardless of phenotype, cod utilize shallow, structured habitats at an offshore site, Cashes Ledge, during the warm periods of the summer and fall. Habitat use is focused on the most complex habitats in the shallowest peaks of the ledge, which often are covered in thick macroalgal stands. The shallow structured habitats preferred by red phenotypes and utilized by all cod are not available to widely used groundfishing gear, thus limiting fishing pressure on these important habitats. At the same time, shallow coasts and ledges will be affected by warming surface waters and increased storm activities associated with ongoing climate change. The importance of these habitats and their utilization by cod relative to their adopted migratory strategies may impact population-scale responses of cod to future management activities.
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Chapter 1: Relationships between diverse migratory strategies, habitats, and phenotypes in marine fishes

Introduction

Adaptation to local geologic and oceanographic conditions may be common among marine fishes, with evidence for parallel evolution to environmental gradients in disconnected populations of some marine species (Conover et al. 2006, Bradbury et al. 2010). Marine population structure in open ocean environments is partly mediated by migratory behavior throughout critical life history stages (Frisk et al. 2014, Gahagan et al. 2015). Migrations of marine fishes provide links between spawning, nursery, and foraging grounds that maintain populations, while diversity in these behaviors may enhance long-term sustainability and ensure connectivity between reproductive groups (Secor 2015). Because the marine environments that fish reside within are dynamic, migratory behaviors must be adaptive and conform to changing biological, geographical and oceanographic features (Harrison & Taylor 1997).

The adaptive nature of migration leads to variability in spatial behaviors within marine fish species, as some populations will travel greater distances and exploit different habitat types in the pursuit of growth, maturation, and maximized reproductive output (Chapman et al. 2011, Chapman et al. 2012). In some species, differences in spatial behaviors may be stark, resulting in largely sedentary and highly mobile populations based on local conditions and climate-level gradients in temperature (e.g., non-migratory and largely anadromous Arctic charr Salvelinus alpinus, Doucett et al. 1999, Klemetsen et al. 2003). In others, separate populations may coexist, and their divergence is supported by behavioral reproductive isolation or other early life-history processes that occur prior to hatch (e.g., assortative mating and physical characteristics of eggs...

Many, if not most, anadromous species either maintain or retain the dormant capacity to support multiple migratory strategies within the same open population, known as partial migration (Chapman et al. 2012). A number of mechanisms may support partial migration, including differences in early growth due to maternal behaviors and feeding conditions (Japanese ayu *Plecoglossus altivelis*, Tsukamoto and Uchida 1992), territorial requirements for reproduction and nursery habitats in some salmonids (Dodson et al. 2013), as well as the interaction of inherited traits and conditional states of individual fish (*e.g.*, rainbow trout *Oncorhynchus mykiss*, Phillis et al. 2016). Regardless of whether they occur within or vary between populations, distinct migratory strategies correlate with and influence important differences in phenotype and productivity with population-scale consequences (Kerr et al. 2010).

An important implication of diverse spatial behaviors is conspecific phenotypic variability, where members of the same species express distinct physical characteristics. These differences can be adapted to particular habitats, such as the additional “armor-plating” of the open-water form of lake-bound threespine stickleback that is lacking in sympatric conspecifics (Vamosi & Schluter 2004). Open-water sticklebacks utilize the limnetic zone, leaving them more vulnerable to predators than the benthic form, and body armor reduces predation risk (Walker 1997). Salmonids in a number of taxa express variability in body morphologies including mouth shape and position (Arctic charr, Skúlason et al. 1989), overall body shape (coho salmon *O. kisutch*, Taylor and McPhail 1985), and fin size (brook trout, *S. fontinalis*, Morinville and Rasumussen 2008). Phenotypic variability in these species align with feeding behaviors,
preferred prey, water current velocity, or the trade-offs between maneuverability and swimming speed and duration.

In other species, habitat use may be driven in part by existing attributes, as in migrant white perch *Morone americana*, whose higher metabolic rates may shape dispersal from natal freshwater to brackish habitats (Kraus & Secor 2004, Kerr & Secor 2009). Dispersal comes with potential costs, as lower stability in migrant white perch groups suggest a potential for very high mortality rates not common in residents (Kraus & Secor 2004); however, migrants typically display higher growth rates, likely due to the combination of the metabolism of migrants and better potential foraging conditions in their new habitats (Kerr & Secor 2009). Conversely, residents never are subjected to the higher mortality risk endured by migrants, but they also fail to reap the productivity benefits of these behaviors. Again, this tradeoff may align with established metabolic rates, as it seems likely that the relatively low growth rates of residents would lead to smaller growth dividends in a more productive environment.

The phenotypic variation associated with behavioral strategies has important implications for the future behavior of individuals and the persistence of their populations. Evidence that habitat specific morphological traits like those expressed by salmonids are reversible is limited and mixed (e.g., Eurasian perch, Olsson and Eklöv 2005); since these changes occur during development and result in physically distinct body forms (Morinville & Rasmussen 2008), they would seem to be maladaptive to other behavioral strategies and habitats. On the other hand, switching between migratory behaviors when divergent morphology is not a factor may be common (e.g., juvenile striped bass *Morone saxatilis*, Conroy et al. 2015), although there are likely specific costs associated with the originally adopted behaviors that are retained (e.g., increased predation risk associated with compensatory feeding, Metcalfe and Monaghan 2001).
The distribution of marine resources may lead to the sympatric exploitation of food sources or use of spawning grounds by members of the same species expressing alternative migratory tactics. This is obviously the norm in partially migratory populations that share common reproductive (i.e., breeding partial migration, Chapman et al. 2011) or feeding habitats (non-breeding reproduction). Regardless, overlap by conspecifics expressing alternative migratory tactics may provide important insights into how resident or migrant strategies affect habitat requirements, reactions to environmental conditions, and trophic ecology.

Variability in the physical characteristics and behaviors of Atlantic cod *Gadus morhua* has been recognized by fishermen and scientists since at least the 19th century. In his overview of United States fisheries, Goode (1887) details a number of terms locally applied to cod in the Gulf of Maine and Georges Bank, including those used to define recognized groups based on appearance or behavior. Fishermen used terms that referred to physical appearance—such as red and brown cod, location—shore and bank cod, and their preferred prey—herring cod and shad schools (Goode 1887). These descriptions were, in part, economically motivated, as some forms of cod would yield higher (e.g., George’s cod from Georges Bank) or lower (e.g., red or rock cod found on kelp-covered ledges close to shore) prices at market depending on color, size, and other factors (Goode 1887, Grenfell 1924). Evident in these early descriptions is the recognition that behavior, physical appearance, habitat, and diet are connected, presaging future research on life history diversity and its consequences in exploited fishes.

The variance in cod phenotype and spatial behaviors recounted by fishermen in Massachusetts and other coastal fisheries has since been observed across the species range, as more than a century of research has revealed an array of cod migratory strategies (Robichaud & Rose 2004). The red cod Goode (1887) described as limited to vertically structured hard
substrates close to the shore in the Gulf of Maine matches the physical characteristics of cod found in bays featuring similar substrates elsewhere throughout the North Atlantic, including the coasts of Canada (Gosse & Wroblewski 2004) and Scandinavia (Dannevig 1953). The site fidelity observed on the coast of Massachusetts has mostly been ascribed to early life history stages in cod, after which the importance of specific habitat characteristics is thought to decrease as predation risk decreases as fish grow larger and invest in reproductive effort (Lough 2004). The early reliance on specific substrates and habitat features in cod eventually gives way to general substrate preferences, that interact with other important factors such as temperature to influence cod distributions (Methratta & Link 2006). However, there is evidence for the periodic importance of structure to cod spawning (Dean et al. 2014) and foraging behaviors (Reubens et al. 2013).

The home range of red cod found elsewhere in the North Atlantic is surprisingly limited for a species known for the length of its seasonal migrations (Green & Wroblewski 2000, Robichaud & Rose 2004, Kuparinen et al. 2015). The movements of these individuals are limited to coastal embayments, where local spawning, currents promoting retention of pelagic eggs and larvae, and foraging conditions support largely closed populations and resident spatial strategies (Green & Wroblewski 2000, Wroblewski et al. 2005, Kuparinen et al. 2015). The distinct coloration of these residents is accompanied by growth rates, body shapes, and diets that also differ from those of more migratory cod (Morris & Green 2002, Kuparinen et al. 2015). The persistent correlation of these resident attributes in partially closed coastal bays appears to lie at one extreme of a gradient of migratory behaviors, shaped by oceanographic conditions, bathymetry, prey availability, and many other potential factors (Robichaud & Rose 2004). However, the importance of specific habitats, local environmental conditions, and prey
communities to observed behaviors has not been explored in depth. These factors often affect the persistence of diverse migratory behaviors in other fish taxa (Chapman et al. 2012).

Although managed as a single stock, cod in the Gulf of Maine comprise several spawning components maintained through spatial and temporal segregation during reproduction (Zemeckis et al. 2014). Migratory behaviors between and within groups vary, although spawning components are generally migratory or sedentary (Howell et al. 2008, Gröger & Fogarty 2011). Residents along Stellwagen Bank on the edge of Massachusetts Bay co-exist with non-residents as well as migrants traveling between spawning grounds in the Gulf of Maine and foraging habitat south of Cape Cod (Gröger & Fogarty 2011). At some locations along the bank, cod remain in close proximity to isolated boulder reefs throughout the year (Lindholm et al. 2007), expressing site fidelity and limited spatial ranges more often associated with residents in semi-enclosed bays in other regions (Green & Wroblewski 2000, Kuparinen et al. 2015). Although Stellwagen Bank residents have not been associated with a distinct phenotype, red pigmented cod are found in a kelp forest covering an offshore seamount in the central Gulf of Maine (Sherwood & Grabowski 2010). These red cod appear to be limited to this isolated habitat and differ in morphology, growth rate, and diet from co-occurring cod that express more typical pigmentation and body shapes (Gosse & Wroblewski 2004, Sherwood & Grabowski 2010). Cod expressing these distinct phenotypes in an open bay (Goode 1887) and at an offshore seamount (Sherwood & Grabowski 2010) within the Gulf of Maine provide an opportunity to determine biological and ecological differences through direct comparison, and ultimately to explore potential factors that contribute to their maintenance.

The research reported in this dissertation focuses on local and regional scale expression of diversity within Gulf of Maine cod. Pigmentation was used to group cod in coastal and
offshore areas into 2 phenotypes that would have been familiar to the fishermen interviewed by Goode (1887) - red and olive phenotypes. Physical characteristics, including morphology and growth, and diet were assessed to determine divergence between the phenotypes that relate to development, trophic ecology, and behavioral strategies (Chapter 2). Both red and olive phenotypes “tagged” with acoustic transmitters revealed aspects of cod spatial ecology and habitat associations both at an offshore seamount (Chapter 3) and in an open coastal bay (Chapter 4). Finally, I used habitat suitability modeling to identify cod essential fish habitat and predict the extent of EFH on Cashes Ledge (Chapter 5).

The physical characteristics of cod were found to correlate with important differences in vital rates, trophic ecology, and spatial behaviors, but there were limits to this divergence, as some of the most important differences in sampled cod existed between inshore and offshore cod rather than between phenotypes. The spatial behaviors of these groups differed in ways both apparent and subtle, indicating both distinct migratory strategies within Robichaud and Rose’s (2004) sedentary-migrant spectrum, as well as overlapping responses to seasonally variable oceanographic conditions. Although I found that shallow rocky and kelp-covered habitats are utilized by both migrants and residents, residents were far more prevalent in these habitats. These macroalgal-covered habitats featured higher densities of cod overall compared to deeper habitats, regardless of structure, further suggesting the importance of productivity associated with the shallow kelp forest.

Recent research findings have highlighted the need to incorporate the diversity of cod spatial behaviors into fisheries and marine habitat management (Le Bris et al. 2013, Sherwood & Grabowski 2015). It remains unclear whether cod spatial behaviors are mostly population-scale phenomenon (Svedäng & Svenson 2006), the result of conditions during development
(Tsukamoto & Uchida 1992), or the product of inherited traits interacting with environmentally influenced physiological states (Dodson et al. 2013). The mismatch between spatial management and population structure is believed to have contributed to previous cod stock collapses (Smedbol & Stephenson 2001), and may be leading to the incorrect stock assessment estimates of overall productivity in the Gulf of Maine and Georges Bank stocks (Kerr et al. 2010). Regardless of the cause, the association of resident spatial behaviors with low productivity and reduced growth rates could have important effects on the Gulf of Maine cod stock and influence its recovery.

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Chapter 2: *Life-history implications of color variation and region in Gulf of Maine Atlantic cod (Gadus morhua)*

Abstract

Trait variability of conspecifics often correlates with differences in life history that can influence population dynamics and consequently fisheries productivity. Distinct forms of Atlantic cod *Gadus morhua* that occupy unique habitats in New England waters have been reported by fishermen for at least 130 years. One form that features red pigmentation was recently confirmed to have specific preferences for shallow, structured habitats. A previous study conducted within the Gulf of Maine found that this red phenotype differs from the more mobile sympatric olive phenotype (*i.e.*, holotype) in body shape, diet, and growth. Here I explored the body and otolith morphology, growth, and trophic ecology of cod in the context of morphometry and region (inshore/offshore). Cod were collected from both the offshore location of the previous study, Cashes Ledge, and an inshore embayment, central and western Massachusetts Bay. As in the previous study, body shapes differed between red and olive phenotypes, although discrimination between groups was less successful in Massachusetts Bay. Otolith shapes diverged between regions more than phenotypes. Although diets did differ overall, there was substantial overlap in targeted prey and no difference in gut fullness. Based on the results of related migratory behavioral studies, the overlap in diet may be seasonal. Overall, phenotypic divergence likely occurs early in ontogeny, but whether behavioral and phenotypic differences are the result of reproductive isolation is unclear. Differences in growth are not likely due to diet composition alone, as both phenotypes targeted a high proportion of available benthic prey. The relationship between reduced productivity and resident behavioral strategies is consistent across the range of the species, regardless of physical isolation from conspecific migrants or region.
Cod residents tend to select structured habitats, leaving them less susceptible to fishing pressure and mortality. The combination of these spatial behaviors and habitat preferences could lead to selective pressures resulting in more resident life-history strategies, which could affect the overall productivity of cod in the Gulf of Maine and beyond.

**Introduction**

The environments occupied by mobile marine species are surprisingly diverse, shaping their behaviors and vital rates (Conover & Present 1990, Harrison & Taylor 1997). Within species, expressed phenotype and spatial behaviors may vary widely, as local environmental conditions, geography, and other factors influence the process of maximizing individual fitness (Winemiller 1991). These combinations may affect different populations of the same species, leading to vastly different life histories that vary latitudinally. In partially migratory species, these effects are observed within a single population, as the behaviors of individual are influenced by external factors (Chapman et al. 2011). Both of these phenomena may be observed in the anadromous Arctic charr *Salvelinus alpinus*. Individual spatial behaviors vary between lake and stream dwelling residents and ocean-going migrants depending on early conditions and, most likely, inherited traits (Adams & Huntingford 2004, Adams et al. 2008). In addition to behavioral diversity between individuals of a population, the proportion of residents:migrants varies by latitude, as more southerly populations may be entirely resident and more northerly populations mostly migrant (Nordeng 1983). Physical characteristics vary along with these differences in migratory tactics, as migrants are larger and feature distinct pigmentation not observed in residents (Jonsson & Jonsson 2001). These behaviorally-specific phenotypes are common across fish taxa, as residents tend to grow at slower rates, reach smaller maximum sizes, and are generally less productive than migrants (Jonsson & Jonsson 1993); differences in body
morphology are also common, both as a result of residents’ lower growth (Griffiths 1994) and adaptation for local retention rather than longer migrations (Morinville & Rasmussen 2008).

Regardless of whether the physical differences that correlate with migratory strategies occur within or between populations, they have important influences on the productivity of commercially important species and the sustainability of their catches. This variation in life history strategies can have important implications for the catchability of different types, thereby impacting their relative importance to associated fisheries.

Atlantic cod (Gadus morhua) adopt a range of migratory strategies, with effects on vital rates generally adhering to the familiar relationships between mobility, growth, and productivity (Robichaud & Rose 2004). In some locations, the phenotypes of residents have diverged substantially from the familiar cod holotype (Ruzzante et al. 2000, Gosse & Wroblewski 2004, Sherwood & Grabowski 2010). Some of these phenotypically distinct resident groups are maintained, in part, through reproductive isolation (Kuparinen et al. 2015), with at least one case where partial physical isolation may have led to the observed divergence (Green & Wroblewski 2000). However, in other regions the mechanisms that help maintain visibly distinct forms of resident cod are less clear, such as in the Gulf of Maine and other regions of the North Atlantic where the spatial overlap between migrants and residents is substantial (Lindholm et al. 2007, Gröger & Fogarty 2011). Further efforts to characterize how resident and migrant cod differ, as well as what maintains these behaviors, will enhance our understanding of partial migration in open populations while also improving our ability to promote the sustainability of both types.

Variation in color pattern and pigmentation is common across cod populations (Bigelow & Shroeder 1953, Gosse & Wroblewski 2004). Although color has been shown to be malleable, changing with diet and location throughout a single cod’s lifetime (Bigelow & Shroeder 1953,
Fox & Vevers 1960, Gosse & Wroblewski 2004), red coloration has been found to correlate with distinct vital rates and reproductive potential throughout the species range (Morris & Green 2002, Sherwood & Grabowski 2010). Cod that express red pigmentation have typically been found in shallow, structured habitats within bays, inlets, and fjords in Labrador (Green & Wroblewski 2000) and Scandinavia (Dannevig 1953, Kuparinen et al. 2015), with the only known offshore exception being the kelp forest at Cashes Ledge in the Gulf of Maine (Sherwood & Grabowski 2010). Subsequent telemetry studies in some of these locations have revealed behavioral strategies and habitat preferences that diverged from cod expressing more typical counter-shade patterning and coloration (Green and Wroblewski 2000, Chp. 2,3).

In this study, I attempted to extend Sherwood and Grabowski’s (2010) existing description of the red phenotype at Cashes Ledge to a large, nearshore embayment that features similarly pigmented cod long believed to be resident by local fishermen (Goode 1887). I explore whether cod phenotype influences growth and diet. I predicted that the body morphology of the red phenotype would differ from that of the olive phenotype across the region. Specifically, I hypothesized that the red phenotype would feature a larger head, deeper body, and thinner tail, characteristics that have often been associated with persistent resident strategies in fish (Morinville & Rasmussen 2008, Sherwood & Grabowski 2010). Otolith morphology would similarly differ between these phenotypes, suggesting their divergence occurred sometime early in their development (Hüsey 2008, Vignon 2012). In addition, I predicted that the red phenotype would attain smaller maximum size and grow more slowly than the olive phenotype. I predicted that the diet of the red phenotype would primarily feature benthic species, and large crustaceans would comprise a greater proportion of consumed prey when compared to the diet of the olive phenotype. Ultimately, I predicted that morphology, growth, and trophic ecology diverge
between observed color phenotypes and their distinct migratory tactics across the region, and that inshore residents will be more similar in their shape, growth, and diet to offshore residents than to migrants caught in their immediate vicinity. Sedentary behavioral modes in cod are likely adopted at some point early in life history, permanently influencing development, growth, trophic ecology, and behavior.

**Methods**

**Cod Sampling**

Cod were collected from two regions within the Gulf of Maine where either previous studies (Sherwood & Grabowski 2010) or ecological knowledge of local fishermen indicated the consistent presence of distinct cod phenotypes (Goode 1887, G. Veprek pers.comm.). Offshore sampling occurred between May and October 2013 and was concentrated along an offshore seamount, Cashes Ledge, which is known to feature both red and olive phenotype cod in close proximity and in large numbers (Witman and Sebens 1992, Sherwood and Grabowski 2010, Fig.1). Nearshore sampling was conducted within Massachusetts Bay from May to December 2014-2015 between Boston Harbor and Cape Ann, an area highlighted in the earliest scientific description of red phenotype cod by Goode (1887). All offshore and most in-shore sampling consisted of hook-and-line fishing with weighted, clam-baited hooks. Once hooked, samples were reeled slowly to the surface to prevent gas bladder rupture, which may affect morphometric and diet analyses. A limited number of samples were collected from central Massachusetts Bay via longline in 2014. Each cod was measured for total length (cm) and photographed using a digital camera (sRGB color format); depths and coordinates (latitude and longitude) of capture locations were also recorded. Samples were tagged with a unique identification number, placed
in plastic bags, and kept on ice until returned to the lab. Within 12 hours of capture, samples were frozen at -10°C.

Color and Morphometry

Collected samples that had not become visibly discolored or suffered substantial damage to their opercula were assigned to either red or olive phenotypes based on the results of a quantitative color analysis. This assignment was the basis of phenotype definition for subsequent analyses. Pictures of the right or left lateral side of each sample taken immediately upon capture were used to quantify pigmentation and scaled morphometry (i.e., body shape). Using the sRGB (red, green, blue) color profile format of digital picture files, I used the image processing software ImageJ (Rasband 2012) to enumerate the quantity of red and green pixels within a 10cm² circle superimposed on the operculum (posterior to the eye, Fig.2a). The ratio of red to green pixels (RGR) provides a quantitative measure of a physical characteristic that has been shown to vary with population, behavior, and diet (Morris & Green 2002, Gosse & Wroblewski 2004, Sherwood & Grabowski 2010). Pictures found to have severe glare, skin lacerations, or alternating light and shadow on the sample’s operculum were not included in RGR analysis. I applied the same value used by Sherwood and Grabowski (2010) to identify the threshold between red and olive phenotypes, RGR = 1.3; samples with RGR < 1.3 were assigned olive phenotype, while those with RGR ≥ 1.3 were assigned red phenotype.

Phenotypes assigned during color analysis were further assessed using both whole body and whole otolith morphometry. Pictures not taken directly above the sample subject or featuring a cod that was at an angle were not included in morphometric analysis; cod in spawning condition were also eliminated from analysis due to the influence of full gonads on body shape (Cadrin et al. 2014). Whole body morphometry was quantitatively analyzed using the box-truss
method, which uses standardized distances between a predetermined set of homologous points to efficiently characterize shape (Strand & Huse 2007). The box-truss method is widely employed in morphometric studies, including those focused on cod body shape in the context of spatial management policy consequences (Sherwood & Grabowski 2015) and cod life history diversity (Sherwood & Grabowski 2010). For this study, I used the same 11 features and 17 distances identified by Sherwood and Grabowski (2015, Fig.2b). The distances measured between homologous points were used as inputs in a multivariate analysis to characterize morphometry quantitatively. Whole otolith morphometry was assessed using scaled digital pictures of right sagittal otoliths, distal surface facing the camera and rostrum oriented to the left (i.e., lengthwise, Fig.3a); when right otoliths were not available, pictures of the left otolith were inverted horizontally to ensure appropriate orientation. Otolith outlines were drawn automatically using the statistical program R via the package shapeR (Libungan et al. 2015). Due to small errors associated with automated outlining, a local moving average was used to smooth pixel noise in digital representations of the outline (Haines & Crampton 2000). Mean otolith outlines were reconstructed from wavelet coefficients and plotted to visually assess group otolith shapes. Wavelet coefficients were used to quantitatively assess differences in otolith shape between phenotypes and regions.

Growth

Following morphometric analysis, otoliths were baked on porcelain tiles heated to 400°C for ≤45 seconds, which darkens and discolors translucent zones to aid in age determination. Baked otoliths were mounted in clear epoxy resin and allowed to set for ≥24 hours. Mounted otoliths were sectioned using a water-cooled low-speed circular saw equipped with 2 diamond-tipped wafering blades set <1mm apart. Sectioned otoliths were secured to labeled petrographic
slides using thermoplastic glue. Otoliths were aged by counting continuous translucent (i.e., hyaline) zones, beginning at primordium and continuing to the otoliths edge. If the outermost translucent zone did not coincide with the otolith edge, a final age was assigned based on the amount of otolith material (opaque zone) between the translucent zone and the edge of the otolith. Otolith ages and previously measured fish total lengths were used to construct von Bertalanffy growth models (see Analyses section below).

Trophic ecology

Differences in the diets of phenotypes were examined using stomach contents. Stomachs of the samples used in morphometric and color analysis were removed, dissected, and assessed for regurgitation or eversion. Samples with signs of regurgitation or stomach eversion were excluded from further trophic analyses. Prey items were identified to the lowest taxon, weighed, and enumerated. Prey items were assigned to 1 of 6 categories: benthos, crab, lobster, fish, unidentified biomass, and unidentified inorganic mass. Benthos consisted of amphipods, isopods, shrimp, echinoderms, gastropods, and cephalopods. The importance of prey categories was determined using the partial fullness index (PFI, Lilly and Fleming 1991). Total fullness index (TFI, Paz et al. 1993), the sum of all PFI categories for each individual, was used to assess gut fullness. PFI was used to assess the presence and importance of particular prey groups to cod diets, while TFI was used as a measure of overall foraging success.

Analysis

The measurements derived from the box-truss approach were used as inputs in a multivariate analysis to both characterize body shape and assess group differences between phenotypes collected at inshore and offshore locations. Since overall fish size contributes much
of the variance in body dimensions for any group of conspecifics, I tested raw measurements for the influence of total length using a combined principal components analysis (PCA) and linear model approach used in past morphometry studies prior to further analyses (Cadrin 2000, Sherwood & Grabowski 2010). The 17 distances were log-transformed due to their non-normal distributions and used as separate variables in a PCA. Since size accounts for most of the variance in the dataset, the 1st principal component (PC1) largely expresses this variance (Cadrin 2000). To assess differences in body shape rather than overall size, distance measurements were standardized. This process consisted of separate linear models for each of the 17 log-transformed box-truss measurements distance data sets with PC1 as the explanatory variable. The residual values resulting from these models were then used in a stepwise discriminant function analysis (DFA) to assess group differences in body shape.

Wavelet coefficients derived from otolith outlines were used to assess group differences in otolith morphology. The first 3 wavelet coefficients, derived via the discrete wavelet technique, were not used for analysis due to their correlation to size and polar orientation (Lleonart et al. 2000). Additionally, linear models featuring the interaction total length x phenotype, were constructed for each wavelet coefficient to identify and remove any additional influence of overall size from further analysis. Remaining coefficients were used to quantitatively assess differences in otolith shape between phenotypes. Canonical analysis of principal coordinates (Anderson & Willis 2003) and a permutational ANOVA (2000 permutations) using wavelet coefficients were used to assess overall differences in otolith shape by region, phenotype, and region x phenotype. Specific polar coordinates where otolith outlines differed between phenotypes were determined via plots of mean wavelet coefficients +/- standard deviation (SD) and intraclass correlation coefficient (ICC) by degree polar coordinates. High SD indicated
regions where variance was high in otolith outlines, while high ICC indicated regions where variance within group was low relative to total variance, indicating separation between groups. Due to the identical orientation of all otoliths, polar coordinates (measured in degrees) were interpreted as standard morphological characteristics: 0° was aligned with the postrostrum, 90° midway along the dorsal edge, 180° the excisura major between the antirostrum and rostrum on the anterior side, and 270° midway along the ventral edge (Fig.3).

von Bertalanffy growth models (VBGM’s) were fit to red and olive phenotype length-at-age data. Initial coefficient values of asymptotic length ($L_{inf}$), Brody growth coefficient ($K$) and theoretical age at length = 0 cm ($t_0$) were determined using the Ford-Walford method and used as inputs in an iterative non-linear model fitting process (nls function in R). Growth models consisting of all combinations of common and separate coefficients were estimated and model fit was assessed using both Akaike information criterion (AIC) and hierarchical $F$-tests. Upper and lower 95% confidence intervals were estimated for $L_{inf}$, $K$, and $t_0$ through bootstrapping (999 resampling iterations). Growth trajectories of red and olive phenotype were compared using modeled maximum size ($L_{inf}$) and the rate at which this size is reached ($K$).

The diets of red and olive phenotypes both inshore and offshore were assessed using two approaches. Distinctions in the overall diets of identified groups were assessed using a permutational multivariate analysis of variance (PerMANOVA; Anderson 2001) including all 6 prey categories as responses and phenotype, inshore/offshore, and their interaction as explanatory variables. Instead of the raw values of responses, PerMANOVA uses a matrix of dissimilarity indices that must be computed prior to analysis (Anderson 2001). Due to the existence and importance of “double zeroes” (i.e., stomach samples featuring none of the 6 prey categories), I avoided the process of removing records with missing values from the dataset, a
step common in community analyses (Field et al. 1982, Anderson et al. 2006). Instead, I employed a dissimilarity measure, Gower’s index, capable of computing dissimilarities of double zero records (Gower 1971). Unlike other simple dissimilarity indices, such as Euclidean and Mahalanobis methods, the Gower index is not prone to overemphasizing large or underemphasizing small dissimilarities, which may result in biased variance estimates during analysis (Gower 1971, Clarke & Warwick 1994). Prior to conducting the analysis, I compared the multivariate dispersions of the inshore and offshore phenotype groups matrices using a beta diversity test to ensure PerMANOVA assumptions were met (Anderson et al. 2006).

The presence and importance of individual prey categories were analyzed using a hurdle modeling approach, which incorporates separate analyses for prey presence and abundance to account for the different processes at work during prey selection and consumption (Stefánsson & Pálsson 1997, Buchheister & Latour 2015). For each prey category, generalized linear mixed effects models (GLMM) with binomial error distributions and logit link functions were constructed to assess potential effects on prey presence. Prey category abundance (i.e., PFI ≠ 0) was non-normally distributed (positively skewed), which is common for diet analysis datasets (Stefánsson & Pálsson 1997). I log-transformed abundance data prior to analysis, following the log-normal approach often used in hurdle models of gut contents (Steinhausen et al. 2006, Buchheister & Latour 2015). GLMMs with Gaussian error distributions and identity link functions were constructed to assess the factors influencing log-transformed prey category abundance. Fixed effects in both binomial and log-normal GLMMs included phenotype, region (inshore/offshore), and two-way interactions on prey category presence, while depth at capture was included as a random effect; length was included as a fixed effect in the models assessing prey presence, but excluded from analyses of prey abundance due to its inclusion in the formulae
for responses PFI (Lilly & Fleming 1981) and TFI (Paz et al. 1993). Corrected Akaike
information criteria (AICc, Burnham and Anderson 2002) were used to assess model fit. Best fit
models were tested for multicollinearity (i.e., variance inflation; Dormann et al. 2013) and
bootstrapped 95% confidence intervals for all model factors were estimated (n_{iterations} = 999) to
assess the importance of individual fixed effect levels to prey category presence. Finally, effect
sizes (i.e., $R^2$) determined using fixed effects (i.e., marginal effect size) and both fixed and
random effects (i.e., conditional effect size) were used to aid in interpretation of best-fit models
(Nakagawa & Schielzeth 2013).

Results

The discriminant model constructed using samples collected from Cashes Ledge
successfully classified color phenotype in 81% of cod included in the analysis. Stepwise DFA
identified body depth, the measured distance (M08, Fig.2) between the anterior edges of the 1\textsuperscript{st}
dorsal fin (L05) and pelvic fin (L11), as the most important variable in discriminating between
red and olive phenotypes. Also important were the size of the caudal peduncle (M13: L07-L09)
and head (M03, L01-L04). The discriminant model constructed using samples collected from
Massachusetts Bay, however, was far less discerning, resulting in 69% of included cod assigned
to their color-based phenotype. Stepwise DFA identified both body depth (M08) and the distance
(M09) between the anterior edges of the 2\textsuperscript{nd} (L06) and 3\textsuperscript{rd} dorsal fins (L07) as the most important
measurements in discriminating between phenotypes in Massachusetts Bay samples.

Otolith shape analyses revealed little difference in otolith morphology between red and
olive phenotypes across sample locations (phenotype permutational ANOVA $F_{1,189} = 1.48, p =
0.16$), with phenotype explaining less than 1% of the variance in wavelet coefficients derived
from otolith outlines, as determined using the CAP approach (Fig.3,4,5). Although partitioning otoliths by phenotype and region did result in the identification of distinct group-specific otolith shapes (phenotype x region $F_{3,187} = 2.79, p < 0.01$), further analyses of the otolith shapes of phenotypes in Massachusetts Bay ($F_{1,189} = 1.20, p = 0.26$) and at Cashes Ledge ($F_{1,189} = 1.18, p = 0.27$) revealed inconsistent differences in shape. Dividing otoliths into inshore and offshore groups did reveal distinct otolith shape by region (region $F_{1,189} = 2.88, p < 0.01$). Regional differences accounted for 5% of detected differences in otolith shape, with most of the between group variance occurring along the dorsal otolith edge (65-85°, Fig.3,4), at the antirostrum and excisura major morphological features (145-160°), and, to a lesser extent, the anterior ventral edge and postrostrum (325-340°). Much of the variance in otolith shape between Massachusetts Bay and Cashes Ledge appears to be due to substantially greater variance among otoliths of cod from Cashes Ledge compared to Massachusetts Bay, rather than an inshore-offshore division.

Age distributions of cod collected both inshore and offshore were truncated, with olive but not red phenotypes exceeding age-5 (Fig.6). Attempts at modeling growth of inshore and offshore sample sets led to model convergence issues; therefore, the two phenotypes were analyzed separately. Modeled red and olive phenotype growth differed, as the common model fit pooled age-length data poorly (Table 1). Modeling growth using separate estimates of maximum size, Brody’s coefficient, or theoretical age at size 0 resulted in similar model fits. The best fit models suggested that olive phenotypes grow more quickly than red phenotypes and attain larger sizes, although the inclusion of older red and olive phenotypes would likely improve coefficient estimates.

Based on tests of all prey categories, red and olive phenotype diets were distinct (PerMANOVA $F_1 = 4.49, p = 0.02$). Region influenced diet overall ($F_1 = 5.58, p = 0.03$), but did
not affect the differences in between the phenotypes (i.e., no interaction, phenotype x region $F_I = 2.26, p = 0.10$). As predicted, crabs (Table 2, Fig.7) were an important food source for red phenotypes, occurring more often in red phenotype (71.3%) than olive phenotype diets (56.3%), although abundance was consistent when present ($PFI_{\text{red}} = 0.66 +/-0.09, PFI_{\text{olive}} = 0.64 +/-0.10$). The importance of benthic prey generally followed expectations, occurring at similar rates in diets regardless of region or phenotype (inshore FO = 73.1%, olive FO = 55.7%; offshore red FO = 74.5%, olive FO = 63.9%) with most of the variance in prey presence due to diet changing as fish increase in size (i.e., benthos occurrence decreased with size, $\beta = -0.06$). Unlike previous diet analyses of these phenotypes conducted at Cashes Ledge, I did observe more benthic biomass in the stomachs of red phenotypes ($PFI = 0.21 +/-0.05$) that targeted this prey than olive phenotype ($PFI = 0.13 +/-0.04$). In Massachusetts Bay, however, there was no difference between phenotypes in the amount of benthic biomass present ($PFI_{\text{red}} = 0.08 +/-0.03, PFI_{\text{olive}} = 0.09 +/-0.02$). Fishes were found more often in inshore stomachs (FO = 58.6%) than those sampled from offshore (FO = 32.4%). Fishes may contribute less to red phenotype diets at Cashes Ledge ($PFI = 0.15 +/-0.05$) than those of olive phenotypes ($PFI = 0.88 +/-0.31$) or cod sampled in Massachusetts Bay ($PFI_{\text{red}} = 0.83 +/-0.32, PFI_{\text{olive}} = 0.76 +/-0.16$), but high variance in biomass may have confounded modeling efforts. Lobster were rare in cod diets, found in only 7.7% of sampled stomachs. Although previous sampling found no evidence of olive phenotypes feeding on lobster (Sherwood & Grabowski 2010), 6.5% of those sampled during this study had evidence of having recently consumed lobster. How frequently lobsters were consumed by red and olive phenotypes depended on region. Specifically, lobsters were found in red phenotype diets more often at Cashes Ledge ($FO_{\text{red}} = 12.8\%, FO_{\text{olive}} = 3.3\%$), whereas they were more prevalent in olive phenotype diets in Massachusetts Bay ($FO_{\text{red}} = 3.9\%, FO_{\text{olive}} = 9.8\%$). A
lobster was found in only one red phenotype stomach in Massachusetts Bay. When present, lobster dominated stomach contents (offshore $PFI_{\text{red}} = 1.52 +/-0.37$, $PFI_{\text{olive}} = 2.56 +/-0.43$; inshore $PFI_{\text{red}} = 3.98$, $PFI_{\text{olive}} = 1.09 +/- 0.85$), with contents limited to a single lobster or even lobster claw. Despite these differences, best fit models indicated no important effects of either phenotype or region, which may be due to the low prevalence of this prey category in dissected samples (Table 3). As predicted, cod targeted fishes and lobster more often as they increased in size ($\beta_{\text{fish}} = 0.07$, $\beta_{\text{lobster}} = 0.05$). Stomach fullness did not differ between phenotypes and/or regions (offshore $TFI_{\text{red}} = 1.23 +/-0.17$, $TFI_{\text{olive}} = 1.20 +/-0.14$; inshore $TFI_{\text{red}} = 1.87 +/-0.33$, $TFI_{\text{olive}} = 1.10 +/- 0.16$).

**Discussion**

The phenotypes of fish often vary within the same species, differing with preferred habitats and migratory behaviors. Although fish pigmentation may change to match the color of occupied substrates (Sugimoto 2002), body shape is more likely to vary with habitat and general behavioral strategies (Wood & Bain 1995). Overall morphology or the shape of specific body parts often reflects water conditions like flow, such as the fusiform bodies and smaller fins of brook trout *Salvelinus fontinalis* occupying fast currents (McLaughlin & Grant 1994). Phenotype may also vary with patterns of habitat use, like the large fins of resident brook trout thought to enable finer spatial movements in complex habitats (Morinville & Rasmussen 2008), or migratory strategies, for example the streamlined forms and small fins of migrant coho salmon *Oncorhynchus kisutch* (Taylor 1991) The correlation of morphology and behavioral strategies has been identified in a range of freshwater and anadromous salmonids, including cutthroat trout *Salmo clarki* (Bisson et al. 1988), steelhead trout *O. mykiss* (Bisson et al. 1988), and Atlantic salmon *Salmo salar* (Riddell & Leggett 1981), and coho salmon *O. kisutch* (Taylor 1991).
Across these species, resident phenotypes express deeper bodies and thinner caudal peduncles, as well as larger medial fins, compared to the more fusiform shapes and larger tails of migrant conspecifics (Langerhans 2008, Morinville & Rasmussen 2008). Morphological differences are theorized to align with behavioral strategies, as migrants benefit from reduced drag as they swim across long distances and through rapid currents, while resident maneuverability in small complex habitats is aided by larger fins (Bisson et al. 1988, Webb 1988).

Distinct cod phenotypes expressing resident migratory strategies may be found throughout the species range, generally remaining within shallow structured habitats (Green & Wroblewski 2000, Kuparinen et al. 2015). Descriptions of these phenotypes, however, are generally limited to color. Where the body shapes of cod that are characterized by red pigmentation have been studied, however, their morphology has been found to follow the familiar resident form typical among salmonids. Discriminant models based on quantified morphometrics of resident and migrant cod at Cashes Ledge achieved successful classification rates (81%, Sherwood and Grabowski 2010, 86% this study) similar to those found in studies of salmonids such as brook trout (87%, Morinville and Rasmussen 2008). Yet the shapes of resident and migrant phenotypes caught in Massachusetts Bay were largely similar, where reclassification resulted in nearly 1 in 3 sampled cod incorrectly assigned phenotype. The focus of this study was on phenotype as defined by pigmentation and associated with strict habitat preferences and resident migratory tactics, which are on one end of the wide spectrum of cod spatial behaviors (Robichaud & Rose 2004). Although I predicted distinct morphology aligning with observed pigmentation in Massachusetts Bay, Goode (1887) lists a variety of “cod types” recognized by Massachusetts coastal fishermen in the late 19th century; these variants were thought to have
different habitat preferences and spatial behaviors based on when and where they were encountered.

Studies elsewhere investigating variation in cod migratory strategies found that groups of cod considered resident or sedentary may move within “home ranges” that could encompass much of the Gulf of Maine. Coastal cod along the southern coast of Iceland occupy relatively shallow (< 200 m) waters across a 3000 km$^2$ expanse of Icelandic shelf, their spatial behaviors defined as sedentary relative to morphologically distinct frontal cod that leave adjacent spawning grounds along the shelf for deeper waters, traveling thousands of kilometers before returning to spawn the following year (Pálsson & Thorsteinsson 2003, McAdam et al. 2012). Within the Gulf of Maine, conventional tagging and telemetry studies have identified different migratory strategies, including the sedentary tactics of specific spawning components (e.g., Ipswich Bay, Howell et al. 2008). Additionally, telemetry studies focused on cod use of large-grained substrates and boulder reefs at depths ≥ 60 m on Stellwagen Bank identified strict site fidelity (Lindholm & Auster 2003, Lindholm et al. 2007). Although morphometry was not examined in these studies within the Gulf of Maine, other efforts have identified the robust body shape characteristic of red phenotypes in coastal Massachusetts and at Cashes Ledge in deeper regions of the Gulf of Maine and Georges Bank (Sherwood & Grabowski 2015). If morphology is linked to behavior, the existence of cod in the Western Gulf of Maine expressing a range of migratory tactics, including strict residence at relatively deep structures and less restrictive residence leading to limited home ranges ~ 65 km$^2$, could result in increased variance in body shapes sampled across a range of depths throughout Massachusetts Bay and confounding analyses of body shape based on binary color assignments.
Otolith morphology and structure have been used to discriminate between fish stocks for nearly a century (Cadrin et al. 2014). Otolith shape is the combined result of genotype (Libungan et al. 2015) and conditions during early development (Hüssy 2008, Vignon 2012), as well as foraging success (Feet et al. 2002, Fox et al. 2003), growth (Folkvord et al. 2000), and metabolism throughout life history (Vignon 2012). At the scale of the Gulf of Maine, the most pertinent influences on otolith morphology are the combined contributions of spawning component and the ontogenetic influence of growth and development during the larval and juvenile stages. Spawning components of the Gulf of Maine’s cod population complex utilize overlapping spawning grounds (Zemeckis et al. 2014), which might be predicted to limit variance in otolith shape. However, differences in otolith morphology between spawning groups are not necessarily related to distance between spawning aggregations, as observed in the Irish and North Seas (Galley et al. 2006).

Across Atlantic herring (*Clupea harengus*) populations in the North Atlantic, spawning season explains the majority of otolith shape variance rather than gene flow (Libungan et al. 2015). The importance of hatch date to otolith shape lies primarily in early environmental and feeding conditions, as fish hatched in spring tend to experience conditions favorable to early growth, leading to marked differences in otolith morphology. The timing of spawning may influence otolith shape through seasonal effects on growth rates, since growth and otolith deposition are proportionally related (Pilling et al. 2007). Favorable feeding conditions and high prey density during development have been shown to result in changes to otolith material deposition, leading to an increasingly rectangular, less elongated outline (Hüssy 2008). Off the southern coast of Iceland, sedentary coastal cod experience more favorable foraging conditions leading to higher growth rates on the Icelandic shelf in comparison to migratory frontal cod that
move to deeper colder waters (Pállsson & Thorsteinsson 2003). A recent study focused on these two cod ecotypes found that faster growing coastal cod had rounder otoliths than frontal cod, aligning with previous experimental findings (Bárðarson 2015). Interestingly, the otoliths of cod sampled at Cashes Ledge were slightly rounder than those of cod collected from Massachusetts Bay. Based on the demonstrated factors influencing otolith morphology of cod and other marine fishes, these could be grounded in genetic differences or diverging conditions during early life history. Southern spawning components in the Gulf of Maine tend to reproduce in winter or early spring, while northern spawning components tend to form aggregations later in the spring and early summer (Zemeckis et al. 2014). If cod collected at Cashes Ledge were members of the offshore southern complex, then they may have experienced a long growing season post-hatch, while Massachusetts Bay cod collected during this study were not feeding until early summer and subsequently grew less during their first year. Samples collected during this study displayed no evidence of regional trends in gut fullness, suggesting foraging conditions from summer through early fall were not substantially different between Massachusetts Bay and Cashes Ledge.

A genetic basis for the observed differences in morphology is also possible, as the “inshore” northern coastal and “offshore” southern spawning components in the Gulf of Maine have only limited connectivity (Kovach et al. 2010, Zemeckis et al. 2014). Interestingly, variance in otolith shape within the Cashes Ledge otolith set was higher than that of those from Massachusetts Bay. This could indicate the presence of multiple spawning components at Cashes Ledge, increasing variability in group otolith shape characteristics without differences splitting along the lines of phenotype as I have defined it in this study (based on color). Despite significant differences in the shape of otoliths from inshore vs. offshore cod, regional distinctions accounted for only 3% of the variance in wavelet shape coefficients. For comparison, recent
efforts have led to 90% predictive discrimination between coastal and frontal cod in Iceland (Bárðarson 2015), suggesting the differences I have identified are small compared to the variance in otolith morphology that exists among cod populations from across the North Atlantic.

Regardless of heritability, the strong relationship between body morphology and migratory tactics suggests both the early adoption of behavioral strategies and their persistence throughout life history. Distinct morphology indicates divergence in body shape at some point during development, as is observed in morphologically distinct salmonid residents and migrants (Varian & Nichols 2010, Vehanen & Huusko 2011). In addition, growth models revealed regional differences in growth that emerged during the juvenile stage, rather than trajectories that eventually diverged at some later age. These findings align with resident cod in other regions (Gilbert Bay, Labrador, Morris and Green 2002; fjords along Norway’s Skagerrak coast, Kuparinen et al. 2015). The early divergence in growth rates of sedentary and mobile cod at each of these locations suggests the establishment of each phenotype is rooted in early life history. Across fish taxa, the early adoption of migratory or resident behavioral strategies is often related to metabolism, which is closely linked to growth (Jonsson & Jonsson 1993). As observed in salmonids (e.g., brook trout Morinville and Rasmussen 2003, Atlantic salmon, Forseth et al. 2003) and moronids (e.g., white perch Morone americana, Kerr and Secor 2009), migrants often have higher inherent metabolic demands during ontogeny, influencing time spent foraging as well as growth efficiency. Although greater energetic requirements may be related to reduced condition in juveniles under certain circumstances (Morinville and Rasmussen 2003), higher metabolic rates translate to increased scope for growth under favorable foraging conditions and high prey densities (Kerr & Secor 2009). The adoption of resident or migratory tactics is further influenced by conditions experienced during ontogeny. Migration rates of brown trout Salmo
are influenced by prey availability in nursery habitats (Wysujack et al. 2009). Following settlement, juvenile cod growth and behavior varies depending on available substrates (Renkawitz et al. 2011), environmental conditions (Tupper & Boutilier 1995, Ottersen & Loeng 2000), and density of conspecifics (Laurel & Brown 2006). In addition to the influences of nursery habitat and conditions on growth in early cod, inherent differences in growth rates between populations have been demonstrated experimentally (Purchase & Brown 2000) and may also occur within populations (Gjerde et al. 2004). Although no direct link between conditions during ontogeny and the adoption of migratory tactics in cod has been established, early growth has important consequences for survival in cod (Tupper and Boutilier 1995). Across taxa, behavioral strategies tend to be determined during development, as environmental conditions, prey availability, and other conditional factors interact with genotype to influence behavior (Dodson et al. 2013). Once established, these behaviors generally persist, as their adoption is related to metabolic development and other lasting characteristics. The morphological and modeled growth differences among phenotypes, the divergence of phenotype sometime during the first few years of life, and the correlation of phenotype and behavior suggest the early adoption of alternative life history strategies by cod.

Across the North Atlantic, migrant cod generally grow to larger sizes than resident or sedentary conspecifics (Morris and Green 2002, Sherwood and Grabowski 2010, Kuparinen et al. 2015), while reproductive groups that feature higher proportions of migrants tend to be more productive overall (Robichaud and Rose 2004). In some resident groups, lower prey availability and the dominance of invertebrates in the diet have been identified as major contributors to reduced size, slower growth, and red pigmentation (Morris and Green 2002, Gosse and Wroblewski 2004). Gut fullness of red and olive phenotypes in Massachusetts Bay and at Cashes
Ledge did not differ, suggesting little difference in foraging conditions faced by these phenotypes at inshore and offshore locations, while prey composition overlapped substantially. Although red phenotypes targeted crabs more often, large crustaceans were still abundant in olive phenotype stomachs and constituted a substantial proportion of their overall diets. The overlap between red and olive phenotypes in Massachusetts Bay and at Cashes Ledge during summer months may be linked to available prey communities, as seasonal oceanographic conditions at these locations support enhanced productivity (western Massachusetts Bay, Butman et al. 2007; Cashes Ledge, Witman et al. 1993). The results of telemetry studies conducted at Cashes Ledge revealed that olive phenotypes largely abandon shallow habitat in early fall (Chp.3), while a similar study conducted in Massachusetts Bay showed olive phenotypes moving using deeper habitats in the fall (Chp.4). If migrant cod only use these habitats seasonally, then the substantial overlap in crab consumption may end with their move away from the rocky shallows. However, past diet studies conducted in the Gulf of Maine have shown Cancer spp. crabs to be an important prey item for adult cod regardless of size (Link & Garrison 2002). Overall, the effects of size and location were more influential on diet than phenotype, which does not support trophic ecology as the primary source of lower growth in adult resident cod.

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Table 1. von Bertalanffy growth model parameter estimates (SE included in parentheses) for red and olive phenotypes; the 3 best-fit models are included, each featuring separate estimation of one coefficient.
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Table 2. Presence/absence best-fit model results; GLMM fit statistics and fixed effect coefficient estimates are reported; both individual fixed effects levels (*) and interactions (∞) that influence diet (as indicated using 95% bootstrapped confidence intervals) are indicated by symbol.
### Table 3

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Presence/absence best-fit model results; GLMM fit statistics and fixed effect coefficient estimates are reported; both individual fixed effects levels (*) and interactions (∞) that influence diet (as indicated using 95% bootstrapped confidence intervals) are indicated by symbol.
Figure 1. The inshore (Massachusetts Bay) and offshore (Cashes Ledge) locations where samples were collected.
Figure 2. (a) Location of morphometry landmarks (⊙) and color analysis (○) on pictured of cod tagged during telemetry study conducted at Cashes Ledge; (b) orientation of morphometric landmarks (L01) and measurements (M01).
Figure 3. (a) Picture of a whole cod otolith used for morphometric analysis, with major morphological features identified and general polar coordinates labelled; (b) mean shapes of inshore ( — ) and offshore ( — ) reconstructed from smoother wavelet coefficients; otolith morphological features where shape differed between inshore and offshore are circled ( O, O, and O).
Figure 4. Mean ± SD of standardized wavelet coefficients across groups (primary y-axis) and (ICC, a measure of between to within group variance in coefficients); groupings as defined in each panel (top to bottom): phenotype, phenotype within Massachusetts Bay, phenotype at Cashes Ledge, and inshore (Mass. Bay) and offshore (Cashes Ledge); circled (O, O, and O) ICC peaks correspond to otolith morphological features circled in Fig.3.
Figure 5. Canonical components analysis (CAP) scores derived using redundancy analysis of all groups; CAP1 explains 4% and CAP2 1% of variance in otolith shape.
Figure 6. Length-at-age (boxplots) and modeled growth (VBGM, lines) of red and olive phenotypes collected from Massachusetts Bay and Cashes Ledge; common $K$ and $t_0$ (Table 1) model estimates used for modeled growth.
Figure 7. (a) Biplots of % frequency of occurrence and abundance (PFI, ± SE) of prey categories from red and olive phenotypes collected from Massachusetts Bay (inshore) and Cashes Ledge (offshore).
Figure 7. (b) Boxplots of gut fullness (TFI) of red and olive phenotypes from Massachusetts Bay and Cashes Ledge.
Chapter 3: Distinct responses of sympatric migrant and resident phenotypes of Atlantic cod (Gadus morhua) to temperature at a remote Gulf of Maine seamount

Abstract

Many motile species adopt alternative life-history strategies, resulting in differences in diet, growth, morphometry, habitat use, timing of reproduction, and often migratory tactics among types. Atlantic cod (Gadus morhua) in the Gulf of Maine have been found to display marked variation in a number of life-history traits, exemplified by differences in body color (i.e., red vs. olive). And while differences in migratory behavior are suspected among these color types, this has yet to be definitively shown. Here, I combined the use of an acoustic telemetry system with fine-scale benthic habitat maps, and revealed that the red phenotype adhered to an isolated kelp forest covering < 2 km² of a remote seamount in the central Gulf of Maine. Meanwhile, the olive phenotype adopted diel vertical migratory behavior, possibly in response to a temperature gradient. Use of shallow, structured habitat was influenced by temperature and may be enabled by dynamic thermal conditions related to internal waves that persist at the study site throughout the summer and early fall. Detections decreased in response to changing thermal conditions, although phenotypes reacted to these changes in distinct ways: the olive phenotype abandoned available shallow habitat prior to peak summer temperatures, while the red phenotype remained until mid-fall when temperatures declined and temperature variability decreased.

These findings support a link between morphometry, color, behavioral strategies, and habitat preferences that may be widespread in Atlantic cod.
Introduction

Migrations of marine fishes are inherently collective movements, undertaken to satisfy important aspects of life history, such as reproduction or feeding. However, a growing body of research has placed important processes contributing to shared migratory behaviors at the level of the individual rather than the group across taxa (Nathan et al. 2008, Chapman et al. 2011, Pulido 2011, Chapman et al. 2012). The ubiquity of broadcast spawning and pelagic early developmental stages, the lack of obvious barriers to wide dispersal, and the results of early molecular studies targeting limited microsatellite loci has led to assumptions of low diversity in marine fish species (Sinclair & Iles 1988, Conover et al. 2006, Levin 2006). However, the application of new techniques in genomics and spatial ecology have revealed surprising diversity in the form of local spatial structure and diverse behavioral strategies in some marine fishes (Hauser & Carvalho 2008). Variance in migratory strategies has long been recognized in some species, such as Atlantic cod, but due to the unique genotypes and phenotypes that correlate with atypical spatial behaviors in some locations, populations with spatially segregated migratory types were assumed to be the appropriate unit for aggregating and studying migratory tactics (Robichaud & Rose 2004, Svedäng & Svenson 2006). The identification of sympatric partial migration within some marine fish populations (e.g., European plaice (Pleuronectes platessa) in the Irish Sea, Dunn & Pawson 2002) demonstrates the possible scales at which behavioral diversity may persist.

Atlantic cod (Gadus morhua) express a variety of migratory behaviors, with consequences for productivity, connectivity, and persistence (Robichaud & Rose 2004). Migrations vary throughout the species’ range, as the movements of some populations span hundreds of kilometers while the entire life history of others are contained within coastal
embayments as small as 60 km² (Morris & Green 2002, Neuenfeldt et al. 2013). With the exception of spatial behaviors during the juvenile stage (Cote et al. 2004), migratory strategies employed by cod are most often studied at the scale of populations or, more recently, spawning groups within populations (Pálsson & Thorsteinsson 2003, Robichaud & Rose 2004, Neat et al. 2006, Howell et al. 2008, Grabowski et al. 2011), although variance in individual movements have been documented, such as size-dependent diel vertical migration (Freitas et al. 2015). Adherence to particular strategies has most often been attributed to conditions that affect populations, such as oceanographic regimes (e.g., dominant currents, Robichaud & Rose 2004; local currents promoting retention, Knutsen et al. 2007) (Robichaud and Rose, 2004) or environmental conditions (e.g., temperature, Pampoulie et al. 2008, Grabowski et al. 2011), although the genotypic differences between behavioral groups have supported this focus on populations (Karlsen et al. 2013). Recent modeling efforts focused on cod diversity within stocks, and the effective size of cod populations suggest that populations and spawning components may be structured at finer scales than those recognized in current management regimes (Robichaud & Rose 2004, Bradbury et al. 2008, Kerr et al. 2010).

Historical accounts describe surprising diversity in phenotypes, behaviors, and habitat affinities of cod arrayed along the western coast of the Gulf of Maine (Goode 1887, Perkins et al. 1997, Ames 2004), with some local groups seeming to match descriptions of highly resident cod populations found in Labrador and coastal Norway (Dannevig 1953, Gosse & Wroblewski 2004, Kuparinen et al. 2015). During the 19th century, Massachusetts fishermen reportedly avoided shoal rocky reefs and kelp stands inhabited by small red-colored “rock” cod that commanded lower prices than large offshore “white-belly” cod (Goode 1887). More recently, resident behaviors have been identified at 65 m deep boulder reefs along Stellwagen
Bank (Lindholm et al. 2007), while a wide survey comparing cod inside and outside of Gulf of Maine and Georges Bank closed areas was able to infer a higher degree of resident behavior for cod within closures compared to adjacent open areas, by employing body shape analysis (Sherwood & Grabowski 2015). A common theme in these studies is the presence of individuals that either display (Lindholm et al. 2007) or are suspected to adhere to residence strategies in close proximity to known or suspected migrants (Sherwood & Grabowski 2015). Although sympatry of migrants and residents is observed in other regions (e.g., Northern cod seasonal use of Gilbert Bay, Labrador, Morris & Green 2002), those behaviors are reinforced by reproductive isolation and distinct genotypes (Ruzzante et al. 2000). Spawning components within the Gulf of Maine display similar genetic differentiation to that seen in other parts of the Northwest Atlantic, but weaker differentiation at a locus often associated with migratory behaviors in other regions (pantophysin I, Kovach et al. 2010). This provides strong evidence for local spatial structure within the Gulf of Maine, but leaves the selection along environmental gradients often associated with different migratory tactics an open question.

Although cod are capable of migrating over great distances (Neuenfeldt et al. 2013), concentrating on relatively fine-scale movements over limited geographic areas has the potential to reveal important factors that influence the adoption of migratory strategies by individuals. At this scale, resident strategies commonly emerge that are seemingly adapted to local conditions and shaped by varying degrees of reproductive isolation (Green & Wroblewski 2000, Grabowski et al. 2011, Karlsen et al. 2013). Oceanography and bathymetry that promote high retention rates of eggs and pelagic larvae, consistent availability of prey for all developmental stages, and isolated spawning and nursery grounds are associated with resident populations featuring substantially reduced productivity, distinct morphology and
often coloration, and strong preferences for specific habitats (e.g., Gilbert Bay “golden cod”, Green & Wroblewski 2000, Wroblewski et al. 2005). These “persistent residents” are often limited to shallow coastal embayments that allow very little dispersal to other grounds or populations and are usually genetically distinct from adjacent spawning components (Karlsen et al. 2013). Meanwhile, “offshore residents” in areas lacking these retentive and isolating characteristics tend to express less distinctive phenotypes and physiology, behaviors and ranges that substantially overlap with migrants, and periodic straying evident in lower genetic differentiation from proximate spawning groups (e.g., “coastal” Icelandic cod, Barðarson 2015, Pampoulie et al. 2008, Grabowski et al. 2011). Concentrating on common conditions that are associated with resident life-histories follows conventional understanding of cod migration strategies as population-level responses, while also providing testable combinations of habitat characteristics (e.g., geomorphology, temperature) and observed behavior that could reveal how these factors influence migratory behavior in individuals.

Recently, a cod phenotype, previously thought limited to coastal bays, featuring deep red to purple coloration and lacking the counter-gradient patterning typical in migrant cod (Gosse & Wroblewski 2004) was identified at a seamount in the central Gulf of Maine, Cashes Ledge. This phenotype is only caught in close proximity to an isolated kelp forest that persists on the shallowest peak within the Cashes Ledge formation (Ammen Rock, Sherwood & Grabowski 2010, Fig. 1). While “red” phenotypes are limited to a small area, cod expressing more common pigmentation, “olive” phenotypes, are caught throughout Cashes Ledge across all depths. Based on their color and apparently limited range (including depth range), smaller size at age, and more benthic diet, red phenotypes are predicted to be highly resident at Cashes Ledge (Sherwood & Grabowski 2010). These potential residents also
express distinct morphometry, featuring deeper bodies, larger heads, and thinner caudal peduncles than sympatric olive phenotypes (Sherwood & Grabowski 2010), possibly following relationships between body shape and behavioral strategies demonstrated in other fish taxa (e.g., salmonids, Morinville & Rasmussen 2008). Although the limited spatial range observed from sampling and the physical characteristics of this distinct phenotype provide circumstantial evidence of residence, this migratory strategy and such strict site fidelity have yet to be identified in cod at offshore locations that may lack many of the isolating features of coastal bays and fjords. Explicitly testing the degree to which these red phenotypes are resident and whether they prefer specific benthic habitats is an important step in describing the causes and consequences of migratory strategies and determining how cod spatial behaviors fit into the larger paradigm of partial migration across fish taxa.

During this study, I used fine-scale spatial and temporal observations of these cod phenotypes at Cashes Ledge to explore conventional hypotheses concerning migratory strategies and factors that influence the spatial behaviors of cod. Using acoustic telemetry and fine scale bathymetric and benthic habitat maps of Cashes Ledge, I tracked the presence and habitat use of red and olive phenotypes at Ammen Rock during the summer and fall of 2013. Vertically-stratified substrates allowed us to make inferences about cod habitat preferences using recorded depths, while the availability of these substrates and depths at Cashes Ledge permitted us to translate detections into general behavioral strategies. In addition to substrate type, I examined the influence of temperature, season, and time of day on habitat affinity and residency, providing a range of factors that could relate to and differ between migratory strategies. I predicted that (1) red phenotypes would be restricted to the highly structured shallow kelp forest and remain resident on Ammen Rock for the duration of the study. (2)
Conversely, olive phenotypes would use habitat in proportion with availability and spend far less time within the array, resulting in consistently greater depths than red phenotypes; these movement patterns would suggest a migrant strategy and reduced habitat selectivity. Finally, I predicted that (3) olive phenotype presence within the array would decrease with the onset of high temperatures in late summer/early fall, following Gulf of Maine seasonal distribution patterns, while red phenotype preference for highly complex habitats would preclude their abandonment of the kelp forest regardless of environmental conditions. Consistently differing spatial behaviors correlated with phenotype at a shared offshore location would provide additional context for partial migration in cod and other mobile marine fish.

Methods

Study Location: Cashes Ledge and Ammen Rock

Cashes Ledge is a seamount ~ 90 km east of Cape Ann, MA, consisting of a series of granite bedrock peaks and domes < 40 m in depth, interspersed by deeper depressions and valleys (Uchupi & Bolmer 2008). The shallowest of these peaks, Ammen Rock, reaches < 10 m depth (Fig. 2). Cashes Ledge is a highly productive and varied marine system that contains a range of bottom types (Witman & Sebens 1988, McGonigle et al. 2011) and diverse benthos (Witman 1985). Benthic habitats experience frequent temperature fluctuations and receive suspended particle subsidies (phytoplankton, zooplankton) throughout the spring and summer as internal waves pass through the area multiple times per day (Witman et al. 1993, Pile et al. 1996, Witman et al. 2004). Although there is substantial variance in dominant grain size across depths, geologic substrates are generally vertically stratified (Fig. 3c). The shallowest portion of Ammen Rock consists of intermittently broken granite ledge covered with algae to a depth of ~ 40 m: a Laminaria zone featuring fronds up to 2 m in length with intermittent
Sacharina spp. blanketing the ledge to 25 m, which then transitions to a sparse mixture of Agarum spp. and foliose red algae (Vadas & Steneck 1988, McGonigle et al. 2011). Beginning in the Agarum zone, isolated boulders and boulder piles among smaller grained sediments and bare granite ledge eventually give way to gravel and fine-grained sediments at depths > 75 m (Witman and Sebens 1988, Calvert, unpublished data). Ammen Rock’s steep slopes, varied habitats, and vertical stratification of substrate types create a mosaic of habitats within a small geographic footprint.

Acoustic Array

Ammen Rock, to a depth of ~ 60 m, was selected as the location of the acoustic telemetry array. Deploying the array at this depth provided detection coverage of the kelp forest on top of the ledge and other, deeper substrate types (Fig. 2,3). Four VR2W (Vemco) acoustic receivers were deployed at depths of 28-33 m to the northwest, northeast, southwest, and southeast of Ammen Rock on 31/05/13 and retrieved on 05/11/13. Receivers were deployed on low-profile, sub-surface moorings consisting of 11” hard plastic trawl floats, Subsea Sonics AR-60-E acoustic releases, and ~100 lbs of steel weight connected via torque-free 12 strand synthetic line and marine-grade chain. V13P acoustic transmitters reported identification (unique to each transmitter and fish) and depth (m) every 60 to 90 s, which were recorded by receivers whenever transmitters were within detection range (~ 400 m). Receiver locations and the power of the V13P transmitters ensured overlapping detection ranges for all four receivers, creating an array detection area of 2 km² under 95% of oceanic conditions observed during the deployment period (based on 5 years of wave height observations from NOAA buoy 44005, NOAA 2016, and tag manufacturer’s range estimates, Vemco 2016); array detection area was confirmed using a V16 range-testing transmitter. From 22/07/13 to
Onset TidbiT data loggers attached to the NE and SW receivers recorded bottom temperatures (~ 1.5 m from bottom) at 5 minute intervals. The coordinates of the four receivers and their estimated detection areas were used to extract depth and substrate data from bathymetric and habitat map rasters, providing depth distributions of substrates at a resolution of 5 m² (Figs. 2,3).

In addition to temperature measured within the array, I obtained wave height (m) and wind speed and gust (m/s) data from NOAA and NERACOOS environmental monitoring buoys to identify any storm activity that could affect both detection range and cod behavior. Since the Cashes Ledge buoy (NOAA station 44005) was not functioning during the period of this study, I used data from monitoring buoys deployed at Jordan Basin (NOAA station 44037), Jeffreys Ledge (NOAA station 44098), and central Maine Shelf (NERACOOS station E01). Weekly mean and max wave height, wind speed, and wind gust were used to assess sea state and identify storm events in the central Gulf of Maine.

Tagged fish

Atlantic cod used in this study were caught via hook-and-line (frozen clam bait) from Ammen Rock at depths < 40 m. Once hooked, cod were slowly reeled to the surface to avoid barotrauma. Following hook removal and initial visual assessment for barotrauma, cod (n = 25) were transferred to an onboard livewell (900 L seawater, 100% changeover every 20 minutes) and allowed to acclimate for ≥ 30 minutes. At the end of the acclimation period, cod were again assessed for barotrauma as well as overall condition. Based on visual observation of body coloration and overall size, five red and five olive phenotypes representing available size ranges were chosen at random for this study. Although field designations of cod as red or olive were based on visual analysis alone, I confirmed phenotype assignments by measuring...
the ratio of red to green pixels (RGR) in a small (10 cm²) patch of operculum posterior to the eye. The threshold RGR value identified by Sherwood and Grabowski (2010) was employed in this study (i.e., RGR ≥ 1.3 indicated red phenotype, RGR < 1.3 indicated olive phenotype). Pictures of tagged cod (sRGB color profile) were analyzed using the image processing program ImageJ (Rasband 2012).

V13P acoustic transmitters were implanted following the protocol described in Conroy et al. (in review). Following the procedure, tagged individuals were transferred to a livewell filled with fresh seawater and allowed to recover during an observation period ≥ 15 minutes. If the cod recovered from surgery (remained upright, swam normally, and responded to stimulus, Woody et al. 2002) by the end of the observation period and transmitters were confirmed as functioning (checked using a receiver connected to a laptop), they were released within the array. If they had not recovered, tagged individuals were sacrificed and transmitters were recovered and cleaned for reuse. As a final confirmation of recovery, immediate post-release behavior was visually assessed.

Analysis

Since cod spatial behaviors change throughout ontogeny (Howe et al. 2002, Lough 2004) and size is a reasonable measure of age in fish (Macdonald & Pitcher 1979), I compared the total length distributions of phenotypes included in the study using a t-test. To account for the demonstrated differences in red and olive phenotype growth rates at Cashes Ledge, I used Sherwood and Grabowski’s (2010) phenotype-specific von Bertalanffy growth models to estimate the age of study subjects from measured total length; estimated ages were then compared using a t-test.
Detection and depth records for each tagged cod from throughout the period of the study (158 days, 31/05/13 to 05/11/13) were compiled into profiles. Reported depths were adjusted to account for change in tidal elevations using the DTU10 global ocean tidal model (Cheng & Andersen 2011). Depth profiles of each tagged cod were aggregated into day and night mean depths and detection profiles were summarized as hours per day and night period using date and coordinate-specific sunrise and sunset times provided by NOAA’s Solar Calculator (NOAA Earth System Research Laboratory 2016). These aggregation steps were introduced to ensure sufficient temporal overlap in highly unbalanced (i.e., more than an order of magnitude difference between phenotypes in number of detections) repeated measures data for analysis while still retaining enough resolution to describe cod movements on temporal scales relevant to known behavioral characteristics (e.g., swimming speeds lower at night than during the day, Løkkeborg & Fernö 1998). The effects of phenotype (red, olive), period of the day (day, night), time (time steps through entire study), season (summer, fall), receiver (NE, NW, SE, SW), size (total length), and bottom temperature (daily mean, maximum, and minimum, limited to 22/07/13 to 05/11/13) on depth were analyzed using linear mixed models (LMMs) with cod/receiver as the random effect. Next, the influences of phenotype, period of the day, time, season, size, and bottom temperature on presence were analyzed using generalized linear mixed models (GLMM) with binomial link functions and including time and cod as random effects. To account for autocorrelation in the repeated measures data, I employed 1st order autoregressive (AR(1)) covariance structure in LMMs and included observation level random effects (i.e., time and cod) in the GLMMs. Prior to inclusion in models, fixed effects were tested for multicollinearity (i.e., variance inflation, Dormann et al. 2013); any combination of fixed effects as individual explanatory variables or within interactions that were found to be
collinear were excluded from models, using a variance inflation factor (\textit{VIF}) threshold of 10 (\textit{e.g.}, \textit{VIF} = 11 would lead that variable combination or interaction to be excluded from analyses, Dormann et al. 2013). Since bottom temperature records were limited to late July through the end of the study, additional LMMs and GLMMs were constructed incorporating data from the period for which bottom temperature was available. Following restricted maximum likelihood model fitting, a subset of models were selected based on AICc (Burnham and Anderson 2002). The contribution of individual model terms (\textit{i.e.}, fixed effects) were tested via likelihood ratio tests; model term estimates were compiled via Wald tests. Models were further assessed using both marginal (fixed effects) and conditional (fixed and random effects) effect sizes (\textit{i.e.}, \(R^2\), Nakagawa & Schielzeth 2013).

Due to the gradient of decreasing structural complexity with depth that exists across the varied biological and geological substrates of the study site, I interpreted observed depth distributions in the context of available bottom types. Tagged cod and substrate depth distributions were assessed qualitatively and through summary statistics. Additionally, the proportion of shallow, structured habitats covered the by the array (\textit{e.g.}, > 67% of \textit{Laminaria} kelp forest) and the lack of similar habitats for a distance of > 60 km allowed us to interpret detection rates within the array as preference for particular habitat types as well as residence behavior.

All statistical analysis, data manipulation, and mapping were conducted in the R statistical program (R Core Team 2016).

\textbf{Results}
Collected cod that passed initial visual assessment for barotrauma and condition (n = 25) were 35-75 cm TL. Although experimental subjects were selected to be representative of the range of observed sizes for both phenotypes, tagged red phenotypes (39.8 - 57.5 cm total length [TL] range) were significantly smaller than olive phenotypes (49.5 - 66.5 cm TL range; \( t_8 = 2.78, p = 0.02 \)). However, modeled ages of tagged red and olive phenotypes did not differ (predicted age ranges of olive phenotype 2.47 - 5.34 years and red phenotype 1.31 - 5.40 years, \( t_8 = 1.35, p = 0.21 \)). Red phenotype RGR values were between 1.89 and 3.50, while olive phenotype RGR values were between 1.11 and 1.26, confirming initial classification to phenotype at the time of tagging.

Over the period of the study 53,281 detections of tagged cod were recorded, 51,585 of red and 2,236 of olive phenotypes (one olive phenotype, GM13-SRG-03, was not detected despite being released within the array; summary statistics and analyses of depth and presence exclude this individual). Daily maximum vertical movement at times exceeded daily tidal depth range for all 9 detected cod following release, indicating the post-surgical survival of these individuals. Tagged cod were detected 37.4 ± 2.5 times per day (mean +/-SE, used throughout unless otherwise indicated), with red phenotypes being detected over an order of magnitude more frequently (64.5 ± 4.2) than olive phenotypes (3.5 ± 1.0). Red phenotypes remained within the array for more than ¼ of each day throughout the study (6.6 ± 0.34 hours), while olive phenotypes were present less than 1 hour per day (0.9 ± 0.10 hours, Fig. 4). Mean detections of all tagged cod were nearly evenly divided between day and night, 18.7 ± 1.3. However, presence within the array differed depending on phenotype (phenotype*time of day, \( \chi^2 = 215.90, p < 0.01, \) Table 1), with olive phenotypes spending more time in detection range during the day (2.36 ± 0.7 detections/period, 0.5± 0.06 hours/period) than at
night (1.1 ± 0.4 detections/period, 0.4 ± 0.04 hours/period) while red phenotype behaviors were not affected by time of day (day: 31.8 ± 2.1 detections/period, 3.3 ± 0.17 hours/period; night: 32.7 ± 2.3 detections/night, 3.3 ± 0.18 hours/period). Both phenotypes spent more time within detection range during the summer (red 8.8 ± 0.5 hours/day; olive 1.3 ± 0.16 hours/day) compared to the fall (red 3.5 ± 0.4 hours/day; olive 0.3 ± 0.06 hours/day), although the reduction in time spent within the array was larger for olive than red phenotypes (phenotype x season, χ² = ratio = 64.65, p < 0.01); this may reflect, in part, the 67% of total olive phenotype detections that occurred within 7 days of tagging, that contrasts with red phenotypes whose detections in any single week never exceeded 10% of total detections (Fig. 5).

Throughout the period of the study, red phenotypes (19.43 ± 0.21 m) remained shallower than olive phenotypes (25.49 ± 0.32 m) while within the array (phenotype, LRT = 5.78, p = 0.02, Table 1, Fig. 3), with little difference in depth between daytime (17.7 ± 0.03 m) and nighttime periods (17.2 ± 0.02 m). Olive phenotypes moved shallower from day (32.6 ± 0.20 m) into night (28.5 ± 0.24 m; time of day, LRT = 21.86, p < 0.01). The depth distributions of tagged cod throughout the study were mostly limited to the shallower depths available within the array, with 96% of red and 89% of olive phenotype depth observations being shallower than the overall median depth for the array (31.2 m). These shallows consisted of kelp (63% coverage) and rock (32% coverage). The maximum (25.0 m) and median depths (18.0 m) of available Laminaria kelp habitat aligns with the 95th % and median (17.4 m) observed depths of red phenotypes (Fig. 3). While 23% of observed olive phenotype depths occurred within this kelp zone, 76% corresponded to the boulder and rock region extending from the edge of the kelp habitat to 55 m. Overall, olive phenotypes were more
variable in their vertical movements within the array, leading to 35% of all depths being ≥ 1 SD (7.54 m) from mean depth, compared to just 9% of red phenotype observations (SD = 4.01 m).

As bottom temperatures within the array increased from mid-July (daily mean 8.3°C) to late August (daily mean 9.6°C), all tagged cod spent increasingly more time in the shallow habitats surrounding Ammen Rock (Fig. 4). The change in red phenotype presence was small, an increase from 8.0 hours/day over the first 1.5 months of the study to 9.4 hours/day in August, while olive presence increased by a factor of 10, from 0.2 to 2.0 hours per day over the same periods of the study. Weekly detections of both phenotypes peaked in early August (week of 02/08/13: red 5179 detections, olive 127 detections), as did mean hours present per day (week of 16/08/13: red 10.14 hours/day, olive 2.89 hours/day). However, once daily mean temperatures reached 10°C and maximum daily temperature exceeded 16°C, both phenotypes reduced the amount of time they spent within the array. Olive phenotypes returned to brief visits (0.2 hours/day), while red phenotype presence was halved (4.2 hours/day). As temperatures declined from their peak during early October, red phenotype presence further decreased to < 0.5 hours/day. This marked reduction in time red phenotypes spent within the array coincided with the steep decline in hourly variance in temperature (Fig. 6). Bottom temperatures in August (Fig. 7a), before the observed behavioral change in red phenotypes, feature both larger patterns of increasing and decreasing temperature that align with the tidal cycle and brief fluctuations in temperature of up to 4°C that may indicate periodic internal waves as described by Witman et al. (1993); bottom temperatures in October (Fig. 7b), following the behavioral change, feature the cyclical tidal signature but lack brief fluctuations, which would align with the seasonal disruption of internal waves at Cashes Ledge (Witman et
The overall relationship between bottom temperature and detection rates was positive, albeit substantially more influential for red (mean bottom temperature $\beta_{\text{red}} = 0.81$, Table 1) than olive phenotypes ($\beta_{\text{olive}} = 0.03$). Interestingly, maximum temperature had a pronounced negative influence on red phenotype detection rates ($\beta_{\text{red}} = -0.44$), reflecting the rapid decrease in red phenotype time spent within the array in late summer, and surprisingly little effect on olive phenotypes ($\beta_{\text{olive}} = 0.04$), despite their decreased detection rates around the same time. Regardless, the influence of temperature on presence was small in comparison to the importance of phenotype (Fig. 8). The relationship between cod behavior and temperature did not extend to the depth preferences of red and olive phenotypes, as observed depths of cod did not change with increases and decreases in temperature throughout the study (Fig. 4). Cod did move deeper as maximum daily bottom temperatures decreased in October, but this did not translate to a consistent relationship between maximum temperature and depth preference.

Ocean conditions in the form of wave and wind energy remained consistent throughout the study (Fig. 9). Maximum wave height, sustained wind speed, and wind gusts during the course of the study occurred at the end of October, two weeks after the noted decrease in red phenotype use of the kelp forest. The timing of this increased wind and wave energy did coincide with the maximum mean weekly depth of red phenotypes. The previous maxima in these measures of storm activity occurred in early August when both phenotypes were spending in excess of $\frac{1}{4}$ of each day within the array, suggesting storm activity did not affect observed behaviors.

**Discussion**

Accounts of deep-bodied and darkly pigmented cod often discuss the limited depths and structural complexity of the habitats they occupy (Goode 1887, Dannevig 1953, Sherwood &
Grabowski 2010). While olive phenotypes utilized all available substrates to some extent, with most of their activities focused on rock habitats, red phenotypes were almost exclusively observed within the boundaries of the kelp forest, that would seem to support my predictions concerning habitat affinity and behavioral strategies. However, changes in the spatial patterns of red phenotypes in late summer indicate their relationship with specific habitat types is seasonally variable. In August, detection rates of both phenotypes decreased over several weeks, resulting in olive phenotypes mostly deserting the shallow habitats covered by the array and red phenotypes halving the time spent within detection range. In early October, red phenotypes again underwent a change in behavior, appearing to largely abandon the habitats covered by the array. As *Laminaria* and *Saccharina* kelps persist in the Gulf of Maine throughout the months covered by my study (Witman 1987, Steneck et al. 2002) and no major storm events occurred during these months, these shifts in movement did not coincide with major changes to physical substrates. The abandonment of extant kelp indicates weaker habitat affinity than I had initially predicted. Over the final 2 weeks of the study, 3 tagged red phenotypes appeared to utilize deeper habitats (≤ 38 m) within the detection range of the southeastern receiver, although observed depths at other receivers remained within the depth limits of the kelp. More than 77% (0.61 km$^2$) of the kelp forest (0.80 km$^2$) was covered by my array, but relatively shallow habitats consisting of rock ledge with intermittent stands of *Agarum* are available across a broad gentle slope that extends beyond the detection range of my array’s southeastern edge to a depth of ~ 40 m (McGonigle et al. 2011). This shallow slope offers structured habitat (Fig. 10), although the structural complexity of large-grained sediments does not match that of macroalgae (Carr 1989, DeMartini & Roberts 1990). At the scale of the Gulf of Maine, cod distributions shift in depth seasonally in response to
environmental factors such as temperature, leading to occupation of large grained sediments at
greater depths in the fall (Methratta & Link 2006). Although this analysis is focused on local
movements rather than regional cod distributions, similar processes may drive fine-scale
behavior as temperature and sea state conditions change.

Major factors influencing divergent sympatric habitat use in fish are complicated and
often conditional (Wysujack et al. 2009), as environmental conditions (Conroy et al. 2015),
predation pressure (Skov et al. 2010), and inherited characteristics (Dodson et al. 2013) may
affect spatial behaviors. However, resident and migrant strategies are often related to
metabolic requirements, as migrants of some species are driven to spend more time and
expend more energy on feeding to meet the demand of higher metabolic rates (e.g., white
Rasmussen 2003); when these efforts payoff, migrants are rewarded with increased growth
rates and greater fecundity in comparison to residents (Kerr & Secor 2010). Although this
connection between metabolism and behavior has not been demonstrated for red and olive
phenotypes at Cashes Ledge, proximate resident and migrant cod often differ physiologically,
with consequences for temperature tolerances (Goddard et al. 1997) and depth preferences
(Pampoulie et al. 2008). Distinct temperature tolerances likely cascade to differences in
metabolic rates (respiration, excretion, specific dynamic action, and assimilation efficiency),
which are temperature-dependent in cod (Claireaux et al. 2000), as typically shallower
residents face a wider range of temperatures than more mobile migrants (Goddard et al. 1997,
Le Bris et al. 2013). Such effects on metabolism could contribute to the lower growth rates
and reduced productivity of red phenotypes at Cashes Ledge (Robichaud & Rose 2004,
Sherwood & Grabowski 2010). Observed differences in fine-scale habitat use at Cashes Ledge
may likewise reflect distinct energetic requirements. The diverging growth rates of red and olive phenotypes may be due to the combined effect of inherently different metabolic rates and diverging foraging efforts, along with the direct influence of other important factors such as temperature, as olive phenotypes seek to meet energetic requirements by utilizing more of the available habitats.

At the level of the individual, temperature has profound effects on the physiology, growth, feeding rates, condition, and metabolic rates of cod (Claireaux et al. 2000, Björnsson et al. 2001, Rätz & Lloret 2003, Freitas et al. 2010), which can directly influence behavior (Claireaux et al. 1995). At the scale of the Gulf of Maine, seasonal distributions follow regional temperature trends as cod move to deeper, cooler habitats in the fall (DeLong & Collie 2004, Methratta & Link 2006). Despite demonstrated tolerance for a wide spectrum of conditions, theoretically optimal temperatures for cod growth and metabolism fall in the range of 7-14 °C (Björnsson et al. 2001, Björnsson & Steinarsson 2002, Freitas et al. 2010). The reduction in use of shallow habitats by both phenotypes as temperature increased through August seems to align with region wide trends in cod distributions (Methratta & Link 2006) and the importance of temperature to cod condition and its effects on behavior (Claireaux et al. 1995). Applying the relationship between cod size and temperature reported by Bjornsson and Steinarsson (2002) to cod tagged during this study (body weight ~1200 g), bottom temperatures recorded at Cashes Ledge in August could translate to a 25% reduction in maximum potential growth rates when compared to optimal temperatures. This substantial cost directly associated with temperature suggests the observed changes in habitat use may have been induced by unfavorable environmental conditions. However, the near-complete abandonment of the kelp forest by red phenotypes did not occur until early October, when
bottom temperatures had fallen from their previous annual maximum, matching daily means observed during the August peak in cod use of shallow habitats. Additionally, the continued, albeit reduced, presence of red phenotypes in shallow habitats throughout the period of highest measured bottom temperatures does not support a simple relationship between temperature and behavior that is mediated by individual physiology.

Behavioral responses to temperature are not limited to avoiding unfavorable conditions or seeking favorable ones. When faced with a stratified water column, recently matured cod may adopt a diel vertical migration strategy, remaining in cold deep waters by day and moving to warm shallow waters by night (Claireaux et al. 1995). This behavioral strategy has been observed along the Norwegian coast, where small adult cod move shallower at night until mid-summer, when surface temperatures reach 15 °C (Freitas et al. 2015). Olive phenotypes tagged at Cashes Ledge, whose sizes fall within the range studied in the Norwegian Skagerrak, moved vertically between day and night throughout the length of the study. In particular, their nighttime activities centered on habitats 2-3 m shallower than those of their daytime activities, a vertical shift in center of activity similar to that identified by Freitas et al. (2015). Past studies have identified a thermocline developing at Cashes Ledge in the spring, its depth fluctuating with the jagged shallow bathymetry of Ammen Rock and other adjacent peaks (Witman & Sebens 1988, Witman et al. 1993), which provides the prerequisite temperature gradient for these behaviors. Interestingly, the presence of a shallow thermocline might also contribute to the shallow distribution of olive phenotypes throughout the summer, a departure from my prediction of no measurable depth preference within the array. Vertical migratory strategies in mature cod may result from several processes, including predator avoidance, foraging, and maintenance of physiological condition (Hop et al. 1992,
Neat & Righton 2007). Although moving to shallow habitats in search of specific prey is an impetus for migratory behavior in some fishes (e.g., spotnape cardinalfish, *Apogon notatus*, Fukumori et al. 2008), this has proven unlikely for the generalist demersal predator cod (Claireaux et al. 1995, Freitas et al. 2015). This would seem to hold true for olive phenotypes at Cashes Ledge, whose diets consist mainly of benthic (*Cancer* spp. crabs) and demersal species (cunner (*Tautogolabrus adspersus*)) not restricted to the shallow confines of Ammen Rock (Sherwood & Grabowski 2010, Ch. 2). Instead, small adult cod may utilize available temperature gradients largely as a means to maximize growth rate and physiological condition, due to increased digestive and metabolic efficiency in warm water and the long-term effects of temperature on essential processes such as reproduction for recently matured cod (Levy 1990, Claireaux et al. 2000, Yoneda & Wright 2005). Once surface waters in Norway reached 15 °C in mid-July, small cod ceased returning to shallow water at night (Freitas et al. 2015). Similarly, tagged olive phenotypes abandoned the shallows around Ammen Rock once a temperature threshold was breached. Although the mean temperature at which this behavioral change occurred (10 °C) was lower than that observed in Norway and within the range of favorable growing conditions, surface temperatures at Cashes Ledge in August are substantially higher than those on the bottom (2008-2012, 18.9 °C ± 0.03, NOAA buoy 44005, NOAA 2016) and daily maximum bottom temperatures exceed 16 °C. Norwegian cod resumed vertical migrations in winter, although the inversion of the thermocline (i.e., higher temperatures in deeper bottom waters) coincided with a reversal in depths that cod occupied during the day vs. night (i.e., deeper during the night, shallower during the day, Freitas et al. 2015). It remains unclear whether olive phenotypes adhere to similar winter strategies around Cashes Ledge following a period at constant depth in the fall.
or, alternatively, simply maintain consistent vertical behaviors at greater depth, beyond the
detection range of my array (~ 55 m), in reaction to the deeper orientation of the thermocline
in late summer (Witman & Sebens 1988). Resolving this question would reveal whether
recently matured cod seek temperature gradients in order to maximize condition via
thermoregulatory behavior or, alternatively, simply avoid unfavorable environmental
conditions that negatively impact their physiology.

Despite the persistence of a temperature gradient, red phenotypes did not adopt vertical
migratory behaviors akin to olive phenotypes. Even as the time they spent away from the
array increased along with bottom temperatures in mid-August, red phenotypes still spent
several hours per day within the kelp forest. Physiology affecting metabolic rate and
temperature tolerance may provide some of the explanation for their continued, though
reduced residence, but other seasonally variable characteristics of Cashes Ledge align with the
timing of red phenotype behavioral changes. During the spring and summer Cashes Ledge is
consistently buffeted by internal waves, depressions in the thermocline caused by thermally
stratified water moving over steeply sloped subtidal features via tidal currents and propagated
over great distances by gravity (Garrett & Munk 1979, Witman et al. 1993). Witman et al.
(1993) determined that wave events typically occur at 1-3 hour intervals, resulting in regular
temperature fluctuations of up to 6 °C and 5-fold increases in nutrient and plankton
concentrations at depth as the thermocline oscillates up to 30 m in the water column (Witman
et al. 1993). Subsequent work has shown these effects impact the landscape level ecology of
the seamount (Witman et al. 2004). The effects of internal waves include extended vertical
ranges of macroalgae (Witman et al. 1993) and sponges (Witman 1985), as well as enhanced
secondary productivity (i.e., invertebrate growth rates, Lesser et al. 1994) and increased
foraging activities (*i.e.*, predation rates on crustaceans, Witman & Sebens 1992). Subsidized benthic productivity likely contributes to the high densities of cod and other demersal species regularly observed around Cashes Ledge (Witman & Sebens 1992, Grabowski 2010) and may make residence within the kelp forest possible. Although no measurements of chlorophyll or plankton concentrations were made during this study, bottom temperature records indicate similar temperature conditions to those described by Witman et al. (1993). Brief changes in temperature of up to 4 °C throughout the summer and early fall decreased abruptly in mid-October at the same time as red phenotype appeared to largely abandon the *Laminaria* kelp forest. Since these events are nonlinear gravity waves propagating along the existing stratified water column, the increased depth of the thermocline in late summer (Witman & Sebens 1988) leads to subsidies further down the slope of Ammen Rock (Witman et al. 2004). This could help explain the decrease in kelp use by red phenotypes witnessed in August, as they visit subsidized benthic habitats outside of the array’s detection range. Internal waves and their effects persist into the fall, eventually ceasing in early October (Witman et al. 1993), coinciding with red phenotype abandonment of shallow habitats within detection range of the array. In addition to the loss of these subsidies, surface conditions in the central Gulf of Maine become more dynamic in October. Seasonal storm activity and wave conditions increase disturbance in shallow sub-tidal benthic habitats via wave-induced and current shear stress and may physically alter macroalgae (Denny 1987, Gardner et al. 2001, Steneck et al. 2002). The effects of seasonal changes in oceanographic conditions likely reduce the relative habitat quality of the kelp forest, which could influence the spatial behaviors of red phenotypes. However, identifying the specific processes leading to red phenotype spatial behaviors would require the application of multi-disciplinary approaches simultaneously tracking the
movements of tagged cod at Cashes Ledge while recording temperature, productivity, and current across available depths throughout the year, observing conditions coinciding with cod abandonment and recolonization of Cashes Ledge’s shallowest habitats.

Although a broad swath of spatial behaviors have been categorized as resident or sedentary, the extensive history of tagging efforts (Robichaud & Rose 2004) and more recently, physiological and molecular approaches (Goddard et al. 1997, Bradbury et al. 2010) applied to cod population structure have identified a few commonly encountered strategies (Green & Wroblewski 2000, Grabowski et al. 2011, Karlsen et al. 2013). The shallow habitats occupied by red phenotypes at Cashes Ledge feature some of the prerequisites for persistent residence summarized by Wroblewski et al. (2005): ample benthic and demersal forage (based on diet analyses of red phenotypes, Sherwood & Grabowski 2010) and the existence of essential habitat for immature cod (Grabowski 2010), but the remaining requirements may not be present. The circulating currents around Ammen Rock are unlikely to retain early pelagic stages (Vermersch et al. 1979, Manning et al. 2009), although recent oceanographic modeling efforts have demonstrated that some self-recruitment to the area is possible and larvae could be supplied to Cashes Ledge from coastal Maine on a consistent basis (Churchill et al. 2016). To this point, no consistent spawning events nor high concentrations of cod eggs have been identified in the vicinity of Cashes Ledge (Berrien & Sibunka 1999); however, red phenotype gonadosomatic index peaks in the fall (G. Sherwood, pers. obs.), which, when considered with the possibility of widespread resident behaviors expressed in offshore closures (Sherwood & Grabowski 2015) as well as current proposals to reduce the extent of Cashes Ledge Closed Area (New England Fisheries Management Council 2014), warrants concerted efforts to locate potential spawning aggregations and eggs. Isolated reproduction and local retention and
survival of early life history stages coincides with widely adopted resident migratory tactics in some populations of cod, but it remains unclear whether or not these dynamics exist for red phenotypes at Cashes Ledge. Whatever processes and conditions contribute to the unique phenomenon of phenotypically distinct offshore residence, they may be a departure from the conventional understanding of cod residence strategies, which provides an opportunity to examine assumptions associated with this life history strategy in cod and its origins.

Sedentary cod behaviors have been variably identified as the outcome of accrued responses to local conditions (e.g., AFGP production, Goddard et al. 1997) and the inherited result of past selective forces (e.g., pan I genotypes, Árnason et al. 2009). Conditions during ontogeny, such as habitat complexity following settlement, have strong and lasting influence over cod habitat use patterns and reactions to predation threats (Salvanes & Braithwaite 2005). In addition to early experiences, inherited genotype at the pan I locus is often associated with environmental and behavioral gradients (Case et al. 2005, Sarvas & Fevolden 2005, Árnason et al. 2009), with the pan IAA genotype most often associated with less mobile inshore/nearshore spawning components in the Gulf of Maine and the rest of the western Atlantic (Beacham et al. 2002, Kovach et al. 2010); although evidence of the non-neutrality of this locus is mixed in other regions (e.g., Iceland, Eiriksson & Árnason 2013). pan I genotype frequency seems to have been influenced by selective pressures in the Gulf of Maine, although differentiation between spawning groups at this locus was low (Kovach et al. 2010). The combined effects of experience and inheritance on residence behaviors in cod have not been explicitly explored. However, similar interactions have been identified in latitudinal gradients in morphology along the western Atlantic- evidence of interacting intrinsic (genotype) and extrinsic (temperature) factors was found during common garden experiments examining
stabilizing selection in early growth and development (Marcil et al. 2006b, a). When it comes to migratory behavior in cod, resident strategies, low growth rates, and distinct morphometry persist in reproductively isolated populations, as demonstrated by Gilbert Bay “golden cod” (Green & Wroblewski 2000, Ruzzante et al. 2000). A threshold model of migration predicts similar outcomes resulting from distinct combinations of genotype and environment (Pulido 2011). Dodson et al. (2013) summarized alternative migratory behaviors in salmonids as threshold traits, continuous attributes that trigger the adoption of different behavioral strategies when they reach threshold values during ontogeny. This approach encompasses processes that could lead to the demonstrated reproductive isolation of residents in Gilbert Bay and the Eastern Atlantic (Ruzzante et al. 2000, Karlsen et al. 2013), as well as the persistence of multiple migratory tactics adopted by distinct phenotypes within the same spawning component. However, the existing evidence for the importance of reproductive isolation to the maintenance of diverse migratory behaviors remains strong. Additional work aimed at determining the genetic components of phenotype and behavior in Gulf of Maine cod would provide insight into the processes that support partial migration in mobile marine fishes.

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### Tables

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**model fixed effects abbreviations:**
- $p$ - phenotype
- $t_b$ - mean bottom temperature
- $t_{mi}$ - min. bottom temperature
- $t_{mx}$ - max. bottom temperature
- $s$ - cod size (total length)
- $dn$ - day/night (period of day)
- $t$ - time step (continuous throughout study)
- $r$ - receiver (NE,NW,SW,SE), *LMM only*

Table 1. Linear (LMM) and generalized linear mixed model (GLMM) results and model diagnostic measures.
Figure 1. Western Gulf of Maine bathymetry with location of study site, Cashes Ledge highlighted; broken line indicates boundary of Cashes Ledge Closed Area.
Figure 2. Categorical substrate (left) and bathymetry (right) maps of Ammen Rock array (black circles) and surrounding area.
Figure 3. Boxplots of (a) olive and red daily mean depths by season and (b) overall depths and (c) categorical substrate depths within the array.
Figure 4. (top) Mean (± SE) hours detected per day per individual aggregated by week for both phenotypes; (middle) boxplots of hourly mean depth aggregated by week; shaded area indicates full range of temperatures within week; (bottom) mean (± SE) depths per day aggregated by week for both phenotypes.
Figure 5. Weekly detections (bars) and cumulative proportion of total detections (line) of olive (top) and red phenotypes (bottom).
Figure 6. Mean daily (solid line), minimum, and maximum (broken lines) temperature recorded at northeast receiver (30.5 m, ~ 1.5 m from bottom), hourly variance in temperature (weekly mean of the hourly moving average temperature) also reported (filled circles).
Figure 7. Temperature (solid line) recorded every 5 minutes at northeast receiver (30.5 m, ~ 1.5 m from bottom) during the weeks (a) 8/18/13 to 8/24/13 and (b) 10/20/13 to 10/26/13; times of high (+) and low (x) tides also reported.
Figure 8. Season-specific predicted probabilities of red and olive phenotypes visiting the array during day or night across temperature conditions (minimum, mean, and maximum daily bottom temperature); predictions derived using best-fit GLMM with temperature (see Table 1).
Figure 9. Weekly mean (solid lines) and maximum (broken lines) (top) wave height (m), (middle) sustained wind speed (m/s), and (bottom) wind gusts (m/s) recorded at stations to west (Jeffreys Ledge), north (central Maine shelf), and east (Jordan Basin) of Cashes Ledge.
Figure 10. Ammen Rock and surrounding area of Cashes Ledge (left) bathymetry, depths < 70 m highlighted; (middle) available benthic habitats; (right) rugosity (standardized roughness, Dartnell 2000); black circles indicate detection range of each telemetry receiver, broken black line and solid black line indicate 40 m and 55 m depth contours, respectively.
Chapter 4: The persistence of resident behavioral strategies of Atlantic cod (Gadus morhua) in Massachusetts Bay

Abstract

Migratory strategies in Atlantic cod range from strict site fidelity to long distance migrations. As in many species that adopt a range of migratory tactics, these strategies are associated with growth and productivity. Resident life history strategies in Atlantic cod are thought to be a function of geographic setting and oceanographic conditions that enforce reproductive isolation. Uniquely pigmented cod thought to be resident on shallow rocky ledges along the Massachusetts coast were first reported by fishermen in the 19th century and have been targeted by recreational anglers more recently. The spatial behaviors of suspected resident as well as co-occurring migrant cod, classified using their distinct pigmentation, were tracked throughout Massachusetts Bay using acoustic telemetry. Site fidelity and range of movements were interpreted on the gradient of migratory tactics. Times spent within arrays spread throughout the bay were interpreted as habitat preferences using fine-scale bathymetry and newly aggregated benthic habitat maps for context. The spatial behaviors of supposed resident and migrant phenotypes differed as predicted, as residents remained relatively shallow and selected structured habitats while migrants utilized deeper habitats and showed little preference for specific bottom types. Despite their strict habitat preferences, resident phenotypes were more mobile than predicted, with individuals traveling south of Boston Harbor and north around Cape Ann. Behaviors of both phenotypes changed in early fall, leading to reduced use of rocky ledges in the western bay as oceanographic conditions changed and wave energy in shallow waters increased. These seasonal changes did not affect depth distributions of resident phenotypes. More than ¼ of tagged cod of both phenotypes returned the following year, demonstrating the
persistence of these behavioral strategies in an open coastal bay that lacks the isolating habitat characteristics most often associated with residence in cod. The habitat characteristics of these shallow ledges are likely to change in the future as surface waters and shallow bays continue to warm. These changes will affect the relative value of these habitats to cod regardless of phenotype, which may have other impacts on behavior and distribution.

**Introduction**

Partial migration is common in animal populations and may be ubiquitous in widely recognized migratory species (Chapman et al. 2011). In unpredictable environments, variance in individual spatial behaviors provides important insurance for the persistence of populations (Schindler 2010). In many taxa, including most instances studied in fish, differences in migratory strategies are related to both inherited traits and conditions experienced during ontogeny (Chapman et al. 2012, Dodson et al. 2013). This combination of inherited and accrued components to behavioral strategies has important implications for partial migration in fish species. Plasticity in development and variance in behavioral responses to early conditions increase stability in stochastic environments for individual year-classes, while also ensuring the maintenance of latent migratory or resident tendencies in populations regardless of which strategy is favored (Kerr et al. 2010, Secor 2015). A range of migratory strategies have been identified in Atlantic cod (*Gadus morhua*) populations across the Atlantic, from cod whose entire life history is spent within a single bay to nearshore groups that utilize shelf waters across expanses exceeding 1000 km$^2$ (Green & Wroblewski 2000, Robichaud & Rose 2004). Particular geologic and oceanographic conditions are known to sustain the reproductive isolation of cod that remain resident within bays and fjords on both sides of the Atlantic (Wroblewski et al. 2005, Kuparinen et al. 2015). However what Robichaud and Rose (2004) referred to as “sedentary”
behaviors are far more diverse, conforming to local conditions and geography regardless of reproductive isolation. The diversity in migratory behaviors within populations revealed by fish tagging studies suggests cod are among the many partially migratory marine, freshwater, and estuarine fish species, despite the existence of entirely resident populations (Robichaud & Rose 2004).

Highly resident cod within certain coastal embayments are spatially isolated from more mobile cod groups and display substantially lower growth rates, distinct phenotypes and environmental tolerances (Goddard et al. 1994, Ruzzante et al. 2000, Morris & Green 2002, Gosse & Wroblewski 2004, Kuparinen et al. 2015). The isolation of these behavioral groups has led to suggestions that cod behaviors are consistent at the population level (Wroblewski et al. 2005, Svedäng & Svenson 2006). Based on these bay resident populations a number of general prerequisites for residence in cod have been developed- local spawning and nursery grounds, consistent forage, and currents that ensure the retention of pelagic egg and early developmental stages. Over the past decade, a number of studies have identified the divergent behavioral strategies persisting within larger population complexes that lack distinct reproductive isolation. For instance, the southern coast of Iceland hosts spawning grounds for both frontal cod, which migrate offshore and move vertically in search of food and temperature refuges, and resident coastal cod, which do not venture beyond the 200 m depths of the coastal shelf regardless of temperature (Pálsson & Thorsteinsson 2003). These distinct behavioral strategies persist with very little straying despite the absence of retentive features found in coastal bays and fjords, although residence in this case refers to an area of shelf exceeding 3000km² (Grabowski et al. 2011, Thorsteinsson et al. 2012). Within the Gulf of Maine, residence strategies over a range of scales have been identified, from limited migrations within the western Gulf (Howell et al. 2008).
to site fidelity at isolated boulder reefs on Stellwagen Bank (Lindholm et al. 2007). Residence behavior in Gulf of Maine cod persists despite the lack of consistent currents promoting retention of eggs and larvae along the western shore (Churchill et al. 2005, Churchill et al. 2011). Genetic studies of Gulf of Maine cod have revealed fine-scale population structure, although the separate genotypes associated with different habitat preferences and migratory strategies observed in other regions (e.g., Iceland, Pampoulie et al. 2008) are far less distinct in Gulf of Maine cod (Kovach et al. 2010). Where these conditions do not conform to Wroblewski et al.’s (2005) prerequisites for nearshore stocks, other factors must ensure the persistence of multiple strategies.

Diverse forms of cod whose physical characteristics and preferred habitats are limited to particular coastal areas have been recognized by Gulf of Maine fishermen since at least the 19th century (Goode 1887). Some of these phenotypes, known as “rock,” “native,” or “red” cod depending on the port, were known to inhabit shallow rocky ledges and kelp stands, and were thought to target specific benthic prey (Goode 1887). These historic descriptions match observed phenotypes of resident cod populations found in coastal bays and fjords on the coasts of Labrador and Scandinavia (Dannevig 1953, Gosse & Wroblewski 2004). Meanwhile, a recently discovered cod phenotype found at Cashes Ledge, a remote offshore seamount in the Gulf of Maine, is seasonally resident within an isolated kelp forest on the ledge’s shallowest peak (Conroy, Chp.3). These residents express deep red to purple pigmentation, feature distinct morphometry characterized by an enlarged head, deep body, and thin caudal peduncle, and targets primarily benthic prey (Sherwood & Grabowski 2010). In addition to color and body shape, these red residents grow at substantially lower rates than co-occurring olive migrants. That cod expressing physical characteristics similar to Gilbert Bay and Scandinavian fjord cod
persist at an offshore location lacking a number of the features typical of such strict residence behavior suggests the role of mechanisms beyond physical retention and isolation in maintaining sedentary spatial behaviors. Whether some combination of conditions enables these resident behaviors to persist at Cashes Ledge remains unclear. However, the reported existence of red pigmented resident cod in Massachusetts Bay provided an opportunity to explore the coincidence of residence and phenotype occurring in close proximity to important spawning grounds.

Cod expressing the familiar red phenotype, distinct body shape, and low growth rates of Cashes Ledge residents are found in Massachusetts Bay during the spring, summer, and fall (Chp. 2), where they are targeted by local recreational fishermen and often wind up in the traps of local lobstermen (personal observations). These red phenotypes may be resident in Massachusetts Bay (or western GOM), that hosts significant cod spawning activities throughout much of the year (Zemeckis et al. 2014). In this study, I focused on a series of rocky ledge habitats in the northwest corner of Massachusetts Bay where this red phenotype co-occurs with olive phenotypes that are thought to be migrants. Samples of these phenotypes were tagged with acoustic transmitters and tracked to determine whether spatial behaviors and habitat preferences aligned with known differences in phenotype, growth, and diet. I predicted that the suspected resident phenotype would remain within the array during the summer and fall. Conversely, I hypothesized that the migrant phenotype would visit the structured habitat within the array infrequently during the summer before completely abandoning nearshore areas during the fall, following larger trends in cod spatial and depth distributions within the Gulf of Maine (Methratta & Link 2006). Across Massachusetts Bay, I predicted olive phenotypes to be undiscerning in their habitat preferences, occupying available depths and substrates in proportion to their availability, while red phenotypes would show strict preferences for shallow, structured hard
bottom. The persistence of these distinct phenotypes in an open coastal bay provides an opportunity to study migratory strategies detached from the isolating mechanisms often associated residence in cod. Through this, I hope to increase our understanding of life history diversity in mobile marine fishes.

Methods

Study Site

Massachusetts Bay forms a semi-enclosed embayment system with Cape Cod Bay at the southwestern extent of the Gulf of Maine. Land encloses the bay to the west and northwest, while Cape Cod Bay to the south and the Gulf of Maine to the east and northeast complete its perimeter (Fig. 1). The bay is relatively shallow (mean depth 35 m) and reaches a maximum depth in the central Massachusetts Bay trough Stellwagen Basin (90 m). Farther east of this depression is shallow (≥ 20 m) Stellwagen Bank, which runs along most of the bay’s mouth to the Gulf of Maine. Stellwagen Bank prohibits the exchange of bottom water between Massachusetts Bay and the greater Gulf of Maine, limited the influx and outflow of subsurface water to deeper channels bracketing the bank to the north and south, and ensures currents in the lower water column remain low (Zhao et al. 2011). Fundamental differences in oceanographic conditions exist between summer and fall, as the highly stratified water column, tidally-forced currents, and consistent upwelling of the bay in summer transition to the well-mixed waters of early fall and increased influence from strengthening winds (Bothner & Butman 2007).

The floor of Massachusetts Bay offers a range of habitats, from hard, complex ledge and boulder outcroppings to flat expanses of soft sediments. The Geologic Mapping of the Massachusetts Sea Floor project, a collaboration between the US Geological Survey and
Massachusetts Office of Coastal Zone Management started in 2003, has published a library of GIS data products, including fine-scale bathymetry (≥ 10 m resolution) and substrate (≥ 5 m resolution) maps of Massachusetts Bay (USGS 2015). The collective results of these efforts are a clear picture of bottom habitats, revealing nearshore benthic habitats characterized by a mosaic of rock, boulder, and cobble with interspersed deposits of gravel and finer-grained sediment. Sediment grain size and complexity decreases with depth to the central bay, which is dominated by sand, mud, and silt with patches of gravel and isolated boulders providing essential fish habitat to a range of crustacean and demersal fish species (Fig. 1b). Massachusetts Bay also hosts two of the four major extant cod spawning aggregations remaining in the Gulf of Maine, as well as important nursery habitat for early developmental stages (Howe et al. 2002, Zemeckis et al. 2014).

Array and Habitat Characterization

Using historical accounts (Goode 1887), the local ecological knowledge of experienced fishermen, and limited sampling conducted during the fall of 2013, I identified rocky ledges along Marblehead Neck and Manchester shore known to host red phenotypes. I deployed acoustic receivers near the top of shallow features where past catch rates had been highest, covering a 5 km$^2$ area of shallow water along Marblehead Neck from June-October 2014 and June-December 2015 and a 4 km$^2$ area off Manchester from June-October 2014 (Fig.1). In addition to this array, 24 receivers were deployed in the bay for other research projects, providing expanded spatial coverage, and increasing the area of the bay within detection range of a receiver.

In order to make inferences about cod behavior using detections within the extended network of arrays, I defined the distribution of depths and substrates available within detection
range of each receiver. Bathymetry of Massachusetts Bay was available through Twomey and Signell’s (Twomey & Signell 2013) digital elevation model (~ 30 m resolution), while comprehensive coverage of substrates required the merging of multiple data products. Three (3) USGS geologic studies covered state waters within Massachusetts Bay using a combination of acoustic backscatter, bathymetry, sediment samples, and photograph/video samples to define sediment grain-sizes and bedforms (Barnhardt et al. 2005, Pendleton et al. 2013, Pendleton et al. 2015). Beyond the state marine boundary, I utilized the USGS’s East Coast Sediment Texture Database, which features samples collected since 1962 (Politis et al. 2014); since this data is available as sediment definitions at sampling coordinates (i.e., spatial points vector), I used sample locations to define a Voronoi grid cell layer, maintaining the existing sediment definitions for each cell. Substrate categories used in each data source differed slightly due to methodological discrepancies, requiring us to reclassify each data source prior to merging. Using a rule-based approach, I identified three general classes of substrate across the data sources: rocky sediments, gravel, and soft sediments. Prior to merging substrate data into a single raster, cells defined as rock, ledge, boulder, or cobble were assigned to the rocky sediments class, sand and small grain sizes were classed as soft sediments, while gravel remained as originally defined. These simple classes retained important habitat information, such as structural complexity and likelihood of attached fauna and flora, while also applying to all original data source substrate definitions. I used the Vemco range calculator, applying 90th % sea state conditions, to define a detection range for each receiver (radius of detection = 500 m). Habitats available to each receiver were provided by distributions of depths and substrate classes extracted from rasters using the 500 m detection range (Fig.2). The combination of 34 total receivers over the 2 years provided detection coverage of a range of depths and substrates that reflected those available
within Massachusetts Bay. Since temperature is an important determinant of regional cod
distributions (Methratta & Link 2006) and influences individual behaviors at local scales
(Claireaux et al. 2000, Freitas et al. 2015), I was interested in investigating its potential influence
on cod movement patterns. Oceanographic conditions at several locations throughout
Massachusetts Bay were used in the analysis of cod spatial behavior. In Massachusetts Bay, a
strong thermocline forms in late spring, isolating cold bottom waters in the central bay (Bothner
& Butman 2007). I viewed temperature as an important consideration when attempting to
described cod spatial behaviors. Hourly Massachusetts Bay water temperatures at the surface
east of Boston Harbor (NOAA buoy 44013) and throughout the water column (1 m, 2 m, 20 m,
50 m, and 52 m depths) southeast of Cape Ann (NERACOOS buoy A01) were obtained through
the National Data Buoy Center and NERACOOS, respectively. Weekly mean temperatures of
the entire water column provided information on the strength and position of the seasonal
thermocline. Hourly bottom current measurements (50 m) at the NERACOOS A01 buoy were
also obtained.

Subjects

Atlantic cod (*Gadus morhua*) were caught via hook-and-line in June 2014 sampling in
depths 18-25 m along the coasts of Swampscott, Marblehead, and Manchester in western
Massachusetts Bay (Fig.1). Once hooked, cod were slowly reeled to the surface to avoid
barotrauma. Cod were visually assessed for barotrauma and condition, then transferred to an
adjacent anchored surface net pen (1.0 m²) for the duration of a ≥ 30-minute acclimation holding
period. Acclimated cod considered in good condition based on monitoring and a final visual
assessment underwent acoustic transmitter (V9) implantation as per the process detailed in
Appendix A. Cod were anesthetized in an aerated tank until fully sedated (no response to
stimulus or ability to orient within tank, opercula still visibly drawing water over gills, Woody et al. 2002). Anesthetized cod were placed vent side up in a v-shaped surgery cradle that featured a constant source of cold seawater run directly over their gills for surgery. Scales were cleared from and incision was made in a small area < 2 cm in length, adjacent to the ventral midline and anterior to the vent. A single V9 acoustic transmitter covered in triple antibiotic ointment was inserted into the incision. Sutures closed the incision, which was then covered in triple antibiotic ointment. Following surgery, cod were tagged with an external t-bar tag for identification and placed in an aerated seawater tank (20 L) and monitored until fully recovered (i.e., able to remain upright and resume active swimming, Woody et al. 2002).

At the time of capture, cod were assigned a preliminary phenotype based on visual comparisons with color pictures of confirmed red and olive phenotypes. Phenotypes were later confirmed using Sherwood and Grabowski’s (2010) approach. Color pictures in the sRGB color profile format were taken at the time of tagging. The number of both red and green pixels within a 10 cm$^2$ circle of operculum were extracted using the image processing program ImageJ (Rasband 2012); the ratio of the number of red pixels to the number of green pixels (RGR) was used as a quantified measure of pigmentation. Following Sherwood and Grabowski (2010) as well as Chapter 3 of this dissertation a threshold value of RGR = 1.3 was applied as the boundary between the phenotypes, so that RGR < 1.3 would indicate olive phenotype while RGR ≥ 1.3 would indicate red phenotype. Each tagged cod was affixed with a V9 acoustic transmitter (i.e., tag) that produced a unique acoustic identification code at an interval of 60 - 120 seconds. Transmitter lifetime was predicted to exceed 21 months (653 days) by the manufacturer.

Analysis
Since cod spatial behaviors change throughout the juvenile period (Howe et al. 2002, Lough 2004) and size is a reasonable measure of age in fish (Macdonald & Pitcher 1979), I compared the total length distributions of phenotypes to ensure distinct spatial behaviors were not influenced by development. Total length of both phenotypes was tested using a t-test.

Although the seasonal thermocline is a consistent and important feature of Massachusetts Bay’s oceanography, the timing of its formation, fluctuations in its strengths and depth, and its eventual dissipation vary annually (Bothner & Butman 2007). I used both linear models and piecewise quadratic spline interpolation on depth-specific temperature data to assess stratification and water column mixing. The weeks of the study when the best-fit model (selected using AICc, Burnham and Anderson 2002) featured spline interpolation, the presence of a thermocline was assumed. During weeks when a linear model fit best, the slope coefficient ($\beta$) was used to determine the presence of a constant temperature gradient ($\beta \neq 1$) or a well-mixed water column featuring constant temperature across depths ($\beta = 1$). Since records are missing from the 2015 dataset (limited to 2 depths June-October), modeling was mostly restricted to linear regression, precluding detection of thermocline dissipation.

I used presence within the available arrays to determine habitat preferences and migration strategies. This required the assumption that detections by a receiver indicated use of the habitat defined by its detection range. Since the subjects of this study were predicted to express diverging migratory strategies, I expected the total number of detections and detection rates to differ. I aggregated detection data by day, then summed days detected per week to provide a rate of presence by receiver for each tagged cod. This aggregation step facilitated analyses and reflected meaningful differences in resident and migrant strategies—chiefly persistence within a limited geographic area. Weekly presence rates were analyzed using generalized linear mixed
models (GLMM) with binomial link functions and feature cod per receiver•week as random effects. Two (2) separate sets of GLMMs were constructed using (1) only detections recorded within the array I deployed, in order to determine residency, and (2) including detections from all available receivers to define habitat and depth preferences in the wider bay. The influence of both universal oceanographic conditions (i.e., spatially consistent across Massachusetts Bay) and receiver specific habitat characteristics were tested as fixed effects: phenotype, substrate, depth, bottom temperature, bottom current, season, and study week (i.e., consecutive time steps). Continuous fixed effects were scaled to ensure model convergence and back-transformed to determine predicted probabilities. Substrate and depth distributions were specific to each receiver, providing a means to measure affinity for specific habitats, while temperature and bottom current were measured as weekly means at the NERACOOS A01 buoy. Substrate influences were reduced to a single sediment class, proportion of rocky sediments, to avoid multicollinearity (due to correlation when ≥ 2 of 3 total proportion variables are retained); rocky sediments (as defined during this study) included complex habitat which I predicted would be preferred by red phenotypes. Receiver-specific depth distributions were summarized as mean depths for analysis. Prior to inclusion in models, fixed effects were tested for multicollinearity (i.e., variance inflation, VIF; Dormman et al. 2013); any combination of fixed effects as individual explanatory variables or within interactions that were found to be collinear were excluded from models, using a VIF threshold of 10 (e.g., VIF = 11 would lead that variable combination or interaction to be excluded from analyses). I compared models using AICc (Burnham & Anderson 2002). Individual fixed effects and interactions were assessed using likelihood ratio tests, while coefficients were evaluated using 95% confidence intervals derived
via profile likelihood (Venzon & Moolgavkar 1988). Fixed effect coefficients and marginal
effect size (i.e., $R^2$, Nakagawa and Schielzeth 2013) were used to aid model interpretation.

All statistical analysis, data manipulation, and mapping were conducted in the R statistical
program (R Core Team 2016).

Results

All cod implanted with acoustic transmitters recovered from the implantation process and
swam actively upon release. RGR analysis confirmed the assignment of red (RGR 1.32-1.72) and
olive (RGR 0.84-1.25) phenotype to tagged cod. Sizes of tagged phenotypes (red 60.2 ± 2.5cm,
olive 55.8 ± 3.6cm) did not differ ($t_{18} = 1.01, p >0.05$), supporting the assumption that
differences in spatial behaviors observed were not due to developmental stage.

Thermocline conditions in Massachusetts Bay followed expected seasonal patterns of
stratification and mixing, although the timing of some changes in thermal conditions may have
differed between years. In 2014, the thermocline was established by the commencement of
tagging efforts in June, as indicated by quadratic splines providing better fits to available
temperature-depth data than linear models (based on AICc, Fig. 3). By the middle of September,
temperatures steadily decreased with depth, resulting in linear models providing best fits to
temperature-depth data during the week of 9/14/14 and suggesting a weakened thermocline. By
the beginning of November (11/3/14) the slope of the best fit linear regression ($\beta = 1, p < 0.01$)
indicated a well-mixed water column of uniform temperature. Although 2015 records were
limited to surface and bottom temperatures from September through early October, a thermocline
was present in late May at the time of receiver deployment. Fall conditions in 2015, again based
on complete records, suggest the water column remained stratified further into November than
the previous year, as the slope of the best fit linear regression was > 1 until the week of 11/22/15 ($\beta = 1, p < 0.01$). These thermal conditions align with three of the four oceanographic seasons of Massachusetts Bay- the thermocline and strong stratification during the summer, rising bottom and falling surface temperatures along with increasing wave and wind strength disrupt the shallow thermocline in early fall, eventually leading to late fall’s well-mixed water column (Butman et al. 2007).

Cod tagged during this study were detected 601,292 times by Northeastern University receivers deployed along the coasts of Marblehead and Beverly, while telemetry equipment deployed by other research groups throughout Massachusetts Bay contributed an additional 1,185 detections. Each individual was detected during an average of 109.1 ± 19.2 days (mean ± SE throughout) over the course of the study, amounting to 2,214 total days when tagged cod were present within established arrays (2,190 days within Northeastern’s array, 24 days within other arrays). Olive phenotypes were detected more often (131.5 ± 32.3 days) than red phenotypes (86.7 ± 19.0 days) along the shallow ledges during this time, although in the first year both phenotypes were detected at similar rates (2014 olive 85.7 ± 17.2 days, red 2014 76.2 ± 14.4 days). In 2015, despite 5 of the 10 red phenotypes tagged in 2014 returning to the array I established along the coast of Marblehead, total detections per individual was only 10.6 ± 6.0 days compared with 46.6 ± 24.2 days of the 3 olive phenotypes that also returned. Beyond the redeployed array, one of these olive returners and two additional olive phenotypes were detected in 2015 at receivers in deeper waters. Due to the infrequent detection of red phenotypes during the 2nd year of the study, I concentrated on modeling results from 2014 data; the 2nd year of the study provided additional context for interpretation of phenotype behaviors at larger spatial and temporal scales.
Contrary to their similar patterns of presence during the study’s first year, red and olive phenotype spatial behaviors differed with respect to available physical habitats and oceanographic conditions. Predicted differences in residence in the highly structured shallows covered by the Northeastern arrays did not occur, as olive phenotypes visited these habitats as often as red phenotypes in both the summer (olive 0.40 ± 0.04 days per week, red 0.36 ± 0.04 days per week) and fall (olive 0.32 ± 0.05 days per week, red 0.27 ± 0.04 days per week) of 2014. However, during the summer red phenotypes were more prevalent in shallower habitats (best-fit GLMM using detections from NEU receivers only: phenotype • depth $\chi^2 = 2035.9, p < 0.01$, Table 1) displayed strong affinity for structured bottom types, specifically those that offered a mosaic of grain sizes (i.e., providing a mixture of substrate types), while olive phenotypes revealed little preference for specific benthic habitats (phenotype • rock $\chi^2 = 3918.6, p < 0.01$, Fig.4). In addition to the physical characteristics of shallow benthic habitats, Bay-wide bottom temperature (phenotype • temperature $\chi^2 = 118.2, p < 0.01$; Tables 2, 3) and current (phenotype • current $\chi^2 = 32.4, p < 0.01$) also influenced patterns in habitat use. During the summer, both phenotypes spent more time within the array when bottom temperatures were moderate ($6^\circ$C) and bottom currents were low (0.08 m/s), although the effects of temperature and flow on red phenotypes were larger than those on olive phenotypes. With the onset of fall (season $\chi^2 = 20.1, p < 0.01$), tagged cod spent less time in shallow water covered by the arrays (olive 17.2 ± 0.02 days per week, red 14.3 ± 0.02 days per week), as predicted. While the mean depth of receivers frequented by olive phenotypes did not change with season (summer 34.4 ± 0.3 m, fall 34.2 ± 0.4 m), red phenotypes occupied slightly shallower habitats in fall, as the mean depth of receivers they frequented decreased from 32.0 ± 0.4 m during the summer to 29.7 ± 0.5 m in the fall, although this change in vertical position must be considered in the context of
the limited range of depths included in this particular analysis (26.7-36.8 m, Northeastern University deployed receivers). In addition, red cod were detected in areas with more complex substrate, as the proportion of total substrates classified as rocky in the immediate vicinity of the receivers these cod frequented increased from 0.69 ± 0.02 in summer to 0.82 ± 0.01 in fall. In contrast, olive phenotypes did not display any preference for specific habitats, as the proportion of rocky substrates around the receivers they visited matched availability (olive summer 0.72 ± 0.02, fall 0.75 ± 0.01; available 0.75). As in summer, time spent within the shallow habitats covered by the array decreased as bottom currents increased, while higher bottom temperatures corresponded with higher detection rates (Figs. 4,5). However, cod detection rates decreased as temperatures increased throughout the fall (Fig. 6). Despite this, the combination of fixed effects (phenotype-specific proportion of rocky substrate, depth, bottom temperature and current, and season) in the best fit model (AICc = 100127) accounted for most of the variance in detection rates (i.e., marginal $R^2 = 0.79$), supporting its efficacy for explaining cod spatial behaviors.

The addition of detections from receivers throughout Massachusetts Bay reinforced the habitat and depth preferences of red phenotypes, as well as their reaction to oceanographic conditions. These receivers covered diverse substrates (0 - 93.7% rocky) across a range of depths (20.8 m - 81.5 m), resulting in better representation of benthic habitats available within Massachusetts Bay. Regardless, the inclusion of this habitat diversity did little to affect the best-fit model (AICc = 118151, Tables 1-3), which featured the same fixed effects as the previous model that was restricted to only data taken from the Northeastern array (save for the inclusion of a weak interaction resulting from the smaller decrease in rates of red phenotype presence in the fall compared to those of olive phenotypes, Fig. 6). Receivers beyond my array provided important information on movement patterns throughout Massachusetts Bay, resulting in
detections of red phenotypes moving south across Broad Sound to shallow nearshore waters south of Boston Harbor and north around Cape Ann. These arrays also recorded tagged cod at deeper receivers, resulting in detection of olive phenotypes at depths >45m where red phenotypes were absent (Fig. 7).

**Discussion**

Although the movement behaviors of cod are incredibly diverse and range from small-scale movements for foraging and predator evasion as juveniles to larger-scale migratory behavior as adults, general characteristics of more sedentary cod have emerged across populations beyond the physical isolation associated with strict bay residence (Robichaud & Rose 2004). Resident movement behaviors tend to correlate with habitat limitation, often in the form of limited depth ranges or specific temperature preferences. These features are common across the species’ range and spatial scales of home range, present in strict bay residents as well as more open nearshore populations (Wroblewski et al. 2005, Thorsteinsson et al. 2012).

In nearshore locations throughout the species range, depth is often a proxy for important oceanographic and geologic habitat features that influence cod behavior. Steep temperature gradients are common features of temperate shallow nearshore environments, as relatively calm late spring and summer conditions result in the warming of stable surface layers (Geyer 1992, Miller & Wheeler 2012). This process may be enhanced by spring freshwater inputs from rivers and estuaries in coastal areas, as reduced salinity of surface water enhances density stratification (Bothner & Butman 2007). Increased temperature, lengthened photoperiod, and the supply of nutrients from water column mixing over winter drive large increases in primary production above the thermocline, enhancing overall productivity (Nybakken 1993). Additional nutrient subsidies can occur in shallow benthic habitats as a consequence of coastal upwelling (Townsend
1991). In addition, internal waves when present promote vertical mixing of surface nutrients and productivity, which occur when relatively deep stratified water is forced over shallow subtidal areas, thereby by driving the thermocline down in the water column (Garrett & Munk 1979). All of these factors can provide favorable foraging conditions over relatively narrow depth ranges, which have been shown to attract high densities of cod and other predators (e.g., seamount at Cashes Ledge, Gulf of Maine, Witman and Sebens 1993). In other regions, cod use prey concentrations at temperature gradients as important food sources. Such is the case for migrant frontal cod off the coast of Iceland that seek the dense prey aggregations associated with deep thermal fronts where Arctic and Atlantic waters meet (Pálsson & Thorsteinsson 2003).

Temperature gradients exist within Massachusetts Bay in the form of a strong annual summer thermocline around ~15 m depth (e.g., temperature differences between 1m and 20m depths reached 9°C in July 2014), while upwelling is consistent in the western extent of the bay covered by the receivers deployed for this study (Bothner & Butman 2007). Additionally, internal waves, generated as deeper Gulf of Maine water flows over Stellwagen Bank during flood tides, buffet the bottom along the coast with relatively high temperatures and increased concentrations of plankton when the water column is stratified (Scotti & Pineda 2004). Olive phenotypes may use these conditions during summer months, leading them to occupy shallower habitats within the Gulf of Maine. Foraging conditions are enhanced by periodic internal waves, which displace plankton vertically by up to 30 m and contribute to benthic productivity along the shore in summer months and may contribute to the shoreward transport of zooplankton and small fish (Kingsford & Choat 1986, Shanks & Wright 1987). The seasonal reduction in primary productivity and weakening of the thermocline, and subsequent loss of subsidies from internal waves and upwelling coincided with an increase in depth of the habitats visited by olive
phenotypes in the fall, suggesting the importance of these thermal conditions to their shallow
distribution throughout the summer.

Temperature is also intimately related to growth, maturity, and reproduction in cod, with
optimal temperatures generally occurring within the range 7-14 °C (Claireaux et al. 2000,
Björnsson et al. 2001, Rätz & Lloret 2003, Freitas et al. 2010), although tolerances often differ
between populations (Goddard et al. 1994). Experiments and theoretical derivations have
identified temperatures that coincide with optimized physiology, however costs are associated
with uniform temperature conditions regardless of whether or not they fall within optimal ranges
(Claireaux et al. 1995). When presented with a temperature gradient, cod move between thermal
habitats as they likely balance efficient digestion of prey in relatively warm layers and reduced
metabolic costs associated with cooler temperatures (Claireaux et al. 2000). Small adult cod
migrate vertically across steep temperature gradients in fjords along the Skagerrak coast during
the summer, their vertical movements ceasing with the seasonal disruption of the thermocline in
the fall and reemerging over the winter at depth in response to the deep inverted thermocline
typical of their fjord habitat (Freitas et al. 2015). And at a shallow seamount within the Gulf of
Maine, recently matured cod migrated between depths in stratified thermal conditions, likely
moving to greater depths along with the deepening thermocline in late summer (Ch. 3). Cod
tagged in Massachusetts Bay were similar in size to cod that express vertical migratory behavior
in response to temperature gradients elsewhere in the Gulf and across the species’ range.
Measurement of movements at fine scales were precluded by the study design (i.e., tags did not
report depth). However, the consistency with which cod in the Gulf of Maine and elsewhere
actively seek and utilize steep temperature gradients and suggests the importance these behaviors
across the species range.
While olive phenotypes moved to deeper areas of the bay following changes in thermal conditions, red phenotypes remained in shallow coastal waters. In addition to decreasing productivity, surface and near-surface waters provide additional challenges in the form of steadily increasing wind and wave energy throughout the fall and increased storm intensity (Denny 1987, Gardner et al. 2001). The adherence of red phenotypes to a limited range of depths at nearshore locations aligns with resident cod habitat preferences across the species’ range. Differing migratory tactics of resident coastal and migrant frontal cod around Iceland likely reflect distinct foraging strategies that may offer alternative balances between the costs associated with higher temperatures and access to consistent supplies of food, resulting in higher growth rates for coastal cod and better condition for frontal cod (Pálsson & Thorsteinsson 2003, Pardoe & Marteinsdóttir 2009). These strategies may satisfy diverging metabolic requirements, ensuring both coastal and frontal cod of the ability to reproduce annually (Grabowski et al. 2011). Resident phenotypes of cod at Cashes Ledge remain shallower than sympatric migrants throughout the summer, staying within the boundaries of a shallow kelp forest, until they move deeper down the ledge slope in mid-fall (Ch.3). Despite this habitat limitation, residents do not suffer reduced condition compared to sympatric migrants; this may be due, in part, to inherent physiological differences between these phenotypes, expressed most clearly in the substantially reduced growth rates of residents (Sherwood & Grabowski 2010). Although some physiological cost associated with their behavior would be expected as a response to decreasing productivity and increasing energy in shallow waters, red phenotypes collected during this study appeared to have similar access to prey as olive phenotypes regardless of season (i.e., no difference in gut fullness, Ch. 2). Following regional trends in resident cod (Ch. 3), red phenotypes sampled in Massachusetts Bay have lower growth rates than olive phenotypes.
In addition to temperature, physical substrates generally vary with depth and distance from shore. In Massachusetts Bay, sediment grain size decreases with depth, from rock ledge and cobble dominated nearshore shallows to soft sediment covered deeper areas of the central bay and Stellwagen Basin (Fig. 1). Habitat complexity decreases with sediment grain size, as the craggy ledges characteristic of the hard bottom near the coast transition to the gently sloped sandy bay bottom. In addition to the structure provided by rocky and cobble substrates, seasonal macroalgal stands and other attached flora and fauna contribute to the structural diversity of benthic habitats in the shallower western portion of the bay (USGS 2015). Habitat complexity is often correlated with increased predator presence, as high concentrations of prey provide an ample source of forage despite the availability of refuge and increased survival of prey (Heck & Crowder 1991, Grabowski & Powers 2004). Although complex substrates are considered essential habitat for cod (New England Fisheries Management Council 2014), temperature, depth, and prey availability are thought to provide the largest influences on cod distributions (Methratta & Link 2006). However, the observed strict residence at solitary structures and spatially limited complex habitats as well as the diversity of behavioral strategies expressed by cod throughout their range provide evidence of both persistent and seasonal site fidelity more commonly associated with reef fish (Robichaud and Rose 2004, Lindholm et al. 2007, Ch. 3).

Although only occurring in shallow waters, red phenotypes did not express the expected strict residence behaviors I predicted, utilizing the complex rocky ledge habitat demarcated within the Northeastern University array at rates similar to olive phenotypes. Red phenotypes were far more mobile than expected, moving distances of at least 30 km over the course of the summer.

However, regardless of their mobility, red phenotype occupied coastal shallow water where rocky substrates are prevalent and connect Plymouth Bay to Cape Anne and Ipswich Bay with
nearly continuous stretches of hard bottom (Pendleton et al. 2013, Pendleton et al. 2015). For resident cod at Cashes Ledge, differences in preferred structural complexity and spatial behaviors correlated with distinct trophic ecology and a diet focused on benthic prey abundant in these habitats and lower growth rates than co-occurring migrants (Sherwood and Grabowski 2010, Chp.2). Similarly, relatively slow growing red phenotypes in Massachusetts Bay were limited to shallow structured habitats, which are home to high concentrations of benthic prey that composed most of their diets, suggesting a link between behavior, trophic ecology, and metabolic rates.

Early divergence in growth and condition are correlated with metabolic rates and spatial behaviors during ontogeny in a number of salmonid (e.g., Arctic charr *Salvelinus alpinus*, Klemetsen et al. 2003) and moronid species (e.g., white perch *Morone americana*, Kraus and Secor 2004). Distinct energetic requirements and growth trajectories are common among partially migratory fish species (Secor 2015). The alignment of residence strategies with inherent physiology may provide a reinforcing mechanism for the persistence of these migratory strategies in the absence of physical isolation throughout the species’ range and at multiple scales, form Cashes Ledge, to Massachusetts Bay, to the Icelandic shelf.

The differences in behaviors and habitat preferences of red and olive phenotypes observed during this study correlated with distinct body shapes, diets, and growth trajectories. Similarly diverging physical characteristics and behavioral strategies between co-occurring phenotypes has been identified at an offshore seamount in the central Gulf of Maine (Ch. 3), while fully or partially isolated residents in other regions across the species range express similar habitat preferences and truncated depth distributions (Green & Wroblewski 2000, Kuparinen et al. 2015). These examples are characteristic of partially migratory fish populations identified in a
range of aquatic, estuarine, and marine species (Secor 2015). Across these species, distinct migratory tactics develop during ontogeny in response to early environmental conditions, their influence on individual vital rates such as growth and condition, and interactions with inherited traits (Chapman et al. 2011, Dodson et al. 2013). Within this partial migration framework, a population may become fully migratory or resident due to consistent or changing conditions, but still retain some latent plasticity in spatial behaviors due to the conditional adoption of migratory strategies (Dodson et al. 2013).

To place cod within the larger paradigm of partially migratory fish species, more work is required. The mechanisms influencing the adoption of migration tactics and the developmental stage at which they are set must be identified, as do candidate liability traits related to spatial behaviors that may be explicitly tested through rearing experiments (Dodson et al. 2013). Whether or not residence in cod is the result of ontogenetic and genetic interactions similar to those identified across a range of fish taxa, it is clear that cod may adopt a number of migratory strategies in the absence of the isolation once proposed as necessary for resident spatial behaviors.

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Table 1. GLMM diagnostic statistics for best fit models constructed using detections limited to the (1) Northeastern array and (2) all available receivers.

<table>
<thead>
<tr>
<th>model fixed effects abbreviations:</th>
<th>model</th>
<th>AICc</th>
<th>max VIF</th>
<th>R²</th>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>mar.</td>
</tr>
<tr>
<td>1) Northeastern Receivers Only</td>
<td>$p \cdot r + p \cdot d + p \cdot t_b + p \cdot c_b + s + w$</td>
<td>100127</td>
<td>3.3</td>
<td>0.79</td>
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<tr>
<td>2) All Available Receivers</td>
<td>$p \cdot r + p \cdot d + p \cdot t_b + p \cdot c_b + p \cdot s + w$</td>
<td>118151</td>
<td>4.1</td>
<td>0.83</td>
</tr>
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</table>

$p$ - phenotype
$d$ - mean depth
$c_b$ - bottom current
$s$ - week of the study
$r$ - proportion of rocky substrate
$t_b$ - bottom temp
$s$ - season

Table 1. GLMM diagnostic statistics for best fit models constructed using detections limited to the (1) Northeastern array and (2) all available receivers.
<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>1) Northeastern Receivers Only</th>
<th>2) All Available Receivers</th>
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</thead>
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<tr>
<td></td>
<td>$\chi^2$</td>
<td>p-value</td>
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<td>phenotype • depth</td>
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</tr>
<tr>
<td>phenotype • rock</td>
<td>3918.6</td>
<td>&lt; 0.01</td>
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<tr>
<td>phenotype • season</td>
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<td>–</td>
</tr>
<tr>
<td>phenotype • temperature</td>
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<tr>
<td>phenotype • current</td>
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<tr>
<td>season</td>
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<td>&lt; 0.01</td>
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<tr>
<td>week (time step)</td>
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<td>0.03</td>
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</table>

Table 2. Fixed effect likelihood ratio test results for best fit models constructed using detections limited to the (1) Northeastern array and (2) all available receivers.
Table 3. Level-specific coefficient estimates for fixed effects included in best fit models constructed using detections limited to the (1) Northeastern array and (2) all available receivers; 95% confidence intervals derived using profiled likelihood included in parentheses; significant coefficients are reported in *italics*; coefficient values are reported as log-odds of scaled variables.
Figures

Figure 1a. Massachusetts Bay bathymetry (depth in m) and receiver locations during tagging study; black ellipses delineate Northeastern array along the coasts of Marblehead and Beverly.
Figure 1b. Massachusetts Bay substrates and receiver locations during tagging study; black ellipses delineate Northeastern array along the coasts of Marblehead and Beverly.
Figure 2. Proportion of substrates (left) and distribution of depths (right) throughout Massachusetts and Cape Bod Bays, within detection range of receivers in 2014 (c,d) and 2015 (e,f).
Figure 3. Daily mean recorded (●) and weekly modeled temperature in the Gulf of Maine across depths in 2014; predicted temperature profiles resulting from either linear models or quadratic spline interpolation; (left) thermocline present (spline), (middle) constant temperature gradient (lm, \( \beta \neq 1 \)), and (right) well-mixed water column (lm, \( \beta = 1 \)).
Figure 4. Phenotype-specific predicted probabilities of presence across available substrates and depths under different bottom temperature and current conditions within the 2014 Northeastern receiver array in summer and fall; predictions derived using best-fit GLMM using Northeastern receivers only (see Table 1).
Figure 5. Boxplots of weekly wind speed (m/s), bottom temperature (°C) and current (m/s) during 2014 summer and fall; vertical line indicates beginning of fall (9/1), gray horizontal bands indicate the temperature and current that contribute to maximum predicted probability of presence from GLMM modeling.
Figure 6, phenotype-specific predicted probabilities of presence across available substrates and depths under different bottom temperature and current conditions in 2014 in summer and fall (including all receivers); predictions derived using best-fit GLMM using all available receivers (see Table 1).
Figure 7. Distributions of habitat depths at the receivers where red and olive phenotypes were detected.
Chapter 5: Suitability of available shallow habitat for Atlantic cod (Gadus morhua) around an offshore kelp forest in the Gulf of Maine

Abstract

Despite the recognized importance of essential fish habitat (EFH) to the productivity of important commercial species, definitions rely on limited knowledge and poor habitat maps. A previous study used a video survey to examine cod habitat use at a remote offshore seamount in the Gulf of Maine, Cashes Ledge, was used to assess the importance of specific habitat characteristics to cod. Videos were used to identify cod presence and visitation rates (cod/hour) as well as geological substrates and macroalgal coverage. Resulting data were analyzed using linear and generalized linear models to assess the influence of habitat characteristics on cod behaviors. Additionally, data were used as known presences in presence-background predictive species distribution modeling (SDM) efforts. Background data were sampled from fine-scale benthic habitat and bathymetry maps of the area. Both additive (generalized additive models) and ensemble tree (boosted regression tree and random forest) approaches were used. Model results were used to assess relative habitat importance and predict suitability at Cashes Ledge. Cod presence was mostly explained by geological substrate and depth, while visitation rates increased with kelp coverage and decreased with depth. Combined predictive power of habitat and depth were high across predictive SDM approaches. Although depth was consistently the largest contributor to model fit, separate models of substrate and depth revealed a gradient in the probability of cod observations with habitat complexity with cod favoring more complex habitat types. Resulting predictions of suitable habitat consisted of vertically structured habitats across depths. Surprisingly, even on a shallow ledge known for the high complexity of its habitats, the most suitable kelp and boulder habitats were not ubiquitous. If the results of these models may
be considered more widely applicable, they suggest the need to consider vertically structured habitats in future spatial management decisions. These habitats may provide important services even when surrounded by less complex substrates. The extent and distribution of these habitats suggests that the majority of Cashes Ledge < 60 m depth is cod EFH. The use of an existing cod habitat preference study in predictive SDM suggests the potential benefits of applying these techniques more widely. Extrapolating similar habitat models to the entire stock area for cod and other species could be valuable in both spatial management efforts and stock assessments.

**Introduction**

Persistent environmental, geological, and biological characteristics of fishes’ environments are needed to ensure survival, recruitment, development, and productivity of populations and stocks. Identifying and protecting the habitats essential to the persistence and productivity of managed fish stocks is a major concern of fisheries managers (Fluharty 2000). The Magnuson Stevens Reauthorization Act (Magnuson-Stevens Fishery Conservation 2007) requires both ecological definitions and physical delineations of essential fish habitat, as well as measures to ensure its protection, to be included in fishery management plans. The recent amendment process (Omnibus Habitat Amendment 2; OHA2) undertaken by the New England Fisheries Management Council (NEFMC) included the development of new EFH guidelines for managed species that are currently under review by the National Marine Fisheries Service (New England Fisheries Management Council 2014). These revisions used extensive sampling records, published literature, and expert opinion to provide sufficient detail to ensure all aspects of life history were included in EFH definitions (New England Fisheries Management Council 2014). Due to the wide distribution and mobility of many of its managed species, the NEFMC conducts most management actions in the Gulf of Maine at a scale of 10’ cells (i.e., 1/60th of a degree, ~
240 km$^2$). Even at such coarse scales, the influence of habitat characteristics on the distribution of mobile marine species may be apparent (Methratta & Link 2006a), but this approach may obscure local effects of habitat that provide important contributions to productivity and persistence and may influence distributions.

At the coarse scales used in the management of Gulf of Maine fisheries, the spatial distribution of Atlantic cod *Gadus morhua* is positively influenced by the availability of substrates featuring cobble-sized sediments (Methratta and Link 2006). However, cod utilization of their benthic habitats occurs at finer scales where substrates in the Gulf of Maine are far more variable (Auster et al. 2003, Auster & Lindholm 2005). Cod use vertically structured features within benthic habitats as foraging locations due to the higher densities of prey often concentrated in and around complex habitats (Laurel et al. 2003, Auster & Lindholm 2005). Shipwrecks and pilings of offshore windmills in the North Sea host higher densities of cod than surrounding, less complex habitats during the summer when prey densities are also high (Reubens et al. 2013). Along the Skagerrak coast of Scandinavia, cod densities are greater on rocky bottoms than nearby soft sediments during the late summer through early fall (Stål et al. 2007). This preference is reflected in their diets, which are dominated by prey items associated with hard benthic habitats (Stål et al. 2007). In the Gulf of Maine, cod utilize boulders and boulder piles on Stellwagen Bank for forage and, possibly, for refuge from high currents (Auster & Lindholm 2005, Lindholm et al. 2007); these patterns are thought to be seasonal, although some individuals tracked within acoustic telemetry arrays appeared to be year-round residents (Gröger et al. 2007, Lindholm et al. 2007). These examples of cod demonstrating their preference for structured benthic habitats are compelling, but spatially constrained to the scale of
telemetry studies (tens of square kilometers) and direct observations (tens of meters) rather than that of fisheries management activities (hundreds of square kilometers).

Species distribution models (SDM) use spatial data associated with known observations of organisms to gain insight into the specific habitat characteristics and ecological mechanisms underlying observed and predicted distributions (Elith & Leathwick 2009). The relationships between described habitats and the target organisms, determined quantitatively through any number of modeling approaches, are used to test specific aspects of species ecology influenced by environmental conditions (Diez 2007) and predict distributions of species onto maps of known habitats (Guisan & Thuiller 2005). In recent years, SDMs or related approaches have been used in a range of fisheries contexts, including assessing definitions of EFH for immature groundfish at coarse geographical scales using survey data (OHA2 Vol.5 Ap.F, New England Fisheries Management Council, 2014), predicting future changes in groundfish distributions using species niche models and predicted climate scenarios (Hare et al. 2012), and developing a process-driven mapping approach for predicting continental shelf benthic environments (Kostylev & Hannah 2007). SDMs provide a quantitative process for scaling findings to wider geographic scales through either model-based interpolation to unsampled sites within the immediate geographic area of the existing samples or through forecasting to completely new regions or environmental settings (e.g., effects of future climate change on species distributions). Studies focused on fine-scale habitat associations may be more informative to fisheries managers if their findings can be extrapolated to the scale of fisheries management activities using the SDM framework.

In marine systems, fine-scale benthic habitat characteristics beyond bathymetry are particularly difficult to collect (Kostylev et al. 2001). This can limit available inputs, which will
inevitably restrict the resulting scope of inference— the conclusions that may be drawn from modeling results. If the choice of predictor variables are not ecologically relevant and the goal of modeling efforts is not supported with evidence, then results may be misleading (Elith & Leathwick 2009). However, the location of this study has been the focus of benthic research and mapping efforts since the 1980’s (Vadas and Steneck 1988, Witman and Sebens 1992, McGonigle et al. 2011). These efforts have yielded a large amount of data on the geologic and biotic substrates that are present on Cashes Ledge at scales relevant to video observations. Recent efforts have produced fine-scale a habitat map depicting geologic and biotic substrates. Both this map and an existing bathymetry map of the area provide habitat data at a resolution ($\leq 5$ m$^2$ resolution) relevant to cod use of structured habitats like boulder piles. By using these maps and the results of an extensive video survey consisting of fish observations in different benthic habitats across a range of depths (5 - 60 m) at Cashes Ledge as inputs into SDMs, I hope to predict the suitability and relative importance of available habitats at Cashes Ledge, assess new EFH definitions for Atlantic cod in the Gulf of Maine, and explore the possibility of using spatially explicit habitat use and preference data from existing studies to predict patterns of habitat use.

Here, I used both descriptive and predictive modeling approaches to assess the importance of particular habitats to cod presence and habitat use. I applied three modeling approaches using the predictive SDM framework: generalized additive models (GAM), random forest models (RF), and boosted regression tree models (BRT). These models are commonly used in SDM applications due to their ability to incorporate interactions amongst habitat variables that are common in ecological studies (Elith & Leathwick 2009). I hypothesized that these modeling efforts would identify substrate and structural as the most important predictors of
presence, abundance, and habitat suitability. I further predicted that kelp bottom is the most favorable habitat for cod on Cashes Ledge, followed by boulder habitat. The results of this modeling effort will highlight the need to incorporate benthic habitat definitions at finer spatial resolutions than are currently considered by fisheries managers.

Methods

Video Data Collection and Analysis

Video surveys of benthic habitats were conducted at Cashes Ledge in 2006 and 2007 (Grabowski 2009). Video survey equipment consisted of Sony Handycam Camcorders (model DCR-SR100), each fit with a 0.5x lens, external battery with 7.5-hour capacity, and a 30 Gb hard drive. Video cameras were secured within PREVCO polycarbonate housings (9.2 x 11.9 x 19.7 cm) and attached to “blank” lobster traps (i.e., lacking caging) so that they would face slightly downward to capture the immediate substrate within view. A 2.5 cm PVC pipe was mounted to the lobster trap so that the pipe extended 1 m from the trap directly into the field of view of the camera. At the end of the pipe, an additional 1 m PVC pipe was attached to it, forming a T. Holes were drilled in both pipes at 10 cm intervals and black cable ties attached to qualitatively gauge the size of fish in the field of view. A buoy line with sinking pot warp was attached to each trap so that each unit was able to be deployed and recovered from a small vessel. Traps were deployed in July through August 2006 and June through September 2007 during daylight hours (between 09:30 and 20:00 EDT) for durations of between 30 and 90 minutes of bottom time. Deployments targeted depths across 3 strata- shallow (5-20 m), mid (20-40 m), and deep water (40-60 m)- although actual depths varied due to the complex, steep bathymetry of Cashes Ledge. The video survey consisted of a total of 167 separate videos taken during deployments at depths between 9 m and 55 m.
Prior to inclusion in analysis, each video was reviewed for equipment issues, visibility, physical obstructions, and video camera and trap orientation. If it was not possible to identify fish species at a distance of 3 m from the camera across 25% of the field of view, the video was eliminated from inclusion for analysis. Videos were used to identify and count cod and other vertebrate species as well as define benthic habitats. Each fish identification was accompanied by a start and end time of observation, distance from camera, location on the screen (position within a standardized 5x3 cell grid overlaid on the computer screen), estimated total size (if close enough to the reference T-bar), and notes on behavior (e.g., interactions with another species). An observation was considered new if ≥ 30 seconds had passed since the end of the previous observation that possibly featured the same individual. Distance from the camera was standardized to 4 zones: zone 1 directly in front of the camera lens (<0.5 m), zone 2 at and immediately beyond the reference T-bar (0.5-2 m), zone 3 immediately beyond the bar to a distance of 4 m, and zone 4 greater than 4 m away from the camera. Due to limited visibility at distance, any observations > 4 m from the camera (zone 4) were not included in cod presence or quantity data. To account for the likelihood of individual cod visiting the location multiple times during a deployment and the varying duration of videos, separate observations were summarized as a standard hourly visitation rate (cod visits per hour). All observations of cod were confirmed by a 2nd expert reviewer.

Benthic habitats were defined using biotic and geological characteristics visible in videos. Macroalgae were characterized by percent cover and presence of dominant taxa (*Laminaria* spp., *Agarum* spp., and other). Geological substrate was identified to five general categories using the Wentworth scale (1922) and textural characterizations (Harris & Stokesbury 2010): mud/sand, gravel, cobble, boulder, and rock/ledge, and percent coverage of each category estimated. A
A topographical complexity score between 0 and 3 was assigned to each video based on a qualitative estimate of rugosity. Zero was assigned to flat bottom, while 3 was assigned to highly rugose boulder and ledge habitat. While topographical complexity was limited to structural characteristics on a scale > 1 m, the fine-scale spatial complexity visible in each video was assigned a qualitative score based on available interstitial space on sub-meter scales. Specifically, 1 indicated a complete lack of interstitial space, while 3 was reserved for highly complex surfaces, such as boulder piles and kelp stands. The benthic habitat in each video was summarized as primary and secondary dominant substrates using six substrate types, kelp and the five geologic substrates. Macroalgae was considered dominant if kelp covered ≥ 15% of the field of view, while boulder was considered dominant if it composed ≥ 10% of visible habitat and macroalgae < 15%; these percent cover thresholds were established to account for the vertical structure provided by these specific habitat features and their demonstrated influence on cod behavior (Lindholm et al. 2007, Ch. 3) In the absence of macroalgae and boulder substrate, the most represented substrate was identified as primary and the next most as secondary.

Additional Sources of Benthic Habitat Data

In order to expand the scope of inference beyond the immediately vicinity of each video, I used benthic habitat maps focused on macroalgal composition and extent (McGonigle et al. 2011) and essential habitat of groundfish (Calvert 2015, Fig. 1). These maps were developed using acoustic survey datasets and groundtruth still pictures and video of the area. McGonigle et al.’s (2011) fine scale map of the Laminaria and Agarum zones on Cashes Ledge was used to define the extent of the kelp forest during the development of the larger, benthic habitat map (Calvert 2015). I modified the existing benthic habitat map using McGonigle et al.’s (2011) reported depth ranges for the Agarum zone (30-40 m), as well as the relatively narrow band
where shotgun kelp and *Laminaria* spp. were mixed (25-30 m). At depths exceeding the bottom of the *Agarum* zone, I used the existing substrate definitions from the benthic habitat map. I cross-checked the depth ranges of the *Laminaria*, mixed, and *Agarum* zones with kelp presence across depths in video surveys to ensure these revisions reflected observed habitats. The categories of possible habitat types in the updated map matched those used during video analysis: *Laminaria*, mixed kelp, *Agarum*, boulder, rock, gravel, and mud/sand.

I used this benthic habitat map and a bathymetry map created using one of the acoustic datasets as sources of background data for the habitat suitability models (see below). Due to the limited range of depths targeted during the video surveys, I clipped these maps to depths ≤ 60 m. Data was extracted from randomly generated coordinates within the extent of these clipped maps and combined with known presence records for presence-only habitat suitability analysis. The size of the background dataset ensured ~ 0% of the records in the dataset would be known presences. Extracted background data consisted of bathymetry and defined substrate category from each of the background coordinates. Although other potentially relevant habitat characteristics were available using the fine-scale bathymetry data, such as habitat complexity measured via topographic position index (TPI, Wilson et al. 2007) and the standard deviation of slope (Grohmann et al. 2011), steep bathymetry and abrupt ledges in the area where the video survey was conducted precluded the use of on-board GIS to identify the coordinates associated with the video. Since there were no accurate coordinates to use for data extraction from the bathymetry and habitat rasters, my analysis was limited to habitat variables available in both the video survey and benthic habitat map datasets.

*Analyses*
The proportion of videos in the survey featuring cod observations was high (> 80%), leading to a limited number of absences (n = 20 videos) for analysis. In order to increase the available number of records for modeling, I used a presence-only approach typical in SDM (Pearce & Boyce 2006). Presence-only modeling consists of using known observations (in this case, cod observations from the video survey) as presences, while replacing absences with “background” data. Background data, or pseudo-absences, are randomly selected sites where habitat characteristics are known but presence of the target species is unknown. Presence-background modeling incorporates the uncertainty associated with absence records of mobile species (Manly et al. 2007). This approach affects model interpretation, as background data represents the range of available habitats and presence records feature the habitat characteristics associated with greater probability of presence and observation of the study organism (Phillips et al. 2006). The inferences possible from presence-background modeling align with the goals of this study- identifying suitable and preferred habitats at Cashes Ledge.

The observations of cod presence and habitat characteristics resulting from the video survey were used in descriptive and predictive habitat suitability models. The video survey was specifically designed to sample depth ranges and habitat zones equally (Grabowski 2009). However, the depth and substrates are not equally distributed on Cashes Ledge (Figs. 2,3). Therefore, the video survey provided a dataset that was informative for assessing habitat preferences, but its inherent biases complicated efforts to predict habitat suitability on Cashes Ledge. The benthic habitat variables derived from the video surveys provided sufficient detail for modeling presence/absence and visitation rates within the dataset. However, constructing models that are capable of predicting habitat suitability on Cashes Ledge required habitat characteristics available in both the video survey records and the benthic habitat and bathymetry maps. In this
case, the background, or pseudo-absence, data consisted of randomly sampling the bathymetry and benthic habitat maps. Due to the limited depths targeted during the video survey, these maps were clipped to depths < 60 m prior to data extraction. The resulting data were further limited to substrate categories represented in the video survey, resulting in the elimination of gravel and mud-sand data. The number of background data samples extracted from the maps was selected to ensure ~20% presence in the combined presence-background dataset that was used to construct and test models. Once the known presence and background data were compiled, records were randomly assigned to 1 of 5 groups used to identify test and training datasets in iterative, cross-validated modeling. During this process, a modeling step consisted of identifying one group as the test dataset, and the remaining four groups as training data. Each of the five groups was used as a test dataset during modeling, allowing us to evaluate model performance using area under the receiver operating characteristic curve (AUC) and correlation coefficients derived from multiple model runs. AUC provides a measure of predictive accuracy by assessing the successful assignment of class (true positive rate) using all possible probability thresholds for prediction and is often used in presence-background modeling efforts (Phillips et al. 2006). The AUC also provides an estimate of an appropriate threshold for predicting presence in the point along the curve that is farthest from the 1:1 line (representing random chance), which corresponds to the maximum of the sum of true positive and true negative rates (i.e., the value when false predictions are least likely, Hijmans and Elith 2011).

I was interested in assessing the role of macroalgal coverage, geological substrate, depth, and month on patterns in presence and habitat use at Cashes Ledge. The influence of visually described habitat characteristics on cod presence/absence and visitation rates was modeled using
generalized linear and linear models, respectively. I assessed model fit with AIC and used model
diagnostics to assess the effect size of benthic habitats on cod habitat use.

I chose both regression (generalized additive models) and machine learning (random
forest, boosted regression tree) approaches to predict habitat suitability throughout the area.
Generalized additive models (GAMs) are an extension of generalized linear models that allow
individual explanatory variables and their interactions to be fit to a response through additive
smoothing functions (here, thin place regressions) capable of fitting complex non-linear
relationships (Wood 2006). Random forest (RF) and boosted regression tree (BRT) models are
both ensemble methods based on classification and regression trees (CART). The CART process
builds trees by successively partitioning the data into subsets that maximize within group
homogeneity (as measured by the Gini impurity index, Breiman et al. 1984). In RF, the dataset is
divided into a set of subsamples featuring between 60-70% of total records through
bootstrapping (training datasets, n = 500 in these analyses), with each used to build separate trees
(Cutler et al. 2007). RF further randomizes the approach by including a random subset of
predictor variables for each split (Prasad et al. 2006). BRT’s make use of boosting, whereby
additional weight in the overall model is placed on outliers or difficult to model portions of the
original dataset (Elith et al. 2008). This is done by successively building trees based on a random
subsample of the total dataset to partition the residuals from model resulting from the previous
step. At each step, the new tree is added to the existing linear model of existing trees and
residuals recalculated using a new random subset (50% of the original dataset). Both RF and
BRT inherently handle interactions via their hierarchical decision tree structure. Their ability to
effectively model the interactions that are common in ecological studies typically results in more

In both descriptive and predictive analyses, multiple GAMs were fit and evaluated overall using both common model diagnostics (AIC), while the necessity of considering individual variables as non-parametric was assessed using reported effective degrees of freedom (EDF). Initially non-categorical variables were fit using interactive tensor products, then individual smoothing functions (cubic regression splines), and finally parametrically (equivalent to a generalized linear model). Although substrates were categorical, they were considered ordinal in modeling due to their hierarchical complexity (e.g., Laminaria kelp is more complex than Agarum, and kelp in general is more complex than rock), and were included among smoothed variables. Throughout, overall fit was assessed using AIC, while EDF ~1 indicated a smoothed variable could be fit linearly (Clark 2013). Best-fit models were assessed through a cross-validation process. RFs were grown to 500 total trees per model run and were limited to one predictor variable per binary split.

**Results**

The habitats depicted in the revised benthic habitat map were supported by observations from the video survey, their depth distributions matching apart from the absence of small-grained sediments and disparities in median rock and boulder depths (Fig.4). The majority of videos featured benthic habitats dominated by boulder (n=36) or Agarum kelp stands (n=30), followed by rock (n=19) and Laminaria (n=9) or mixed kelp (n=9).

Following the quality control review, 103 of the original 167 videos were available for inclusion in analysis. As has been observed in sampling conducted since the early 1980s
(Witman & Sebens 1992), cod were present in larger numbers at Cashes Ledge, observed in 83 videos (81%) and averaging 5.34 ± 0.66 visits per hour (mean ± SE throughout). Visitation rates decreased with depth (Fig. 5). Although cod were consistently observed in habitats covered by as little as 5% or more by kelp (94% present), visitation rates increased as the proportion of kelp increased (Fig. 6). The best fit linear models included either % kelp coverage (AIC = 665.7, $R^2 = 0.20$, Table 1) or depth (AIC = 665.8, $R^2 = 0.20$). Interestingly, when the combined influence of both these factors were included in models, depth appeared to influence cod habitat usage more than macroalgae. The best fit generalized linear model included both geologic substrate and depth (AIC = 87.6, $R^2 = 0.20$, Table 2). Although my descriptive, ordinal characterization of large-scale complexity was not related to cod habitat preferences, cod were consistently observed in habitats featuring more fine-scale structure (≤ 1 m, interstitial spaces, Fig. 7).

Using the general benthic habitat types that were available for predictive habitat suitability modeling, cod were observed in most videos where benthic habitats featured kelp stands (n = 45 of 48, 94%, Fig. 8), including all *Laminaria* (n = 9, 100%) and most *Agarum* (n = 28, 93%) or both kelp taxa (mixed, n = 8, 89%). Presence in rock habitat was also high (n = 16, 84%), while cod were observed far less frequently in the vicinity of boulders (n = 22, 61%). Cod visited kelp often (8.35 +/- 0.51 visits per hour, mean +/-SE), specifically habitats that featured *Laminaria* (11.03 +/- 1.95 visits/hr, mixed 10.07 +/- 3.47 visits/hr). There were regular visits to *Agarum* (7.03 +/- 1.41 visits/hr), while cod were observed at unvegetated sites dominated by boulder (2.00 +/- 0.51 visits/hr) and rock (4.06 +/- 1.10 visits/hr) less frequently.

Habitat suitability models all performed well based on cross-validation assessments from five modeling runs using unique sub-samples per approach. Both BRT (mean AUC = 0.94, cor = 0.73) and RF (mean AUC = 0.90, cor = 0.61) modeling efforts resulted in high predictive power,
while GAM modeling was slightly less successful (mean AUC = 0.78, cor = 0.39). The complexity of the relationships between presence and both depth and habitat substrate were evident, as the full GAM model required smooth functions for best fit (depth EDF = 4.6, $\chi^2 = 46.7, p < 0.05$; substrate EDF = 2.9 $\chi^2 = 25.1, p < 0.05$). The combined influence of depth and substrate explained 23.9% of deviance in presence-background data, while most of the model’s fit was provided by depth (16.4% deviance explained in the reduced depth-only model, compared to 6.1% in the reduced habitat-only model). The importance of depth to model’s predictive power is greater than that of substrate type, as the high rates of observations in the kelp zones are attributed to depth in the full model, leaving boulder the most favored habitat across depths (Fig. 9). This effect disappears in the substrate-only model, as a steady increase in predicted probability of presence follows the gradient in habitat complexity (from rock to Laminaria, Fig. 10), although a smooth function may not be necessary to fit the relationship (EDF = 1.22.9 $\chi^2 = 25.2, p < 0.05$). In the ensemble tree methods, depth was similarly dominant in its contribution to model fits for the BRT (relative influence = 84.2/100, Figs. 11-13) and RF (% increases in model MSE when depth is dropped from the model, Figs. 14-16).

Across these different modeling approaches, predicted habitat suitability peaked in shallow depths and high complexity substrates, although final predictions did include some surprising differences from video observations. GAM and BRT predicted probabilities of cod observation in the thin band of mixed Agarum and Laminaria were low, considering the relatively high occurrence rates seen in videos within this zone. This substrate featured consistent cod observations (i.e., presence) across videos and high, but variable, visitation rates (i.e., abundance), although predictive modeling was limited to cod presence and did not include visitation rates. Laminaria kelp was selected as suitable cod habitat in all model predictions, but
predicted probabilities were lowest in the BRT results. This was surprising, since BRTs were developed in part to adequately incorporate interactions into model predictions (Elith et al. 2008). The BRT approach focuses trees on residual variance from the previous iterations of the overall linear model, which is meant to reduce the influence of outliers and portions of prediction datasets that are difficult to model (Leathwick et al. 2006). Unlike BRT predictions, the highest GAM and RF predicted probabilities coincided with kelp habitat (*Laminaria* and *Agarum*). Predicted probabilities were higher than expected in boulder habitat across models (Fig. 17). Despite the observed variance in predicted probabilities, high predictive power translated into relatively low threshold probability values across models. This led to the identification of much habitat < 60 m being defined as suitable based on this sensitivity and specificity based approach. When applying the cross-validation determined thresholds to define suitable habitat, all models included *Laminaria*, *Agarum*, and boulder habitats, while none featured rock habitat. This incorporated a large proportion of the available bottom < 60 m depth as suitable habitat.

**Discussion**

As cod grow and mature, the strong habitat affinities observed throughout ontogeny decrease. Despite this, seasonal patterns in preferred depths and substrate grain-size are still generally apparent. At regional scales in the Gulf of Maine and on Georges Bank, cod are associated with large grained substrates, even as their depth distributions change seasonally in response to temperature (Methratta & Link 2006b, a). These observations of cod habitat use and depth preferences are confounded by the depth gradient in the complexity of habitats at Cashes Ledge, and likely influenced these habitat suitability predictions. However, models limited to either depth or substrate. These modeling efforts did not resolve the relative influence of these habitat characteristics, but throughout their range cod have demonstrated preferences for
relatively complex habitats across a range of depths. The focus of this study was on cod distributions within an area much smaller than a single spatial unit defined in previous regional habitat studies (Methratta & Link 2006a, b).

The importance of complex substrates to cod behaviors, especially feeding, is apparent across the species range. However, depth is also an important proxy for a range of seasonally variable habitat characteristics at Cashes Ledge, including temperature (Witman et al. 1993). Although the influence of depth is generally considered at relatively large scales, often in relation to temperature (e.g. Palsson and Thorsteinsson 2003, Nye et al. 2009, Hare et al. 2016), local oceanographic conditions also affect cod vertical distributions and behaviors at fine scales. Cod can display diel vertical migratory behavior when centered on shallow rocky habitats during warmer months (Freitas et al. 2015, Chp.3). Although these behaviors are likely related to foraging conditions and the interaction of metabolism and temperature (Freitas et al. 2015), the combination of dense prey aggregations and temperature gradients are thought to be especially prevalent in shallow hard substrates that feature abrupt changes in bathymetry (Witman et al. 1993). Similar seasonal affinity for shallow structured hard bottom was identified along the Massachusetts coast adjacent to Salem Sound, where similar environmental conditions promoting enhanced productivity also persist throughout the summer (Butman et al. 2007, Chp.4). Due to the depth zonation in substrates and kelp habitats on Ammen Rock, it was impossible to separate the relative influence of these factors.

Extrapolating the findings of this study is complicated by the particular nature of shallow rocky peaks in the Gulf of Maine. Although Cashes Ledge does feature the deepest kelp forest identified in the North Atlantic (Vadas & Steneck 1988), the *Laminaria* and *Agarum* kelp habitats associated with high abundance in this study are depth limited. Similar less shallow
rocky ledges occurring on Cashes Ledge and other ledges and banks in the northwest Atlantic lack the structural complexity of dense kelp stands so important to prey species. Additionally, the interaction of bathymetry, thermocline, and tidal energy that locally disrupts stratification and provides large nutrient subsidies to the shallower peaks of Cashes Ledge are likely greatly reduced, infrequent, or non-existent at deeper peaks (Witman et al. 1993). However, cod were common at habitats that lacked macroalgae in the video survey. Some of these habitats featured high fine-scale complexity- an important characteristic of preferred cod habitats in the video survey. The deep rocky ledges available below the kelp zone likely feature similarly structured substrates, which have been demonstrated to attract cod in other areas of the Gulf of Maine (Auster & Lindholm 2005). To better determine the relative importance of depth, substrate, and habitat complexity, additional locations would need to be included in subsequent sampling.

Separating cod preference for complex non-vegetated substrates, such as boulder piles, within this video survey results from the availability of a highly productive kelp forest nearby. Based on extensive sampling conducted in support of a study focused on the local effects of closed areas on cod abundance, vital statistics, and age distributions, regions of the central Gulf of Maine where fishing is restricted may host relatively sedentary cod that occupy fairly complex habitats (Sherwood & Grabowski 2015). The features associated with these habitats may include smaller peaks within the Cashes Ledge Closed Area’s current border but are outside of suggested boundary revisions (New England Fisheries Management Council 2014). Additional video sampling at these deeper peaks, further removed from the kelp forests on Ammen Rock and other shallow peaks, would provide additional insight on the relative preference for these habitats, as well as seasonal changes in behavior and affinity. The combined effects of seasonal feeding opportunities on rocky peaks across a range of depths in the central Gulf of Maine may provide
important contributions to the overall productivity of the Gulf of Maine cod stocks spawning groups. Cashes Ledge is proximal to both the extant spawning aggregations in Massachusetts and Ipswich Bays to the west and the extirpated spawning grounds along the coast of Maine to the north. Therefore, the peaks within the Cashes Ledge closed area may currently be critical to the population dynamics and eventual recovery of cod stocks throughout the Gulf of Maine.

Efforts to extend the geographic extent of available habitat use data through modeling has become an important tool for ecologists and managers. With the advent of increasingly powerful modeling techniques and their ease of implementation, these techniques have become more common (Elith & Leathwick 2009). The predictive modeling efforts included in this study have allowed us to extend the inferences I made using observations of cod in defined benthic habitats. These spatial extrapolations have provided some context for discussing recently proposed changes to cod management at Cashes Ledge. However, the specific probabilities resulting from the models highlighted some biases in the original dataset that may have affected predictions. The video survey dataset includes a few inherent issues that are relevant to modeling efforts. First, videos in the survey were far more equally distributed than the habitats they targeted. This was an important feature of the original study design, but results in increased representation of relatively rare shallow Laminaria habitat in the video survey data and decreased representation of rock and complete lack of fine-grained substrates. Second, the predictors available to both the video survey and benthic habitat raster datasets consisted of depth and general habitat classifications, which ignores a number of important factors that likely affect cod behavior and habitat use at Cashes Ledge. Perhaps the most important variable not considered in this study was temperature, which has been shown to influence cod behavior at Cashes Ledge at scales relevant to this video survey (Chp.3). Without sufficient functionally relevant predictors in both
training and prediction data, the value of subsequent predictions can be compromised (Elith & Leathwick 2009). Predictor variables available in both existing studies and habitat maps is likely to be a common issue in SDM applications of existing cod habitat use studies and should affect the domain of predictions \(i.e., how far from the original study site results can be reasonably extracted\). These particular biases are unlikely to have affected model results, since the additive and ensemble-tree methods I employed simply used available records to model habitat variable values that influenced cod observations on a site by site basis and prediction results did align with known cod habitat preferences \(e.g., preference for kelp and boulder, Lindholm et al, 2007, Ch. 3\).

As currently defined in the Gulf of Maine, habitat essential to adult cod consists of relatively simple geological substrates, from rock to pebble, across a wide range of depths (New England Fisheries Management Council 2014). Cashes Ledge is dominated by more complex bottom, as were the areas where I observed high visitation rates that these models predicted as the most suitable habitats. The updated definitions included in the new amendment are vast improvements, focusing on complex habitats and hard bottom. These new definitions meet the requirements set forth in MRSA (2007), and exceed definitions of EFH for other, less studied species. However, the relative importance of this broad swath of substrates varies, as use of benthic habitats is related to region (Georges Bank vs Gulf of Maine), season, and depth (Lough 2004). The approach I applied here offers the possibility of satisfying MRSA’s EFH level 2 requirement of habitat-specific densities. Although limited in the Gulf of Maine to a few peaks on Cashes Ledge, the shallow rocky habitats I describe here may in fact be part of a larger, varied ecological habitat utilized by cod across a range of depths. Additional study is needed, however, to identify the specific contributions of geological characteristics \(e.g., substrate grain\)
size, slope) in relation to depth. These efforts might identify additional, deeper peaks along the length of Cashes Ledge that provide important contributions to productivity that are worth preserving.

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Table 1. Best fit linear models of cod visitation rates including explanatory variables and model diagnostics.
Table 2. Best fit generalized linear models of cod visitation rates including explanatory variables and model diagnostics.

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Figure 1. Cashes Ledge maps of (left) benthic habitat featuring seven habitat categories and (right) bathymetry; broken line in both maps indicates the 60 m depth contour, which delineates the domain of habitat suitability predictions for this study.
Figure 2. Distribution of depths available at Cashes Ledge (< 60m) represented in the video survey.
Figure 3. (left) Proportion of available substrates (<60m) by area at Cashes Ledge; (right) proportion of substrates represented in video surveys, records with (presence, black) and without (absence, white) are identified by color.
Figure 4. Depth boxplots of (left) available benthic habitats (< 60 m) and (right) habitats characterized in video survey.
Figure 5. Boxplots of visitation rate (cod visits per hour) by depth.
Figure 6. Proportion of cod observations and mean visitation rate (± SE) by % kelp coverage.
Figure 7. Density plots of (left) topographical complexity and (right) fine-scale complexity at locations where cod were present (dark gray) and not observed (light gray).
Figure 8. Proportion of cod observations and mean visitation rate (± SE) by habitat type.
Figure 9. Probability of cod observations across available substrates and depths as modeled using smoothed substrate and depth in the full GAM model.
Figure 10. Predicted probability of presence (log odds scale) across smoother explanatory variable values resulting from the reduced depth-only (left) and habitat only (right) models.
Figure 11. Probability of cod observations across available substrates and depths as modeled using the BRT full model.
Figure 12. Predicted probability of presence (log odds scale) from the reduced depth-only (left) and habitat only (right) boosted-regression tree models.
Figure 13. Relative influence of each predictor in the full BRT model.
Figure 14. Probability of cod observations across available substrates and depths as modeled using the RF full model.
Figure 15. Importance of variables in full RF model.
Figure 16. Predicted probability of presence (log odds scale) from the reduced depth-only (left) and habitat only (right) RF models.
Figure 17. Predicted probability of observation across available habitats (< 60 m).
Chapter 6: Conclusion

Atlantic cod *Gadus morhua* feature a great amount of diversity in the form of spatial behaviors, physiology, and visible phenotypes throughout the species range. Evidence for important connections between migratory behavior and population productivity is apparent across the North Atlantic (Robichaud & Rose 2004), while at finer scales diet and habitat have been shown to influence physical characteristics like pigmentation (Dannewig 1953, Gosse & Wroblewski 2004). Regardless of migratory tactics, the importance of the physical characteristics of habitat are thought to decrease as cod age, predation risk falls, and their focus shifts to reproduction (Lough 2004). This research focused on the ecological and behavioral diversity associated with visibly distinct Atlantic cod phenotypes, as well as the importance of specific characteristics of benthic habitats to cod spatial distributions and abundance at fine spatial scales.

The connection between morphology, physiology, and behavior places cod within the larger narrative of migration and its consequences across fish taxa. A number of salmonid and moronid species feature morphologically distinct resident and migrant forms with different growth trajectories related to inherent metabolic rates (Jonsson & Jonsson 2001, Kraus & Secor 2004, Morinville & Rasmussen 2008, Conroy et al. 2015). Morphological divergence in salmonids is adapted to behavioral strategies (e.g., fusiform body shapes decrease energetic costs associated with long-distance swims, Crossin et al. 2004), as it is across fish taxa (Blake 2004). The distinct growth rates of white perch *Morone americana* residents and migrants both precede the adoption of migratory strategies and influence later growth rates (Kerr & Secor 2010). In addition to their distinct pigmentation, red phenotype cod feature body shapes and growth rates that align with salmonid and moronid residents (Sherwood & Grabowski 2010, Chp.2). The
diverging responses of red and olive phenotypes to temperature conditions in shared habitats suggests underlying metabolic differences between these resident and migrant cod forms, although more research is needed to identify the connection between behavior and metabolism (Chp.3,4). Regardless, the persistence of both resident and migrant behavioral strategies provides important stability, resilience, and even productivity to fish populations and population complexes (e.g., white perch, Kerr et al. 2009, chinook salmon Oncorhynchus tshawytscha, Satterthwaite & Carlson 2015). Additional research on these and other cod phenotypes is needed to determine their origins during ontogeny and their relative roles in cod population persistence.

Throughout early life history the spatial scale at which cod associate with benthic habitats remains local, as they use large-grained sediments, seagrass, macroalgae, and even sponges for refuge from predation and concentrations of preferred prey (Borg et al. 1997, Gregory & Anderson 1997, Lindholm et al. 1999, Schneider et al. 2008). As cod grow and mature, their reliance on specific substrates decreases, to the point where they are thought to show little preference for any particular benthic habitat (Lough 2004). Although substrate does influence the spatial distributions of adult cod to some extent (Methratta & Link 2006a), other factors such as temperature and depth appear to be more important (Methratta & Link 2006b). The primary importance of temperature to mature cod behaviors has been premised on region-scale biomass distributions in relation to geographically coarse substrate definitions and seasonally changing temperatures (Methratta & Link 2006b). However, small-scale behavioral studies focused on the movements and habitat associations of individual fish have demonstrated the importance of vertically structured substrates to cod behavior and movement patterns (Auster & Lindholm 2005, Lindholm et al. 2007, Reubens et al. 2013, Chp.3,4). The movement of the limited number of fish associated with these studies pales in comparison to the distributional shifts of stock
biomass in space apparent in trawl survey data (Methratta & Link 2006b). However, the distinct differences in apparent habitat limitations of cod expressing divergent growth rates suggests an important connection between local habitat associations and population dynamics. The habitat suitability modelling approach described in Chp.5 provides some possible methods for bridging the gap in spatial scale between the known local relationship of cod and substrate to the regional scale at which management and commercial fishing occur. Through the careful extrapolation of existing behavioral study data, the relationship between phenotype, substrate, environmental conditions, and observed biomass distributions may begin to be pieced together. The results of these efforts may include more informed essential fish habitat (EFH) definitions and maps, possibly integrating phenotype-specific vital rates. These products would provide improved data for the improvement of spatial management of this heavily exploited species.

The results of this research combine with other recent findings to provide some impetus to reexamine the current spatial management of cod in US waters. A recent project focused on the effects of closed areas on cod in the Gulf of Maine and on Georges Bank included the identification of morphological distinctions between fish caught inside and outside of closure boundaries (Sherwood & Grabowski 2015). Inside closures, body shapes were similar to those of red phenotypes, suggesting sedentary migratory strategies based on my results (Chp.3,4). If the connection between morphology and behavior identified in 2 locations in the Gulf of Maine in my research is true of cod in general, closed areas may be protecting inherently less productive resident cod, a possibility the authors considered (Sherwood & Grabowski 2015). In the extreme, the ultimate consequences of this form of spatial management could be permanently less productive stocks. Even if these closure cod are not residents, or if they are relatively more resident than cod outside the closures but no less productive, there is still a risk that inadvertently
reducing the prevalence of a migratory strategy could incur costs in the form of reduced stability or resilience (sensu Central Valley California Chinook salmon, Satterthwaite & Carlson 2015). In instances when management policies have not adequately incorporated cod spatial structure into their design, results have been calamitous (e.g., northern cod, Smedbol & Wroblewski 2002; coastal Maine cod spawning grounds, Ames 2004). In the case of the Gulf of Maine, targeting migrants could lead to higher mortality rates for specific spawning components, as migratory strategies have been found to differ at this scale (Howell et al. 2008). Population models incorporating the existing spatial structure of Gulf of Maine cod have demonstrated the importance of diversity to the productivity of this stock (Kerr et al. 2010b). The gradual decrease in productivity associated with increased fishing pressure could lead to catastrophic consequences in the form of lost biomass and a less stable population overall. The results of the research efforts described in this dissertation provide an important connection between migratory strategies, spatial behaviors, habitat associations, morphology, and productivity that demands further inquiry and focused research.

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Appendix: The effects of intraperitoneal implantation of acoustic transmitters on Atlantic cod (Gadus morhua) in laboratory and field settings

Abstract

During the rapid increase in the popularity of acoustic telemetry as a tool for studying fish movement, behavior, and life history over the past 2 decades, internal implantation has become one of the most preferred attachment methods. Despite this widespread application there have been a limited number of studies that have verified surgical implantation experimentally and provided sufficient detail to allow validated methods to be used in subsequent projects. I developed a protocol for the surgical implantation of acoustic transmitters in the coelom of Atlantic cod (Gadus morhua). These procedures were tested in both controlled laboratory and remote field settings to assess mortality, transmitter security, and effects on Atlantic cod growth. No mortality or tag loss was observed during an 8-week laboratory study and the effects of implantation on Atlantic cod condition and growth were negligible. When implanted in fish at an offshore marine location, the immediate post-procedure survival rate was 91%, with 73% of tagged Atlantic cod detected actively swimming within the array 53 days post-release. The validated protocol offers a simple and effective method ready for use in telemetry and related research initiatives requiring the internal implantation of transmitters in Atlantic cod and other demersal species.

Introduction

Over the past 25 years, the application of acoustic telemetry to the study of fish movement and behavior has accelerated tremendously. A Web of Science (Thomas Reuters 2014; Fig. 1; supplement) search for articles on “acoustic telemetry” and “fish” revealed
exponentially increasing numbers of publications and citations, corresponding to a doubling of publications every 3.6 years and of citations every 3.4 years. With so much emphasis on these and related techniques (e.g., implanted radio frequency identification (RFID) tag), it is no surprise that a number of laboratory experiments and literature reviews have attempted to identify best practices as well as any potentially negative effects of tagging; the focus of these efforts has included impacts on swimming performance (Cote et al. 1999, Cooke & Bunt 2001), growth (Cote et al. 1999), post-operative healing (Moore et al. 1990, Gilliland 1994, Wagner et al. 2000), mortality (Bunnell & Isely 1999, Walsh et al. 2000), and tag retention (Bunnell Jr et al. 1998, Jepsen et al. 2015). However, few studies have tried to provide standardized procedures with relatively long-term monitoring of experimental subjects (Jepsen et al. 2002). Since one of the more popular ways to secure transmitters is invasive surgical implantation within the coelom, there are strong scientific and ethical arguments for developing consistent, validated methods (Brown et al. 2011). Widely applicable and validated surgical methods with enough detail to be repeated are needed to efficiently minimize the effects of transmitter attachment on subject health, behavior, and survival, while simultaneously avoiding complicated and expensive practices that are unrealistic in typical field settings.

In advance of field telemetry projects, preliminary laboratory studies are often carried out to assess tagging-related mortality and behavioral effects as well as rates of tag shedding; in addition, many universities and research institutions require studies to ensure the survival and health of tagged animals over the medium to long term as stipulated by Institutional Animal Care and Use Committees (IACUC). Extended laboratory and field-based experiments may be necessary to assess the effects of surgeries conducted on novel subjects (e.g., unstudied life stages), in new environments, or using recently developed telemetry technologies. However, the
history of successful surgeries involving many species should preclude the need to endlessly repeat simple experiments determining the general effects of transmitter implantation on the health and survival of these taxa. Such wasteful studies drain scarce resources, waste time, and inevitably result in the sacrifice of study subjects while providing a minimum of scientific benefit; unfortunately, as reviewed by Thiem et al. (2011), many potentially useful telemetry studies lack sufficient detail to ensure their utility to other researchers.

Atlantic cod (*Gadus morhua*), has been the subject of many successful transmitter implantations during telemetry studies focused on both the juvenile (Cote et al. 1999) and adult stages (Robichaud & Rose 2001, Windle & Rose 2005, Uglem et al. 2008). However, the surgical descriptions available from these publications differ greatly, and none offer the complete level of detail called for in Thiem et al.’s (2011) suggested reporting guidelines. The goals of this study were to develop a procedure for implanting acoustic transmitters in Atlantic cod and describe these methods with sufficient detail to serve as a model for other telemetry studies. I assessed the efficacy of this procedure during an 8-week laboratory trial, using visual and quantitative measures of survival, condition, growth, and behavior. I considered the surgical protocol effective and ready for application in a field setting if post-surgery survival during the laboratory trial was 100% and measures of growth and condition did not differ from those of Atlantic cod that did not undergo the implantation procedure (*i.e.*, control group). Following the laboratory trial, I applied this protocol in an offshore field setting as part of a 5-month spatial ecology study; here I have used the movements of tagged Atlantic cod as evidence of survival. In addition to the surgery protocol and results of these studies, I have included details of these methods and materials in the context of currently available techniques and technologies (see Discussion). Throughout, I have attempted to provide sufficient information to ensure the
repeatability of these procedures, following Thiem et al.’s (2011) suggestion of including important surgical details, while also adhering to Jepsen et al.’s (2002) recommendation to “keep the procedure as simple as possible.”

Methods

Surgery protocol — Prior to surgery, the surgery cradle, instrument tray, instruments, and materials (including scalpel, sutures, and transmitter) were sanitized using a 2.65% glutaraldehyde solution (*Wavecide*, Medical Chemical Corporation) followed by a freshwater rinse. An individual Atlantic cod chosen for tagging was placed in an aerated tank filled with seawater (50 L volume) and infused with tricaine mesylate or MS-222 (*Tricaine-S*, Western Chemical, Inc.) at a dose of 55 mg MS-222/1 L seawater. Woody et al.’s (2002) four-stage anesthesia assessment was used to determine the depth of sedation. A fish was considered fully anesthetized when it lost the ability to self-right and did not respond to stimulus (*i.e.*, stage 4). Once fully sedated, the individual was removed from the anesthetic tank and placed ventral side up onto a V-shaped surgery cradle where its gills were constantly bathed in fresh seawater (*n.b.*, not anesthetic solution); during surgeries in the field a towel soaked in fresh seawater was draped across the individual’s head at the suggestion of several experienced surgeons (as described in Dean et al. 2014). Scales were removed carefully from a small area of skin < 2 cm in length, adjacent to the ventral midline and anterior to the vent, by carefully scraping in a straight line (anterior to posterior direction) with the tip of a sterile scalpel blade. A small incision (~ 1.5 cm) into the peritoneum (*i.e.*, through the dermal layer, muscle tissue, and coelom epithelium) was made at the area cleared of scales. During the laboratory study, sterile #10 scalpel blades were used; these were exchanged for sterile #12 scalpel blades for the field study. Any discharge of
fluid (e.g., blood) was cleared away using sterile sponges. A single V13P acoustic transmitter (weight in air 12g, volume 4.4 cm$^3$), covered completely with triple antibiotic ointment (Rite-Aid, containing bacitracin zinc (400 units/g), neomycin (3.5 mg/g), polymyxin B sulfate (5000 units/g)), was inserted into the incision; during the laboratory study, “dummy” or non-functioning transmitters supplied by Vemco were used in place of functioning transmitters. The incision was closed with sterile non-absorbable monofilament nylon sutures; 3-0 sutures with NFS-1 reverse cutting needles were used during the laboratory study before being replaced by 4-0 sutures with NFS-2 reverse-cutting needles for the field study. Suturing consisted of interrupted simple sutures (i.e., surgeon’s knot) with an instrument tie (Semer 2001); 2-3 separate sutures were applied ≤ 0.5 cm apart. The closed incision was sealed with veterinary-grade cyanoacrylate (Vetbond, 3M) and covered with a layer of triple antibiotic ointment. Following incision closure, the individual was returned to a recovery tank continuously fed by fresh seawater and monitored closely to ensure immediate recovery (i.e., ability to remain upright and resume active swimming; Woody et al. 2002). Surgeries referenced throughout this paper were performed by the primary author.

Sample collection for laboratory and field studies — Atlantic cod (n = 26; 30-60 cm total length) were collected via baited hook-and-line from nearshore waters (5-15 m depths) off of Marblehead, MA (<3 miles from port; Fig. 2). Collections occurred over the course of 2 non-consecutive days, 10/20/12 (n=16 cod) and 11/7/12 (n=10). Air and surface water temperature were recorded at the time of capture. Caught fish were visually examined for general condition (e.g., signs of barotrauma, open wounds) and hooking location (e.g., jaw hooking, gut hooking) and immediately transferred to an onboard live well. Atlantic cod reached port <1 hour following capture, were transferred to a truck-mounted live well, and reached the holding tank at the
Marine Science Center (Northeastern University, Nahant, MA; Fig. 2) within 30 minutes. Captive Atlantic cod were monitored for 9-27 days prior to commencement of the trial to ensure acclimation to the tank environment and resumption of feeding.

At the commencement of the field study, Atlantic cod (n=25; 35-75 cm total length) were collected via baited hook-and-line from waters (15-30 m depths) around Ammen Rock pinnacle (42° 51.25′ N, 68° 57.11′ W; Fig. 2), 5/31/13. Air and surface temperature were recorded at the time of capture. Caught fish were visually examined for general condition (e.g., signs of barotrauma, open wounds) and hooking location (e.g., jaw hooking, gut hooking) and immediately transferred to a large onboard live well (900 L seawater, 100% changeover every 20 minutes). Atlantic cod were allowed to acclimate to the live well for ≥ 30 minutes prior to undergoing the previously described tag implantation procedure.

Housing and care for laboratory study — Atlantic cod were housed in holding pens (3 m³ PVC cage wrapped in 2 x 2.5 cm mesh polypropylene poultry fencing; n ≤ 13 fish per pen) within a large outdoor seawater tank (24 m³) that was constantly fed with fresh ambient seawater from a pass-through seawater system (pumped directly from Broad Sound, adjacent to the Marine Science Center). Pens were placed into the tank substrate, which consisted of sand and silt. Temperature and salinity were monitored throughout the period of the study. Atlantic cod were fed ~10.5 g frozen ocean clams per cod per day or 1.1% mean body weight (0.97 kg). Effort was taken to ensure all Atlantic cod within each pen were allowed to feed.

Laboratory study — Prior to the laboratory study, Atlantic cod were measured, weighed, and marked with external identification tags (both color- and number-coded; Uline 2” polypropylene fine fabric price tag fasteners). A random subsample of collected fish (n = 20)
were randomly assigned to one of two treatment groups, control (n = 10) and surgery (n = 10). The surgery treatment group underwent the previously described transmitter implantation procedure on 11/16/12. Following tagging, Atlantic cod were photographed and moved to a separate recovery tank filled with fresh aerated seawater for the monitoring period. After recovery, tagged fish were replaced into their original pen. Each step of the implantation procedure (anesthesia, surgery, and recovery) was timed; air and water temperature and salinity were recorded daily both before and after surgeries. Every two weeks throughout the period of the study, both control and treatment Atlantic cod were weighed, measured, and photographed. Eight weeks following surgery, fish were weighed, measured, and photographed a final time before being sacrificed; following sacrifice, cod were dissected to assess internal healing and retrieve organs.

Field study — A subsample (n = 11) of Atlantic cod collected at Cashes Ledge (n = 44) were selected for the field telemetry study based on visually-assessed condition, size (35-75 cm), and phenotype (color; see Sherwood and Grabowski 2010). Selected fish underwent the surgery protocol described above and depth-detecting transmitters were implanted on the same day as collection. Following implantation, immediate recovery was monitored in the live-well for >15 minutes prior to release; upon release, swimming performance was visually assessed. Transmitters were detected using in situ Vemco VR2W acoustic receivers (n = 4) over a period of 5 months. Tagged Atlantic cod survival was determined using transmitter detections: changes in depth and the receiver where the detection was recorded inferred movement and, thus, survival.
Growth, condition, & statistical analyses — The homogeneity of the two treatment groups was confirmed using initial weights and lengths measured prior to the commencement of the laboratory study. Initial differences in the weights and lengths of fish assigned to control and surgery groups were assessed using a multivariate analysis of variance (MANOVA) to ensure no bias in the selection of experimental subjects. If differences were not detected in initial weights and lengths, treatment groups were assumed to have similar size and condition at the outset of the trial.

Changes in condition during and following the completion of the study were assessed using both allometric and physiological approaches. The assessment of condition using weight and length, where greater weight \((W)\) at a given length \((L)\) indicates higher condition, is commonly used in ecological studies (García-Berthou 2001). Here, two allometric approaches were applied to test for the effects of surgery on condition. Fulton’s condition factor \((K=W/L^3)\); Ricker 1975) was used as a measure of condition throughout the period of the study (i.e., measured biweekly). Since this index assumes isometric growth, regression parameters were estimated separately for both control and surgery groups to ensure that the \(b\) parameter was not significantly different from the assumed value of 3 (weight-length relationship modeled conventionally as \(W=a \cdot L^b\); Cone 1989; Gutreuter 1990). The effect of surgery on Fulton’s \(K\) over time was assessed using a mixed effects model with 1st order autoregressive (AR(1)) covariance structure (surgery and time in weeks as fixed effects). In addition to Fulton’s \(K\), a separate mixed effects model with AR(1) covariance structure was used to directly test the effects of surgery on weight over time while controlling for length as a co-variate. In both of these analyses, individual subject intercepts were designated as random effects, since change in condition over time was the primary focus. Using length as a covariate in assessments of
condition has been demonstrated as less biased than testing weight alone (García-Berthou 2001). A physiological measure of condition based on the weight of the liver relative to total weight, the hepatosomatic index ($\text{HSI} = 100 \cdot \frac{W_{\text{liver}}}{W_{\text{total}}}$) was used as a measure of condition at the conclusion of the study (Lambert & Dutil 1997). A $t$-test was used to test whether surgical implantation influenced HSI. All analyses were conducted using the R statistical program (R Core Team 2013).

**Results**

There were no differences in Atlantic cod assigned to the 2 treatment groups ($\text{Wilks' } \Lambda_{2,17}=0.85, P=0.24$) based on initial weights or lengths measured prior to the beginning of the laboratory study. Anesthetization was attained in $\leq 6$ minutes (mean 4 minutes) and surgeries completed in $\leq 10$ minutes (mean 8 minutes). Recovery of fish that underwent transmitter implantation occurred within 10 minutes; however, Atlantic cod were returned to their pens between 21 and 44 minutes (mean 36 minutes) following surgery. On the days when Atlantic cod were collected from Salem Sound (10/20/12 and 11/7/12), water temperatures were 13.0 °C and 11.5 °C and air temperatures were 15.0-22.0 °C (mean 18.5 °C) and 0.5-6.5 °C (mean 4.5 °C). During the laboratory surgeries water temperature was 11.0 °C and air temperature was 5.5-7.5 °C (mean 6.5 °C). Over the period of the study (58 days), water temperature in the holding tank decreased from 12 °C to 3 °C while salinity remained largely constant (range 33-37, mean salinity 34.8 PSU). The highest recorded temperature experienced by Atlantic cod in the tank was 14 °C, which occurred three weeks prior to surgeries. Following transmitter implantation, tank temperatures never exceeded 12 °C.
Through 8 weeks post-surgery, there were no mortalities in either the control or surgery treatment groups. Atlantic cod that underwent surgery were considered fully healed at the conclusion of the laboratory study based on physical examination. No transmitters had been expelled and all had maintained their anteroposterior (i.e., lengthwise) orientation, not interfering with typical organ functioning (e.g., digestion). Some transmitters appeared to have caused minor irritation to the liver; however, when present, this irritation was very limited and livers still appeared to be healthy based on visual assessment. All transmitters were either fully or mostly encapsulated in a layer of what appeared to be mesentery tissue with no evidence of hard, calcitic deposits. For all Atlantic cod, weight change was negligible when controlling for length ($\beta_{\text{week:length}}=-0.001$, $F_{1,77}=5.07$, $P=0.027$). Fulton’s $K$ decreased throughout the period of the study ($\beta_{\text{week}}=-0.13$, $F_{1,79}=18.95$, $P<0.0001$), although only slightly ($\bar{K}_{\text{init}}=0.96$, $\bar{K}_{\text{final}}=0.85$). The assumption of isometric growth was confirmed by fitting the parameters of the conventional weight-length relationship ($W=a\cdot L^b$): the $b$ parameter did not differ significantly from 3 in either the control ($b=2.69$, CI$_{95\%}=2.11-3.32$) or the surgery group ($b=2.60$, CI$_{95\%}=1.67-3.60$). There were no differences in weight ($F_{1,18}=2.19$, $P=0.16$), weight gain (i.e., change in weight over time; $F_{1,25}=0.15$, $P=0.70$; Fig. 3), and Fulton’s $K$ indices ($F_{1,18}=3.11$, $P=0.09$; Fig. 4) between the treatment groups throughout the duration of the study. Similar to condition measures using allometry, the liver-based HSI was not affected by surgery ($t_{18}=0.15$, $P=0.88$).

Anesthetization was attained in ≤ 6 minutes (mean 5 minutes) and surgeries completed in ≤ 10 minutes (mean 7 minutes) during the field study. Ammen Rock Peak water temperature was 11.5 °C and air temperature was 13-16.5 °C (mean 15.0 °C) at the time of tag implantation. One fish expired during the procedure and prior to recovery, resulting in an additional surgery conducted in the field; this increased the sample size of cod undergoing tagging in the field from
the planned $n = 10$ to $n = 11$. All Atlantic cod that successfully survived surgery were deemed recovered (i.e., remaining upright and swimming actively; Woody et al. 2002) within 10 minutes. Tagged Atlantic cod were released 16-76 minutes following the completion of surgery. All released Atlantic cod immediately displayed active swimming in a downward direction.

Atlantic cod tagged and released as part of the field telemetry study were detected $\leq 158$ days following surgery (mean 113 days). One Atlantic cod was not detected within the VR2W array established at Cashes Ledge despite being released after receiver deployment. One Atlantic cod was detected continuously at consistent depth (26-27 m) for 8 hours followed by a brief period of rapid changes in depths (21-30 m); after this initial 9 hour period this transmitter was not detected for the remainder of the study. The remaining 8 tagged cod (73% of tag implantations attempted in the field) were detected at least 53 days after tag implantation (mean 129 days).

**Discussion**

*Equipment, material, & methods*

During the development of this surgical protocol, I used published studies and reviews, technical manuals (e.g., veterinary suturing techniques), manufacturers’ suggestions, and my own experiences to inform decisions. I have included a brief discussion of these choices, with references to the resources I used, to elucidate the reasoning behind the methods employed and materials used in this procedure. This information is included to aid researchers in deciding best practices for future studies.
**Transmitter** — In choosing an appropriate acoustic transmitter for this field study, I considered functionality, battery life, transmission power and frequency, system popularity, and transmitter size and weight. I decided that tagged cod needed to be detected to a range of at least 300 m under average local sea conditions and transmitters had to last more than 12 months. Additionally, vertically stratified substrates and macroalgae at the study site made depth an important aspect of habitat use, justifying the extra cost and added weight that are inevitable concessions for depth-sensing transmitters. Several manufacturers provided products that met these criteria, including Lotek, Sonotronics, Thelma-Biotel, and Vemco. The widespread use of Vemco systems in the region (VR2W receivers are used in federal, state, university, and private research projects within the Gulf of Maine), the manufacturer’s use of universal transmitter coding (unique codes for each transmitter; Vemco 2014), and the manufacturer-supported and user-driven exchange of detection data (e.g., the Atlantic Cooperative Telemetry Network, [http://www.theactnetwork.com](http://www.theactnetwork.com)), increased the effective detection range to other areas of the Gulf of Maine and led us to choose a Vemco telemetry system. The size limit of surgically implanted transmitters has generally been accepted as 2% of whole fish weight (*n.b.*, as weighed in air; Murphy and Willis 1996), although this restriction has been challenged in recent years (Jepsen et al. 2005). I targeted cod 600-4500 g, which corresponds to a maximum tag weight of 12 g based on the 2% rule. After consulting with the manufacturer I chose depth-sensitive V13P transmitters, which weighed 12 g in air while providing a detection range of at least 400m under mean ocean conditions at Cashes Ledge and a battery life of over 3.5 years.

**Transmitter attachment** — Although there are a range of available transmitter attachment methods, surgical implantation is the most widely used method for medium to long term telemetry projects (Cooke et al. 2011b). Transmitters embedded in the coelom are shed less often
than those that are externally attached or intragastically ingested (Liedtke & Wargo-Rub 2012), which is particularly important when conducting prolonged, field-based studies. Additionally, despite the risk of infection and organ damage resulting from exposure of the coelom and introduction of a foreign object, irritation caused by externally attached sensors may continuously disrupt the protective mucus layer covering the epidermis, providing opportunities for infection (Harms 2005). Coating the transmitter prior to implantation (here with antibiotic ointment) has been shown to promote encapsulation and reduce irritation of the coelem (using beeswax, Helm and Tyus 1992; using paraffin wax, Sakaris et al. 2005). When the implantation is conducted by a trained and experienced surgeon, the harmful effects of internal transmitter placement may be smaller over the long term than other seemingly less invasive attachment methods (Liedtke & Wargo-Rub 2012). Since I wanted to track cod over a period of 5 months in a remote field location, I chose to surgically implant transmitters in the coelom.

Anesthesia — Whether sedation is required during handling and surgery depends, in part, on the study subject and nature of the telemetry study being conducted. In some cases, the need to reduce handling-related stress and time to recovery preclude the use of a chemical anesthetic for fear of increased mortality rates or effects on behavior (Damon-Randall et al. 2010). In such cases, non-chemical sedation options may be employed, including tonic immobility (i.e., subject inversion; Kessel et al. 2015) and sensory deprivation (i.e., cold wet towel placed over the subject’s eyes; Dean et al. 2014). Apart from these instances, chemical anesthetics are usually employed and often required by IACUC. With the help of Northeastern University IACUC, I concluded that a chemical sedative would be appropriate for these purposes. Chemical options are limited by current regulations, with only tricaine mesylate (MS-222) approved for use in marine fishes by the Food and Drug Administration (Trushenski et al. 2013). Commonly used
both as an anesthetic and euthanasia agent, MS-222 is lethal to fin fish in high doses or following prolonged exposure (Carter et al. 2011). Additionally, MS-222 is considered hazardous to humans- HIMS & NFPA both rate MS-222 as a level 3 health hazard, able to cause serious temporary harm, necessitating eye, skin, and respiratory protection during use (Western Chemical 2010). For these reasons, I took great care during preparation of the anesthetic bath and throughout the sedation process. However during surgeries in the field, the precautions required for handling and applying MS-222 were cumbersome and difficult to maintain. Additionally, the 21-day hold period required for MS-222 was not feasible for a study at a remote marine location, requiring additional precautions that were only viable due to fishing restrictions at the study site. Although a number of other chemicals have been used as sedatives for fish, including clove oil (Sherwood 2009), dissolved carbon dioxide (Oberg et al. 2015), and benzocaine (Bowker et al. 2014), a general anesthetic for both freshwater and marine fish approved for immediate release has yet to be approved. The FDA and U.S. Fish & Wildlife Service recently designated eugenol-based AQUI-S E and 20E as immediate-release general anesthetics acceptable for used in freshwater fish through its Investigational New Animal Drug program (Aquatic Animal Drug Approval Partnership Program 2016). Despite its restrictions to use in freshwater fish, AQUI-S has been found to be effective in marine fishes (Silbernagel & Yochem 2016) and recent work has suggested that chemical concentration, temperature, fish size, and dissolved oxygen are more importation factors in anesthesia application and effectiveness than taxa (Bowker et al. 2014). The views of regulatory bodies, animal health specialists, and scientists seem to be converging around study-specific decisions on sedation during tagging procedures (Rub et al. 2014), which may lead to more flexibility in determining what anesthetic is appropriate in future studies.
Anesthetic application — As a sedation method, full immersion in anesthetic solution results in efficient uptake via gills and through the skin simultaneously (Neiffer & Stamper 2009). Woody et al. (2002) provide a simple, straightforward assessment of behavior during sedation, tracking opercula movement, equilibrium loss, and finally response to handling. This and related observation-based approaches are predicated on the predictable behavioral pattern displayed by fish during anesthetic induction (McFarland 1959) and have been employed successfully to assess sedation across a range of taxa (Iwama et al. 1989, Keene et al. 1998, Grush et al. 2004, Gullian & Villanueva 2009). Since the intake of any sedatives via the gills and skin is inherently slow, dosage may be adjusted by changing immersion times, which is not possible using other anesthetic administration techniques with set dosages (Rub et al. 2014).

Incision and wound closure — I kept incisions as small as possible (<1.5 cm; transmitter diameter 1.3 cm) in order to limit the number of sutures required for closure, reduce suturing time, and decrease the size of the wound in the event of dehiscence (Mulcahy 2003, Liedtke & Wargo-Rub 2012). Incisions during the laboratory study were made with sterile #10 scalpel blades, commonly used for long, straight incisions in veterinary procedures (Texas A&M Center for Educational Technologies 2013). It was difficult to control the size of incision with these blades, so I employed curved sterile #12 scalpel blades in the field. These blades, which have a fine point and are sharpened on the inside concave edge, require only a quick, shallow puncture into the coelom with the incision created by cutting up (Texas A&M Center for Educational Technologies 2013) — a preferable technique for the dynamic conditions of a ship deck 100 km from shore. Although 3-0 sutures held through the 8 week laboratory study, I decided to substitute 4-0 sutures in the field; I felt that the knots were tighter and more secure immediately upon tying with the smaller gauge sutures, which may be important to wound integrity (Jepsen et
The cyanoacrylate applied to the closed wound failed to remain in place during setting; there is little evidence that these adhesives provide any additional protection against wound opening and may actually compromise sutures (Harms 2005). The layer of triple antibiotic applied to the incision at the end of the procedure may have acted as the protective covering cyanoacrylate was meant to provide. Although Mulcahy (2011) has speculated that antibiotics applied topically to fish are quickly lost to the surrounding water, the ointment itself was still present on most surgical treatment cod at the end of the 8 week laboratory study.

_Surgeon experience and training_ - Surgeon experience is an important and overlooked factor in the outcome of surgeries on fish. Both training and practice are required to ensure the quality and consistency of surgical techniques (Cooke et al. 2011a). Although most of the implantation process may be accomplished by simply following directions (e.g., mixing the anesthetic solution), incising and suturing require training, practice, and experience. These steps demand physically manipulating tissue layers without cutting too deeply or puncturing too forcefully, which would cause unintended damage to tissue and internal organs. In addition to honing the techniques necessary for successful transmitter implantation, experience will also reduce the amount of time required for making incisions and tying sutures, which likely reduces the overall stress of handling (Jepsen et al. 2002). In preparation for these studies, the primary author underwent training by experienced surgeons (the secondary authors), including conducting surgeries on cod already sacrificed for research purposes under direct supervision, and extended practices of instrument ties prior to conducting surgeries on live subjects.

_Outcomes of trials & assessment of protocol_
During these laboratory and field studies, goals were to assess the effectiveness of a surgery protocol for the intraperitoneal implantation of acoustic transmitters in Atlantic cod, including enough detail to provide a repeatable process for future telemetry studies. In the controlled environment of laboratory-based tanks, no lethal or sub-lethal effects were observed in study subjects that underwent transmitter implantation, which suggests the stress of the procedure may not exceed that of captivity and handling. When applied in the field, a conservative estimate of detection data shows at least 73% of cod that underwent the described transmitter implantation survived for at least 53 days, with a confirmed mortality rate of < 10%. Thus, the surgery protocol employed in the studies as described above provides a simple and effective method for implanting transmitters into Atlantic cod in both laboratory and remote marine settings.

All cod that underwent surgery in the laboratory survived and retained their transmitters until the end of the study. Incisions were fully closed and healed at 8 weeks, which agrees with published healing rates of Atlantic cod (Cote et al. 1999). Although some minor irritation of the coelom surrounding transmitters was identified, the hard, calcitic deposits associated with transmitter expulsion, reduced condition, or increased mortality were completely absent (Loher & Rensmeyer 2011). The absence of lethal and sub-lethal effects of transmitter implantation on Atlantic cod has been noted in previously published studies (Cote et al. 1999). However, cod in both of the treatments did not grow and their condition decreased slightly. Although cod were not fed to satiation, they were provided daily rations similar to those consumed in the wild (0.9-1.5% fish body weight; Durbin et al. 1983). Instead, I ascribed this to the stress of being held in a confined environment at densities higher than those typically observed in natural settings, which can negatively impact feeding and growth (Rowland et al. 2006). This stress may have been

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compounded by the biweekly handling required during weighing and measuring. Because the condition of both treatments (i.e., surgery and control cod) decreased but did not differ, the results suggest that this was not caused or exacerbated by surgery.

The effects of implantation in the field study were more pronounced than in the laboratory. There was 1 casualty, suggesting a tag-related mortality rate of 9.1% (1 of the 11 cod that underwent surgery). Although this was the lone confirmed casualty, the detection records of 2 other Atlantic cod that were either detected for a short period of time (<9 hours) or not detected at all could be interpreted as further casualties. Although these limited or non-existent records cannot be used as support for long-term post-surgery survival, they do not suggest immediate mortality, either. The depth record of the individual detected for <9 hours suggested a recovery period of 8 hours at relatively constant depth followed by a return to active movement. The lack of detections for these tagged Atlantic cod may simply reflect the complex, kelp covered rocky habitat present at the study site, which would create areas within the array where detection is not possible (i.e., acoustic shadows; Catipovic 1990), and the limited extent of the detection area in an open marine environment. Indeed, no detections of any tagged Atlantic cod were recorded on 9 days during the entire 158 day period of the study, with the resumption of tag detections suggesting subject mobility rather than mortality. Assessing mortality related to surgery following release into the field is difficult, while determining the extent of sub-lethal effects without the aid of recaptured fish or transmitters equipped with physiological sensors is impossible (Cooke et al. 2011b). However, a conservative consideration of post-surgery survival of 8 cod, 72.7% of those that underwent transmitter implantation in the field, at least 53 days post-procedure is similar to detection rates of Atlantic cod in other studies (Robichaud & Rose 2001, Dean et al. 2014). The prolonged survival of Atlantic cod that underwent implantation
suggests that when applied in the field the protocol is at least as effective as methods employed in other published studies that include fewer details and shorter procedure descriptions.

A number of factors can induce mortality in the field including depth at capture and air temperature during handling. Atlantic cod used in the laboratory study were collected from nearshore rocky ledges, not exceeding 20 m in depth, while those at Cashes Ledge were caught in depths of 15-30 m. Despite being brought slowly to the surface to avoid damage due to changes in depth and temperature, some Atlantic cod still experienced barotrauma in the form of swollen lateral body walls and an inability to swim below the surface of the large live well. Even though physiologically compromised individuals were excluded from the study based on visual inspection, those included in the study may have suffered less severe but still significant barotrauma. In a study monitoring the effects of pressure changes on Atlantic cod swim bladders, Midling et al. (2012) found that all fish experienced swim bladder rupture but retained different amounts of gas within their coeloms, a greater amount of which would provide the visual evidence of barotrauma. In addition to pressure, temperature at the surface is a concern for marine fishes accustomed to life in relatively cold water (Gale et al. 2013). I was not able to lower the temperature in the live well below the surface temperature at the time of the surgeries (11.5 °C). Other telemetry studies have attempted to mitigate the negative effects of warm surface temperatures by pumping water from below the thermocline (Dean et al. 2012) or adding previously chilled seawater to recovery tanks (Sherwood 2009). Although water temperature measured during the laboratory (11.0 °C) and field studies were almost identical, air temperature was drastically different (means 6.5 °C and 15.0 °C, respectively). Even at low temperatures, exposure to air is highly stressful for Atlantic cod, resulting in almost immediate decreases in physical responses to stimulus (Humhorstad et al. 2009). Changes in temperature over even
limited ranges can have profound effects on Atlantic cod (Claireaux et al. 1995), and exposure to extreme temperatures can increase stress (Pérez-Casanova et al. 2008). Additional sources of stress when performing surgeries in remote field locations include less time for study subjects to acclimate to captivity and handling before implantation and less time to recover before being reintroduced to their marine environment. The shortened acclimation period may have also compromised my ability to sufficiently assess condition, possibly leading to study subjects with lower condition being included in the study. The combination of multiple stressors could amplify the negative effects related to transmitter implantation. Despite the precautions taken during this study, some surgery-related mortality may be inevitable for marine demersal fishes during this type of research and should be factored into experimental designs and planning.

This surgery protocol offers a detailed and relatively simple procedure for the surgical implantation of acoustic transmitters in Atlantic cod and could feasibly be applied to other gadoids. The laboratory study, conducted over a period of 8 weeks, might be used in lieu of similar experiments demonstrating the absence of sub-lethal effects related to tagging and, possibly, as an assessment of tag-shedding and mortality rates. Changes such as the use of different anesthetics can be incorporated as developments in fish surgery techniques are introduced without requiring completely new methods. This validated surgical procedure may be used in similar studies of the spatial ecology of gadoids and other marine fishes with similar life history characteristics and morphologies.

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Figure 1. Number of identified (black bars) publications (top) and citations (bottom) reported by a Web of Science (Thomas Reuters 2014) search of the topics “acoustic telemetry” and “fish”; gray lines indicate numbers of publications ($r=0.19$, $P<0.001$) and citations ($r=0.20$, $P<0.001$) predicted for the time series using an exponential growth model ($N=N_0e^{rt}$).
Figure 2. Bathymetry of western Gulf of Maine; cod sampling (Massachusetts Bay and Cashes Ledge), laboratory study (Marine Science Center, Nahant, MA), and field study locations (Cashes Ledge) are identified.
Figure 3. Boxplots of biweekly weights of cod in the control (white boxes) and surgery treatments (gray boxes) over the 8 weeks of the study. Initial weights (week 0) were measured prior to surgeries.
Figure 4. Control (black filled circles and black line) and tagged cod (gray filled circles and gray line) measured and predicted weight at the end of the study (8 weeks following surgery); predicted weight was determined using the relationship $W = a \cdot L^b$ modeled to test for isometric growth.