

1-20-2007

Determining Winter flounder Spawning Sites in Two Connecticut Estuaries

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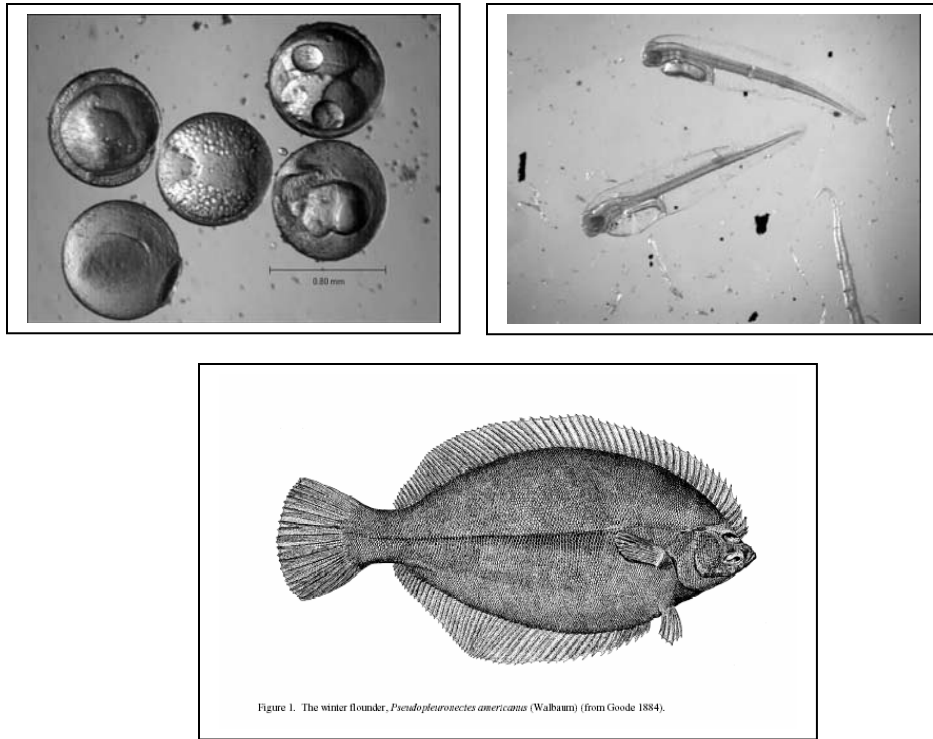
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Recommended Citation

Schultz, Eric T.; Pereira, Jose J.; and Auster, Peter J., "Determining Winter flounder Spawning Sites in Two Connecticut Estuaries" (2007). *EEB Articles*. 19.

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Determining Winter flounder Spawning Sites in Two Connecticut Estuaries



Final Report Submitted to the Connecticut Department of Environmental Protection
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20 January 2007

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Abstract

Winter flounder (*Pseudopleuronectes americanus*) come inshore during the late winter and early spring to lay their eggs in shallow bays and estuaries along the coast. Unlike most fish eggs, which are buoyant, these eggs are demersal and sink to the seafloor. This makes them vulnerable to burial from various types of natural and human-caused disturbances (e.g., storms, mobile fishing gear, maintenance dredging). Our objective was to map spawning areas in two harbors and search for generalities among these sites that would allow us to predict where winter flounder might spawn in other areas. This would allow managers to avoid permitting activities for those times and locations where winter flounder spawn.

We used a modified demersal plankton net (a benthic sled) to collect winter flounder eggs in New Haven and Milford harbors and map their distributions. Most of the eggs were collected at the end of March, when water temperatures were 4-6 ° C. This could vary from year to year depending on temperature. The distributions of eggs were not correlated with sediment type or depth but were related to the prevailing tidal currents in the area sampled. Since the eggs are present in low-current depositional areas, they are vulnerable to burial. Our observations suggest that winter flounder either do not deposit eggs in high current areas, or if eggs are deposited there, they are swept away.

Since early stage embryos (morula, blastula, gastrula) were found in low current areas, it seems unlikely that they were transported there from some other location. These findings have important management implications because any activities (dredging, building breakwaters, installing docks) near spawning areas could have adverse effects if they change the prevailing currents in the area.

Introduction

An understanding of habitat use is an essential part of ecological science and how it is applied to the management of exploited species of plants and animals. The conceptual definition of habitat, the area where an animal lives (Smith and Smith 1998) is intuitive and straightforward. The operational definitions used to define this area, however, are many and varied. Presence/absence data, the concept of the multi-dimensional niche (Pittman and McAlpine 2003), distribution of fish communities (Auster et al. 2001), and population density (Knight and Morris 1996) have all been used as ways to identify habitat. It is also should be noted that individuals are often found in suboptimal habitats and that the mere presence of an organism in a particular habitat does not indicate that the population can sustain itself there (Schultz and Ludwig 2005). The concept of the ecological niche, which is largely based in what habitats species use, has been applied for nearly a century to a variety of ecological issues, particularly in matters of population sustainability and local biodiversity. Somewhat more recently, habitat use and other ecological concepts have become integrated into management of natural resources. This doubtless occurred because there was a growing appreciation for the interconnections within ecological communities, and the limited utility of management approaches that essentially treated species as if they lived in isolation.

This widening of management perspective to incorporate habitat use and ecological interactions is reflected in changes to federal fisheries legislation. Traditional fisheries management techniques relied on adjustments of fishing mortality (via fishing quotas, closed seasons, size limits), with little regard for behavioral characteristics of the species (Hilborn and Walters 1992). To redress this, amendments were recently adopted

to The Magnuson-Stevens Fishery Conservation and Management Act (Public Law 94-265), the federal legislation that controls the offshore fishing industry in the United States. These amendments to the act require that research be conducted to identify “essential fish habitat” (EFH), defined as “those waters and substrate necessary to fish for spawning, breeding, feeding or growth to maturity.” Such federal legislation does not have direct jurisdiction over inshore species such as winter flounder. However, concern for better information on habitat has spread to other management agencies, such as the Atlantic States Marine Fisheries Commission (ASMFC), which represents 15 coastal states and sets goals for the management of coastal species in state waters. The ASMFC recently outlined strategies to promote the goal of enhancing cooperative protection of fisheries habitat; these include “encouraging and facilitating research for determining fish habitat requirements” and “developing effective habitat management, restoration, and protection actions (Stephan et al. 1999). The first strategy is implemented by providing funding for scientists to bring improved research methods to bear on the study of fish species’ autecology; the second strategy is implemented by bringing the data on habitat requirements to bear on management decisions, often via consultations between resource managers and permittees. The need for such data is especially evident when activities are proposed that may have an immediate and direct negative effect on habitat, such as dredging in sensitive spawning areas.

Estuarine habitats are commonly identified as EFH. Estuaries provide nursery habitat for many coastal and anadromous species: ripe adults migrate into estuaries, or larvae migrate in after hatching. Estuaries are relatively productive, providing enhanced opportunities for growth, and may provide some refuge from predation as well (Day et al.

1989). Because they are habitats that harbor early life stages, they tend to play a critical role in sustaining populations. The abundance of a year-class tends to be set during the post-larval stage, because mortality occurs at an especially high rate during the this developmental period (Sissenwine 1984; Houde 1987). This suggests that identification and protection of estuary nursery habitat will be an especially effective way to assure the long-term sustainability of coastal and anadromous fish stocks.

Improved environmental understanding and strong legislation notwithstanding, high-quality estuarine habitats are relatively restricted and are subject to multiple threats associated with coastal development (Howell et al. 1992). The threats include reduced water quality from point source pollution and nonpoint source pollution. Dredging of harbors can have a strong localized effect: the disturbance of water quality and effects on hydrodynamics can influence the ability of larvae to enter or be retained in nursery habitat (Crawford and Carey 1985).

In southern New England, resource managers who are evaluating dredging and development projects often consider the EFH of winter flounder, a species of recreational and commercial importance. The inshore population of winter flounder is known to spawn in estuaries, and in other near-shore, shallow water habitats. There are indications that the amount of estuary habitat is a factor limiting population size (Howell et al. 1992). The early-life stages of winter flounder are susceptible to disturbances in water quality that are associated with dredging and other coastal development projects (Klein-MacPhee 1978; Nelson et al. 1991). Local populations may have low resilience following an episode of habitat disturbance because spawning adults have strong homesite fidelity (Saila 1961; Anonymous 2006). For these reasons, it is important to have complete data

on winter flounder use of estuaries. However, there is scant information on where adults spawn. In Long Island Sound, there is evidence of spawning within harbors and estuaries (Pereira et al. 1994a; Anonymous 2006) and in nearshore, shallow water habitats within the greater estuary (Anonymous 1988)

Winter flounder is an economically significant species in our region. Inshore stocks (all populations except those residing on Georges Banks) had a total economic value, recreational and commercial fisheries combined, of \$19-54 million in 1988 (Howell et al. 1992). The fish is thick and meaty, is regionally one of the more abundant inshore fishes, and is available for exploitation during colder months when other species have vacated inshore regions (Collette and Klein-MacPhee 2002). The commercial fishery mostly uses otter trawls (Howell et al. 1992; Collette and Klein-MacPhee 2002); the fishing season begins after spawning and peaks in spring and early summer. The recreational fishery is most active inshore and the preferred gear is the baited hook (Collette and Klein-MacPhee 2002). Stock assessments of winter flounder provoke serious concern for the future of the fishery. Downward trends in catch rates were observed in the 1980s (Howell et al. 1992). The trend continued in the 1990s; stocks in the late 90s to the present were estimated to be at low biomass levels and were characterized as overexploited (Terceiro 2005). The 2002 year class was the smallest on record (Terceiro 2005).

Inshore stock winter flounder spend a significant portion of their lives in estuaries. Information on their natural history is summarized in (Pereira et al. 1999; Collette and Klein-MacPhee 2002). Larvae are found in the greatest numbers in inshore waters (Lux and Kelly 1981; Scarlett and Allen 1989). Juveniles and adults remain in

shallow waters, even occupying intertidal flats during high tides. Movement to deeper waters, up to 37 m, occurs during extreme winter conditions (pack ice) in northern portions of range (McCracken 1963), or high summer temperatures (>22 degrees) in southern portions of range. In Rhode Island, adults enter coastal ponds in September and feed actively (Crawford and Carey 1985). They remain during the coldest months as gonads develop. After spawning, adults continue to feed inshore but leave in May or June for cooler waters of the open coast in Rhode Island and Block Island Sounds (Crawford and Carey 1985). Juveniles show less preference to migrate and remain in cooler water basins of inshore estuaries (Crawford and Carey 1985). Adults return to spawning areas they used in previous years (Saila 1961; Anonymous 2006)

Inshore winter flounder in our region spawn as the water is just beginning to warm from low winter temperatures. In the Gulf of Maine, spawning begins when water temperatures reach 0° C and ends when waters have warmed above 3.3° C (Collette and Klein-MacPhee 2002). Spawning occurs at higher temperatures in more southern locations (Scarlett and Allen 1989). Timing of spawning can vary from year to year in a given location. Pearcy (1962) reported that March is the month of peak spawning in Long Island Sound populations. Spawning may occur earlier (February) in warmer years and later (late February –March) in colder years (Anonymous 2006)

Historically, location of spawning grounds was inferred from assessing shifts in areas of maximum catch rates in the trawling fishery (McCracken 1963). Interpretation of such studies is difficult because sample efforts are controlled by interest in maximizing catch rather than documenting habitat preferences. Spawning season habitat preference has also been assessed by tracking adults via telemetry (Pereira et al. 1994a); this study

found it difficult to reliably relight adults because they are transient rather than resident. The presence of larvae has also been used to locate spawning areas (Austin 1973; Larson 1973; Perlmutter 1947), but larvae can be moved about by tidal or wind generated currents, and can only give a general sense of spawning location. Some researchers have suggested in fact that current regime may play a role in selection of winter flounder spawning locations. Winter flounder may deposit eggs in areas where the hydrodynamics of the system will tend to retain hatching larvae in the nursery area (Crawford and Carey 1985).

Several studies have targeted eggs in an effort to locate spawning areas (Crawford and Carey 1985; Arnold and Rogers 1972; Austin 1973). The eggs are demersal and adhesive, should be less affected by wind and tide than larvae, and should be found closer, if not immediately on, the area where spawning occurs. Fish eggs are known to vary in degree of adhesiveness (Makayeva 1976; Marliave 1976; Markov 1978; Britz and Cambray 1998; Rizzo et al. 2002; Doi and Aoyama 2006) and winter flounder eggs are moderate in that regard at least when compared to Atlantic herring eggs (James Hughes, NMFS Milford Laboratory, pers. com.).

While the eggs have been reported attached in clumps to substrate or vegetation (Perlmutter 1947; Arnold and Rogers 1972; Crawford and Carey 1985), doing so is detrimental to their development (Smigielski and Arnold 1972) and winter flounder spawning behavior would tend to prevent clump formation (Stoner et al. 1999; Bigelow and Schroeder 1953). Cultured winter flounder eggs are treated with a slurry of diatomaceous earth mixed with seawater to coat the eggs and prevent the formation of clumps (Smigielski and Arnold 1972). Winter flounder eggs collected from the wild

frequently adhere to bits of substrate, which would also help prevent the formation of clumps and make them less buoyant as well. Eggs are rarely stuck together in benthic sled samples (pers. obs.; pers. comm., James Hughes, NOAA Fisheries). We have only observed clumps of eggs in holding tanks at the laboratory when females are kept separated from the males and the females have aborted their eggs. Clumps of eggs in the wild may have become less common as the spawning population declined.

Despite their adhesive nature, winter flounder eggs can be collected in bongo nets fishing in the water column (Lux and Kelly 1981; Pereira et al. 1999) perhaps because some eggs are naturally suspended due to turbulence. An epibenthic sled, however, yields the greatest catch rates of winter flounder eggs (Crawford and Carey 1985; Scarlett and Allen 1989; Hughes 1999; Pereira et al. 2002). These studies show that eggs are most likely found in bottom waters of intermediate salinity (14-21 psu). Spawning may occur in water as shallow as 2 m however (Collette and Klein-MacPhee 2002). Sampling in previous studies has been on a relatively coarse spatial and temporal scale, and does not discriminate between early-stage and later-stage eggs; it is therefore difficult to assess the degree of habitat preference. No study has carefully evaluated sediment characteristics. It is believed that eggs deposited on soft sediments will get insufficient oxygen (Crawford 1990). Eggs hatch 2-3 weeks after spawning at temperatures characteristic of period following most spawning (Crawford and Carey 1985; Collette and Klein-MacPhee 2002).

In summary, we have a good base of data on broad-scale movements of adults during the breeding season. Spawning is thought to take place mostly in small to medium estuaries, rather than in Long Island Sound proper (Richards 1959). We do not know where within the estuaries the flounder leave their eggs; there is scant information on

what bottom type, and depth range, the adults prefer to spawn. Information on their spawning preferences will be useful to coastal resource managers who must judge the ecological impact of such human activities and coastal development and dredging (Crawford and Carey 1985).

Our goals were to sample estuaries for winter flounder eggs in a way that will enable us to evaluate the characteristics of areas where there are concentrations of early-stage eggs indicative of spawning sites. In conjunction with collecting eggs, we evaluated substrate characteristics to look for preferences in sediment type. The main objectives of the study were to: 1) locate spawning areas of winter flounder in two estuaries; 2) determine habitat characteristics associated with the spawning substrate; and 3) determine how widely the eggs disperse from the spawning areas after deposition.

Stated more formally, our goals and objectives can be summarized in two hypotheses:

Hypothesis I: Early-stage winter flounder eggs show no preference with regard to their distribution among depth ranges and substrate types.

Alternate I: Early-stage winter flounder eggs are found in shallow waters with sandy substrate.

Hypothesis II: Later-stage winter flounder eggs show no preference with regard to their distribution among depth ranges and substrate types.

Alternate II: Later-stage winter flounder eggs can also be found in deeper water and mud clay bottom as they spread from the spawning sites.

Methods

Site Selection

Long Island Sound is an atypical estuary with regard to its circulation (Paskausky 1977). Most of the freshwater inputs, rather than occurring at its head at the western end, occur along its northern edge. These inputs from Connecticut rivers form a complex

system of sub-estuaries (referred to as estuaries in the remainder of this document). These estuaries combined with the life history characteristics of winter flounder may contribute to the formation of discrete spawning stocks within Long Island Sound (Crivello et al. 2004) and suggests that focus at this spatial scale is a valid approach to the study of winter flounder spawning habitat.

We selected two estuaries for this project. We wanted to have a pair of estuaries that are of disparate size, and yet are both known to be winter flounder spawning estuaries. For the larger estuary, we selected New Haven Harbor. Prior work in this estuary has examined adult distribution during the spawning season (Pereira et al. 1994b) and on the distribution of eggs (Pereira et al. 2002). We expanded both the spatial extent and intensity of the sampling over that done by Pereira et al. (2002) in order to provide a more detailed map of spawning areas. For the smaller estuary, we selected Milford Harbor, where spawning has been known to occur. An advantage of both these sites is prior information on surficial bottom types (Gayes et al. 1991; Poppe and Polloni 1998; Stone et al. 1998; Knebel et al. 2000; Paskevich and Poppe 2000).

Sampling of the estuaries was conducted as a stratified random design. This design involves random allocation of sampling units (in this case, randomly locating the sampling tows) within predetermined strata, which are combinations of environmental conditions. Stratified random sampling is preferred over simple random sampling when there is evident environmental heterogeneity (variability in predictor variables) that is expected to have some impact on the dependent variable(s) of interest.

We assigned strata within each estuary according to region (inner/outer harbor), depth within the region (1-2 m, 2-4 m, 4-6 m, and >6 m) and time (5 sampling periods at

biweekly intervals). Region was stratified because conditions important to winter flounder vary spatially along the main axis of an estuary, especially sediment types and salinity. We have interpreted geographic features within each estuary that separate inner and outer harbors. Depth was stratified because these fish are known to be depth-selective at other times of the year and are reputed to prefer shallow water for spawning (see above). Finally, we have stratified by time because sampling on multiple occasions minimizes the chance that major spawning activity will be missed, and because it is likely that the distribution of spawning will shift as the season progresses.

Evenly spaced tow sites were fixed within each stratum. Within each combination of region and depth, 2 sites were sampled on each cruise, and 2 replicate tows were conducted at each site. Maps indicating the region, depth strata, and station locations are provided (Figs 1-3). Tables 1 and 2 give latitude and longitude for Milford and New Haven stations respectively. All depth strata were not available in each region. Sampling in inner Milford Harbor was limited to the 4-6 m depth strata. Sampling was not possible outside the channel in the Harbor proper, because of a dense array of fixed moorings. Gulf Pond nominally contained 1-2 m depth strata but was inaccessible and mostly comprised mudflats that emerge on low tide. Sampling in the outer Milford Harbor was limited to the 1-2, 2-4 and 4-6 m depth strata. In inner and outer New Haven Harbor strata, all depth ranges are available. Therefore, the sampling design includes 13 combinations of depth and region. We used the 50-foot F/V Victor Loosanoff of the Milford NOAA Fisheries laboratory for all collection trips. We would expect to collect a total of 220 samples under this sampling design.

Egg sampling methods

Eggs were collected in a modified plankton net. The design, (modified from Yocum and Tesar 1980; Crawford and Carey 1985), has proven effective at collecting benthic winter flounder eggs while minimizing debris (Pereira et al. 1994b; Hughes 1999; Pereira et al. 2002). A 250 micron mesh net (opening = 0.25m², length = 1.5 m) is fitted onto an aluminum sled with runners (Figure 4). The sled includes a Plexiglas shield ahead of the mouth of the net; as the sled is dragged, turbulence behind the bar resuspends lighter material lying on the bottom, such as eggs.

At the selected locations, the sled was towed for 5 minutes, for a distance of approximately 300 m. The heading was set so that the tow remained in the appropriate depth stratum and into the prevailing current if there was one. Salinity and temperature at the bottom were measured at each site, using a YSI, hand-held temperature, salinity and dissolved oxygen probe. Each second replicate tow followed the course of the first tow at a slight distance. Samples were sieved and fixed on board, in 10% buffered formalin. Rose Bengal, a vital stain, was added to the sample in the field. This colored the biological material and facilitates separating specimens from detritus, without interfering with identification and staging.

Egg sample workup

The samples were shipped to the Plankton Sorting and Identification Center in Szczecin, Poland for sorting. The quality assurance protocol at the sorting center calls for 10% of the samples in a batch/shipment to be resorted by a senior staff member. If more than 2 fish eggs or larvae are found in a sample the entire batch was resorted. Larvae were identified to species at the Plankton Sorting Center. Fish eggs were identified to

species and staged by us using standard guides (e.g., Martin and Drewry 1978; Fahay 1983). Staging enabled us to distinguish between eggs that were recently deposited; hence indicative of spawning site preferences, and eggs that are more developed and may have moved from the site of their deposition.

Mapping habitat

Analysis of bottom types was conducted with a system produced by Quester Tangent (Sidney B.C., Canada). This technology has recently been used to successfully classify bottom types with respect to fish habitat (Collins et al. 1996; Freeman et al. 2002). This is a digital seabed classification system, which digitizes return signals from a standard single-beam echo sounder (200 kHz frequency). Seabeds of various types (e.g., mud, sand, sand with shell, gravel, gravel with macroalgae) produce unique waveforms. An on-board computer runs the echo signal through algorithms that produce a set of 166 descriptors, for each record measured. The 166-dimensional variability in the descriptors is summarized using principal components analysis as 3-dimensional variability (“Q-space”). Interpretation of the result with respect to bottom type requires a training or calibration set of recordings in which the bottom type is known. It is possible to develop the training set in advance, and do the seabed classification analysis in real time. However, storing and post-processing the descriptors maximizes quality of the interpretations. This is the approach we used: we collected the acoustic signals while we towed the egg collection gear, storing the descriptors of the echo sounder signal and post-processed the signal using a training set developed after the egg collection cruises. Post-processing used QTC Impact software (Version 3.3).

We developed the training set of acoustic readings by taking underwater video images, using a drop camera at specific ground-truth stations. The drop camera (Figure 5) consists of a submersible color video camera and lights mounted in a frame and tethered to the surface with an electro-mechanical cable. Power for the camera and lights and video imagery from the camera are transmitted through the cable. Video imagery was monitored in real-time and recorded on Hi-8 format videotape for subsequent analysis. In order to obtain video imagery in areas of high turbidity, an optically clear housing, approximately 0.5 m in depth, was fitted to the front of the camera and filled with fresh water to enhance resolution of the seafloor. Cluster analysis was performed on the Q-space data taken during the egg collection tows to locate areas of diverse habitat types. The ground-truth stations were georeferenced using GPS.

The training set, consisting of 12 reference sites and representing a variety of bottom types, was sampled with the QTCView system while simultaneously using video as a ground truthing measure. Over 600 frame grabs were extracted from this data set, saved as JPEG files, and were classified as to bottom features using a modified version of the program Coral Point Count with Excel extensions (Kohler and Gill 2006). In this program, a number of random points (we used 40) were randomly superimposed over the image of the bottom and the features (rock, sand, mud, macroalgae, cobble, sea star, crab) marked by the points are enumerated and used to classify a particular bottom type visually. These were then matched with acoustically distinct signatures provided by the QTCView program so that bottom types can be classified by acoustic signature alone using Discriminant Analysis (SAS 2001). Some of the video ground-truth stations were located near areas where winter flounder eggs were collected and can be used to examine

spawning substrate directly. Additional data on surficial sediments was available through an existing geo-referenced sediment data set for Long Island Sound (Paskevich and Poppe 2000). This USGS open file report contains surficial sediment maps of Long Island Sound, which were constructed from side-scan sonar data ground-truthed with sediment grabs.

Information on current regimes in New Haven Harbor was obtained from a study done by the Army Corps of Engineers of the current patterns in New Haven Harbor. They wanted to study how deepening and widening the channel might effect the current patterns and the shellfish beds in the harbor (Richards 1988). He collected current readings from multiple locations in the harbor and used the data to construct a computer model that generates a vector field representing the current patterns at various stages of the tide. We obtained a copy of the vector field for the full ebb tide and superimposed it over our mapped egg distributions. Additional information on current patterns in New Haven was obtained from McCusker et al. (1977). Information on the current regime in Milford Harbor was obtained from Michael Ludwig of NOAA Fisheries (pers. comm.).

Statistical interpretation

The sampling design was set up so that the effects on egg abundance of time, estuary, region within an estuary, and depth can be assessed. Small sample sizes and a lack of normality in the data necessitated a nonparametric statistical approach and aggregation of the egg counts over time. Egg counts were analyzed via Kolmogorov-Smirnov goodness-of-fit tests; each egg is treated as an independent event in this analysis. We also reduced the results within each estuary to a 2X2 contingency table (with/without eggs, soft/hard sediments) and conducted Fisher's Exact test (Zar 1984),

which thus treats each sample as an independent event. In a third analysis, we estimated the catch of eggs per unit effort as the number of eggs or larvae caught per tow of the benthic sled. We then used a Kruskal-Wallis test to compare the catch of eggs across sediment types and depth strata. Effects of sediment and depth were tested in separate one-way analyses.

The degree to which the distribution of eggs changes with egg stage can be assessed with multivariate methods. We used a principal components analysis to determine if samples vary not only in the overall abundance of eggs, but also in the relative abundance of early- and late-stage eggs. We have used this approach to examine temporal and spatial effects on larval size distributions (Schultz et al. 2003).

Results

Sediment Mapping

We were able to identify 5 distinct acoustic signatures using a cluster analysis but were unable to consistently match them to video classifications of the bottom. Sediment classification done with QTCView IV was apparently unsuccessful because we were often operating in water that was too shallow; recent analysis (Freitas et al. 2005) showed that QTCView IV is not effective in depths shallower than 5 meters. The latest version of the system (QTCView V) is reported by the manufacturer to work in depths as shallow as 0.5 meters and should be used in subsequent attempts to classify bottom habitats in shallow estuaries.

The poor repeatability of our classification is evident in cases where the acoustic track classifications from the benthic sled runs crossed (Figure 6). When we attempted to

repeat the analysis after eliminating all records that were from depths of less than 5 meters, we found we were left with insufficient data for any meaningful analysis.

The video taken in the outer harbor in Milford at station V2 (Figure 7) confirms the soft nature of the sediments there (Figure 8). Video ground-truth stations V6 and V7 in New Haven (Figure 9), confirm the presence of soft sediments in the Long Wharf area (Figure 10). Video sampling at stations V9 and V10 (Figure 9) confirmed the presence of sand at these locations but also revealed some finer scale features not shown on the map. At station V9, ripple marks in the sand are evidence of higher current speeds in this area (Figure 11). At station V10, *Crepidula* (*Crepidula fornicata*) and blue mussel (*Mytilus edulis*) shell cover a large percentage of the bottom and there is less evidence of ripple marks (Figure 11).

Temporal and Spatial Distribution of Winter Flounder Eggs and Larvae

Cancellation of sampling trips due to mechanical problems and bad weather reduced our sampling effort from the 220 planned to 178 samples actually collected. A list of samples taken and stations visited is provided in Table 3. A total of 164 eggs and 122 larvae were collected in both harbors. While a few eggs were collected in mid-February and early March, the majority of the eggs were collected at the end of March when water temperatures were between 4 and 6° C (Figure 12). The first larvae also were collected at this time. The bulk of the eggs and larvae were collected at the end of March. Collections in April consisted almost entirely of larvae.

Thirty-three eggs were collected in the outer harbor in Milford and only three in the inner harbor (Figure 7). All of the eggs were collected on sediments classified as some type of silt or silty sand. Larvae, 31 in all, were collected over sand as well some

type of silt. New Haven Harbor yielded 128 eggs and 91 larvae (Figure 9). The majority of the eggs (95) and larvae (66) were collected in the outer portion of New Haven Harbor. The largest single collection of eggs in the outer harbor (34) was collected at station 36 (Table 4, Figure 9). The remainder of the eggs and larvae from the outer harbor were collected in the sandy substrate to the west of station 36 (Table 4, Figure 9). The inner harbor yielded 33 eggs the majority of which (24) were caught at station 1 located in the Long Wharf area (Figure 9).

Winter flounder eggs (Figure 13) can be identified by their size (0.75-0.85 mm), lack of an oil globule, and the egg's textured surface (Perry 1984) and were classified by developmental stage (Table 4). Dead winter flounder eggs typically could still be identified by their size and textured surface but the chorion was usually more opaque and the contents usually appeared as an amorphous mass. Dead eggs were not staged except in the rare instance (N=3) where the egg appeared to be dead but the contents were clearly visible and identifiable. Hatched eggs were also of appropriate size and showed the textured surface, but were empty and had a small round hole where the larva had emerged.

Staged eggs were divided into two groups. Group 1 contained eggs at the morula, blastula, or gastrula stage, while Group 2 was composed of eggs at the early embryo, tail bud, tail free, late embryo or hatched stage. Principal components analysis identified these two groupings as significant sources of variation. Plots of stations from Milford (Figure 14) and New Haven (Figure 15) are similar; each is dominated by a central cluster of stations with a few stations at the periphery. This indicates that only the

peripheral stations differ markedly in their complement of Group 1 and Group 2 embryos; the stations in the central cluster are similar.

Sediment and Depth Preferences

The distribution of eggs among stations did not simply reflect the distribution of collection sites over the various substrates and was different in the two harbors (Figure 16). The small sample size ($n=36$) and the lack of eggs on the sand substrate at Milford did not allow us to determine significant differences among the three sediment types using the Kolmogorov-Smirnov goodness-of-fit test. The test revealed no significant difference in New Haven where sample size was larger and there were no zero catches on any substrate ($n=128$, $p=0.95$). If the two softer sediments (silt and silty sand) are combined as “soft” sediments while the sand is called “hard”, a Fisher’s Exact test is possible since only two sediment types (sand and silt) are represented in the New Haven data. The results indicate a preference for soft sediments is indicated in Milford but not in New Haven (Table 5). When the distribution of eggs and larvae among sediment types are analyzed on the basis of the average catch per tow, there were more eggs on softer sediments in Milford but not in New Haven when the soft sediments are combined (Figure 17).

We also examined the distribution stations, eggs and larvae among the various depth strata (Figure 18). There was no significant difference in the number of eggs or larvae among depth strata in either harbor. When the data are examined in terms of eggs or larvae per tow (Figure 19), a Kruskal-Wallis test indicates a significant difference in the distribution of eggs across depth strata in New Haven but not in Milford; however, a

test of mean ranks was unable to detect where the difference lay, again probably due to the small sample size.

The result of superimposing the current vector field from Richards (1988) over our mapped egg distributions in New Haven is shown in Figure 20. Additional current information for the harbor from McCusker et al. (1977) is shown in Figure 21. Although there is some discrepancy between these two sources as to the estimate of current flows in the vicinity of station 36 (Figure 9), both suggest that eggs were most abundant in low current areas. Richards (1988) recorded maximum current speeds in that vicinity of 0.6 knots. In Milford, patterns of erosion and deposition in the outer harbor indicate a generally clockwise circulation pattern (Michael Ludwig, pers. comm.) and this is depicted in Figure 22. The majority of the eggs from Milford Harbor (33 of 36) were collected from the center of this gyre.

Discussion

We have essentially met all three of our major objectives for this project. We have located areas in both harbors that held concentrations of developing embryos (Figures 7, 9). We view our egg distribution patterns as reflecting spawning locations. It seems reasonable to assume that eggs would be dispersed less than the larvae. Eggs can adhere to substrates and vegetation (Crawford and Carey 1985), which retards their dispersal. We found larger numbers of early stage eggs (morula and gastrula) in the low current areas on the western side of New Haven Harbor (Figure 9.). Winter flounder embryos reach the morula stage at 24-48 hours post-fertilization and the gastrula stage at 36 hours (Martin and Drewry 1978). This makes it seem unlikely that these eggs were transported here from another location. No high current area produced any eggs so there is no

evidence that they are the source of the eggs in the low current areas. These data are consistent with those of an earlier study which found eggs on the open coast (Anonymous 1988) where tidal currents were 0.8 knots or less (Signell et al. 2000). Our principal components analysis also argues against movement of developing embryos (Figs.14, 15).

In this study, winter flounder eggs were found on both coarse and fine-grained sediments. Crawford (1990) indicated that soft sediments were detrimental to development of the embryos, but gave no references substantiating this statement. Previous workers have reported collecting winter flounder eggs on a variety of substrates including mud (Scott 1929), sand (Bigelow and Schroeder 1953) and gravel (Crawford and Carey 1985). Some evidence exists in the literature that egg burial can have a detrimental effect (Klein-MacPhee et al. 2004) and perhaps this one source of confusion; sediment type is not important so long as the eggs remain on its surface. Contaminated sediments are also known to affect winter flounder embryos (Taibe et al. 2006) and softer sediments tend to be the more contaminated ones.

The inconsistent statistical associations we noted in our study between egg distributions and the sediment types or depth strata, as well as the contradictory evidence obtained by comparing the two harbors, leads us to conclude that depth and sediment type are not the determining factors for winter flounder egg deposition. It is possible that such large-scale habitat mapping is not useful in this case. Flounder may make use of microhabitats not documented by the side-scan sonar mapping.

The video data, while confirming the accuracy of the USGS sediment map, also revealed microhabitat features that could be important in retaining both the eggs and the larvae in nursery. We observed shell with attached algae in the sandy-bottomed area on

the western side of New Haven Harbor, near where the eggs were collected (Figs. 9, 11). This accumulation of shell and vegetation could create a boundary layer of low current speed as well as providing a substrate for the eggs to adhere to. Vegetation has also been observed at winter flounder spawning sites by others (Arnold and Rogers 1972; Crawford and Carey 1985). The presence of vegetation, shell or anything that increases habitat complexity could also help to create microhabitats with lower current speeds that could help to retain winter flounder eggs either by providing a firm substrate to adhere to or simply providing a physical refuge from the current. These areas would also provide a refuge for the hatched larvae during ebb tides. Winter flounder larvae are bottom oriented and negatively buoyant when they stop swimming (Pearcy 1962).

While it is possible that the flounder actually somehow seek out these low current areas, it is also possible that eggs deposited in high current areas are simply swept away leaving the pattern we observed. Although the eggs are sticky and demersal, they have been known to be up in the water column given enough current (Pereira et al. 1999). In their study, Crawford and Carey (1985) rightly pointed out that any project such as the construction of a pier, breakwater, or dredging of the main channel, could have an effect on existing spawning areas if it changed the hydrodynamics of the system. Current speed would seem to be the critical factor. The evidence presented in this study, along with others (Anonymous 1988; Signell et al. 2000), would suggest that current speeds of one knot or greater are required to get winter flounder eggs off the bottom and into the water column.

Winter flounder spawning habitat needs to be addressed at a fine spatial scale. The methods we have developed here, used with updated software, show great promise in

achieving those goals. In addition to mapping substrates, and fine-scale attributes of seafloor habitats, we should also be mapping the current regime on both outgoing and incoming tides. A more closely spaced sampling of water temperature and salinity at different tidal stages would also help to differentiate between water masses that overlay eggs and those that do not. Differences in water density can also form “fronts” along which larvae accumulate. Those considering construction or dredging projects near potential spawning areas must not be allowed to substantially alter the hydrodynamics of the system.

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Table 1. Milford Station Locations. Negative numbers indicate west longitude.

Station			Longitude			Latitude		
No.	Area	Strata	Degrees	Minutes	Seconds	Degrees	Minutes	Seconds
1	INNER	4-6	-73	3	16.48	41	13	5.03
2	INNER	4-6	-73	3	13	41	12	51.25
3	INNER	1-2	-73	2	29.31	41	12	57.9
4	INNER	1-2	-73	2	43.37	41	12	43.94
5	OUTER	1-2	-73	3	37.63	41	12	0.02
6	OUTER	1-2	-73	3	22.16	41	12	12.34
7	OUTER	1-2	-73	3	7.21	41	12	21.82
8	OUTER	1-2	-73	2	51.61	41	12	28.36
9	OUTER	2-4	-73	3	33.63	41	11	56.04
10	OUTER	2-4	-73	3	23.25	41	12	5.33
11	OUTER	2-4	-73	3	9.72	41	12	12.42
12	OUTER	2-4	-73	2	49.25	41	12	18.69
13	OUTER	2-4	-73	3	28.55	41	11	52.9
14	OUTER	2-4	-73	3	17.74	41	12	1.01
15	OUTER	2-4	-73	3	5.57	41	12	7.06
16	OUTER	2-4	-73	2	49.25	41	12	15.23
17	OUTER	2-4	-73	3	26.68	41	11	43.6
18	OUTER	2-4	-73	3	22.53	41	11	50.03
19	OUTER	2-4	-73	3	11.15	41	11	56.63
20	OUTER	2-4	-73	2	48.75	41	12	10.2
21	OUTER	4-6	-73	3	13.16	41	11	45.22
22	OUTER	4-6	-73	2	59.13	41	11	59.82
23	OUTER	4-6	-73	2	36.58	41	12	3.55
24	OUTER	4-6	-73	3	5.35	41	11	42.03
25	OUTER	4-6	-73	2	53.18	41	11	54.68
26	OUTER	4-6	-73	2	31.35	41	11	57.44
27	OUTER	4-6	-73	2	57.62	41	11	38.79
28	OUTER	4-6	-73	2	46.81	41	11	47.01
29	OUTER	4-6	-73	2	25.77	41	11	48.95

Table 2. New Haven inner harbor stations. Negative numbers indicate west longitude.

Station No.	Area	Strata	Longitude			Latitude		
			Degrees	Minutes	Seconds	Degrees	Minutes	Seconds
1 INNER	1-2		-72	54	55.19	41	17	28.11
2 INNER	2-4		-72	54	51.44	41	17	26.65
3 INNER	4-6		-72	54	48.3	41	17	25.2
4 INNER	1-2		-72	55	0.54	41	17	15.18
5 INNER	2-4		-72	54	56.51	41	17	12.71
6 INNER	4-6		-72	54	52.03	41	17	12.7
7 INNER	1-2		-72	55	5.77	41	17	1.89
8 INNER	2-4		-72	54	59.36	41	17	0.14
9 INNER	4-6		-72	54	53.52	41	17	58.56
10 INNER	4-6		-72	54	47.97	41	16	57.83
11 INNER	1-2		-72	55	0.47	41	16	44.2
12 INNER	2-4		-72	54	55.33	41	16	43.78
13 INNER	4-6		-72	54	50.33	41	16	41.9
14 INNER	2-4		-72	54	38.1	41	16	40.64
15 INNER	1-2		-72	55	3.94	41	16	23.34
16 INNER	2-4		-72	54	54.08	41	16	23.24
17 INNER	4-6		-72	54	48.66	41	16	22.82
18 INNER	2-4		-72	54	34.49	41	16	17.68
19 INNER	2-4		-72	54	54.36	41	16	1.75
20 INNER	2-4		-72	54	19.9	41	15	58.39
21 INNER	2-4		-72	55	0.05	41	15	37.95
22 INNER	2-4		-72	54	35.6	41	15	42.56
23 INNER	2-4		-72	54	9.61	41	15	42.56
24 INNER	2-4		-72	54	30.87	41	15	27.67
25 INNER	2-4		-72	54	6.84	41	15	26.1
26 INNER	1-2		-72	54	5.31	41	16	1.22
27 INNER	1-2		-72	53	52.52	41	15	46.75
28 INNER	1-2		-72	53	53.08	41	15	23.79
29 INNER	4-6		-72	54	38.52	41	15	24.63

Table 2 (Cont.) New Haven outer harbor stations. Negative numbers indicate west longitude

Station			Longitude			Latitude		
No.	Area	Strata	Degrees	Minutes	Seconds	Degrees	Minutes	Seconds
30 OUTER	1-2		-72	55	32.99	41	15	20.12
31 OUTER	2-4		-72	55	18.95	41	15	14.14
32 OUTER	4-6		-72	55	2.83	41	15	5.76
33 OUTER	4-6		-72	54	44.49	41	15	1.35
34 OUTER	1-2		-72	55	53.83	41	15	15.3
35 OUTER	2-4		-72	55	36.74	41	14	57.47
36 OUTER	4-6		-72	55	12.42	41	14	45.31
37 OUTER	4-6		-72	54	22.95	41	14	35.24
38 OUTER	1-2		-72	53	55.86	41	14	32.41
39 OUTER	1-2		-72	56	35.65	41	15	7.96
40 OUTER	2-4		-72	56	18.01	41	14	50.65
41 OUTER	2-4		-72	55	54.38	41	14	39.12
42 OUTER	4-6		-72	55	34.38	41	14	18.98
43 OUTER	4-6		-72	55	19.92	41	14	3.04
44 OUTER	1-2		-72	57	16.23	41	14	57.37
45 OUTER	2-4		-72	56	52.61	41	14	52.44
46 OUTER	2-4		-72	56	31.35	41	14	40.9
47 OUTER	2-4		-72	56	9.81	41	14	30.82
48 OUTER	4-6		-72	55	52.3	41	14	10.69
49 OUTER	4-6		-72	55	38.27	41	13	52.86
50 OUTER	1-2		-72	57	47.91	41	14	41.32
51 OUTER	2-4		-72	57	6.78	41	14	44.05
52 OUTER	2-4		-72	56	45.24	41	14	28.53
53 OUTER	4-6		-72	56	23.15	41	14	13.53
54 OUTER	4-6		-72	55	58.83	41	13	52.86
55 OUTER	4-6		-72	67	51.66	41	14	21.81
56 OUTER	>6		-72	57	25.82	41	14	5.03
57 OUTER	>6		-72	56	54.13	41	13	49.51
58 OUTER	>6		-72	56	26.2	41	13	35.55
59 OUTER	>6		-72	58	6.25	41	13	58.95
60 OUTER	>6		-72	57	29.43	41	13	46.15
61 OUTER	>6		-72	56	51.91	41	13	32.83
62 OUTER	>6		-72	54	17.95	41	13	56.74
63 OUTER	>6		-72	55	40.76	41	13	28.22

Table 3. Benthic sled samples taken during the current study. Samples 7 and 26 were lost. Samples 94-97 were taken in another location as part of another study. Also included are temperatures and salinities taken at the time of the sampling.

Sample	Location	Station	Strata	Date	Replicate	Temp. (C)	Salinity (ppt)
1	Milford	1	4-6	2/19/04	A	0.3	15.8
2	Milford	1	4-6	2/19/04	B	0.3	15.8
3	Milford	2	4-6	2/19/04	A	0.3	16.8
4	Milford	2	4-6	2/19/04	B	0.3	16.8
5	Milford	6	0-2	3/3/04	A	2.0	19.3
6	Milford	6	0-2	3/3/04	B	2.0	19.3
8	Milford	5	0-2	3/3/04	A	2.1	24.5
9	Milford	5	0-2	3/3/04	B	2.1	24.5
10	Milford	9	2-4	3/3/04	A	2.2	25.2
11	Milford	9	2-4	3/3/04	B	2.2	25.2
12	Milford	11	2-4	3/3/04	A	2.1	25.2
13	Milford	11	2-4	3/3/04	B	2.1	25.2
14	Milford	26	4-6	3/3/04	A	2.8	24.8
15	Milford	26	4-6	3/3/04	B	2.8	24.8
16	Milford	28	4-6	3/3/04	A	2.6	25.0
17	Milford	28	4-6	3/3/04	B	2.6	25.0
18	Milford	1	4-6	3/3/04	A	2.8	24.8
19	Milford	1	4-6	3/3/04	B	2.8	24.8
20	Milford	2	4-6	3/3/04	A	3.2	24.3
21	Milford	2	4-6	3/3/04	B	3.2	24.3
22	New Haven	39	0-2	3/4/04	A	1.9	26.1
23	New Haven	39	0-2	3/4/04	B	1.9	26.1
24	New Haven	30	0-2	3/4/04	A	2.8	25.7
25	New Haven	30	0-2	3/4/04	B	2.8	25.7
27	New Haven	1	0-2	3/4/04	A	3.4	23.9
28	New Haven	1	0-2	3/4/04	B	3.4	23.9
29	New Haven	4	0-2	3/4/04	A	2.4	25.5
30	New Haven	22	2-4	3/4/04	A	2.4	25.5
31	New Haven	22	2-4	3/4/04	B	2.4	25.5
32	New Haven	29	4-6	3/4/04	A	2.4	26.1
33	New Haven	29	4-6	3/4/04	B	2.4	26.1
34	New Haven	62	>6	3/4/04	A	2.2	26.1
35	New Haven	62	>6	3/4/04	B	2.2	26.1
36	New Haven	63	>6	3/4/04	A	2.7	25.6
37	New Haven	63	>6	3/4/04	B	2.7	25.6
38	New Haven	51	2-4	3/5/04	A	3	25.6
39	New Haven	51	2-4	3/5/04	B	3	25.6
40	New Haven	46	2-4	3/5/04	A	2	26.3

Table 3. (cont.)

Sample	Location	Station	Strata	Date	Replicate	Temp. (C)	Salinity (ppt)
41	New Haven	46	2-4	3/5/04	B	2	26.3
42	New Haven	49	4-6	3/5/04	A	2	26.3
43	New Haven	49	4-6	3/5/04	B	2	26.3
44	New Haven	19	2-4	3/5/04	A	3.6	24.1
45	New Haven	19	2-4	3/5/04	B	3.6	24.1
46	New Haven	32	4-6	3/5/04	A	2.1	26.2
47	New Haven	32	4-6	3/5/04	B	2.1	26.2
48	New Haven	6	4-6	3/5/04	A	3.7	22.8
49	New Haven	6	4-6	3/5/04	B	3.7	22.8
50	Milford	26	2-4	3/26/04	A	4.1	24.6
51	Milford	26	2-4	3/26/04	B	4.1	24.6
52	Milford	22	2-4	3/26/04	A	3.5	26.1
53	Milford	22	2-4	3/26/04	B	3.5	26.1
54	Milford	14	2-4	3/29/04	A	4.8	25.8
55	Milford	14	2-4	3/29/04	B	4.8	25.8
56	Milford	20	2-4	3/29/04	A	4.6	25.6
57	Milford	20	2-4	3/29/04	B	4.6	25.6
58	New Haven	63	>6	3/29/04	A	4.2	26.3
59	New Haven	63	>6	3/29/04	B	4.2	26.3
60	New Haven	36	4-6	3/29/04	A	4.4	26.2
61	New Haven	36	4-6	3/29/04	B	4.4	26.2
62	New Haven	41	2-4	3/29/04	A	4.6	25.7
63	New Haven	41	2-4	3/29/04	B	4.6	25.7
64	New Haven	56	>6	3/29/04	A	4.7	25.9
65	New Haven	56	>6	3/29/04	B	4.7	25.9
66	New Haven	45	2-4	3/29/04	A	4.8	25.7
67	New Haven	45	2-4	3/29/04	B	4.8	25.7
68	New Haven	37	4-6	3/29/04	A	4.3	26.7
69	New Haven	37	4-6	3/29/04	B	4.3	26.7
70	New Haven	19	2-4	3/29/04	A	5.1	25.3
71	New Haven	19	2-4	3/29/04	B	5.1	25.3
72	New Haven	17	4-6	3/29/04	A	5.1	25.6
73	New Haven	17	4-6	3/29/04	B	5.1	25.6
74	New Haven	13	4-6	3/29/04	A	5.3	24.5
75	New Haven	13	4-6	3/29/04	B	5.3	24.5
76	New Haven	12	2-4	3/29/04	A	5	25.8
77	New Haven	12	2-4	3/29/04	B	5	25.8
78	New Haven	1	0-2	3/30/04	A	5.09	25.5
79	New Haven	1	0-2	3/30/04	B	5.09	25.5
80	New Haven	26	0-2	3/29/04	A	4.9	26.4

Table 3. (cont)

Sample	Location	Station	Strata	Date	Replicate	Temp. (C)	Salinity (ppt)
81	New Haven	26	0-2	3/29/04	B	4.9	26.4
82	New Haven	34	0-2	3/29/04	A	4.9	26.2
83	New Haven	34	0-2	3/29/04	B	4.9	26.2
84	New Haven	44	0-2	3/29/04	A	5.1	25.8
85	New Haven	44	0-2	3/29/04	B	5.1	25.8
86	Milford	1	4-6	4/6/04	A	5.3	25.2
87	Milford	1	4-6	4/6/04	B	5.3	25.2
88	Milford	2	4-6	4/6/04	A	4.6	25.9
89	Milford	2	4-6	4/6/04	B	4.6	25.9
90	Milford	6	0-2	4/6/04	A	4.3	26.2
91	Milford	6	0-2	4/6/04	B	4.3	26.2
92	Milford	7	0-2	4/6/04	A	4.5	26.2
93	Milford	7	0-2	4/6/04	B	4.5	26.2
98	New Haven	34	0-2	4/15/04	A	6.5	21.1
99	New Haven	34	0-2	4/15/04	B	6.5	21.1
100	New Haven	30	0-2	4/15/04	A	6.4	19.4
101	New Haven	30	0-2	4/15/04	B	6.4	19.4
102	New Haven	26	0-2	4/15/04	A	6.2	22.2
103	New Haven	26	0-2	4/15/04	B	M	M
104	New Haven	27	0-2	4/15/04	A	M	M
105	New Haven	27	0-2	4/15/04	B	M	M
106	New Haven	5	2-4	4/15/04	A	M	M
107	New Haven	5	2-4	4/15/04	B	M	M
108	New Haven	13	4-6	4/15/04	A	M	M
109	New Haven	13	4-6	4/15/04	B	M	M
110	New Haven	25	2-4	4/15/04	A	M	M
111	New Haven	25	2-4	4/15/04	B	M	M
112	New Haven	29	4-6	4/15/04	A	M	M
113	New Haven	29	4-6	4/15/04	B	M	M
114	Milford	7	0-2	4/16/04	A	6.2	24.4
115	Milford	7	0-2	4/16/04	B	6.2	24.4
116	Milford	5	0-2	4/16/04	A	6	25.6
117	Milford	5	0-2	4/16/04	B	6	25.6
118	New Haven	63	>6	4/16/04	A	5.6	26.2
119	New Haven	63	>6	4/16/04	B	5.6	26.2
120	New Haven	58	>6	4/16/04	A	6	24.8
121	New Haven	58	>6	4/16/04	B	6	24.8
122	New Haven	48	4-6	4/16/04	A	24.3	6.4
123	New Haven	48	4-6	4/16/04	B	24.3	6.4
124	New Haven	42	4-6	4/16/04	A	6.8	21.7
125	New Haven	42	4-6	4/16/04	B	6.8	21.7

Table 3. (cont.)

Sample	Location	Station	Strata	Date	Replicate	Temp. (C)	Salinity (ppt)
126	New Haven	35	2-4	4/16/04	A	6.5	22
127	New Haven	35	2-4	4/16/04	B	6.5	22
128	New Haven	46	2-4	4/16/04	A	8.3	19.3
129	New Haven	46	2-4	4/16/04	B	8.3	19.3
130	Milford	25	2-4	4/16/04	A	7.3	24.2
131	Milford	25	2-4	4/16/04	B	7.3	24.2
134	Milford	1	4-6	4/19/04	A	9.1	23.8
135	Milford	1	4-6	4/19/05	B	9.1	23.8
136	Milford	2	4-6	4/19/04	A	8.8	23.8
137	Milford	2	4-6	4/19/04	B	8.8	23.8
138	Milford	15	2-4	4/19/04	A	7.9	25.5
139	Milford	15	2-4	4/19/04	B	7.9	25.5
140	Milford	14	2-4	4/19/04	A	7.7	25.5
141	Milford	14	2-4	4/19/04	B	7.7	25.5
142	Milford	8	0-2	4/28/04	A	8.1	26.1
143	Milford	8	0-2	4/28/04	B	8.1	26.1
144	Milford	6	0-2	4/28/04	A	8.3	26.1
145	Milford	6	0-3	4/28/04	B	8.3	26.1
146	New Haven	55	4-6	4/28/04	A	7.8	25.8
147	New Haven	55	4-6	4/28/04	B	7.8	25.8
148	New Haven	46	2-4	4/28/04	A	7.9	25.8
149	New Haven	46	2-4	4/28/04	B	7.9	25.8
150	New Haven	40	2-4	4/28/04	A	8.2	26.2
151	New Haven	40	2-4	4/28/04	B	8.2	26.2
152	New Haven	58	>6	4/28/04	A	7.8	26.8
153	New Haven	58	>6	4/28/04	B	7.8	26.8
154	New Haven	62	>6	4/28/04	A	8.5	25.6
155	New Haven	62	>6	4/28/04	B	8.5	25.6
156	New Haven	33	4-6	4/28/04	A	9.1	24.5
157	New Haven	33	4-6	4/28/04	B	9.1	24.5
158	New Haven	25	2-4	4/28/04	A	10	23.9
159	New Haven	25	2-4	4/28/04	B	10	23.9
160	New Haven	4	0-2	4/28/04	A	9.5	24.4
161	New Haven	4	0-2	4/28/04	B	9.5	24.4
162	New Haven	27	0-2	4/28/04	A	10.9	22.6
163	New Haven	27	0-2	4/28/04	B	10.9	22.6
164	New Haven	30	0-2	4/28/04	A	9.6	25.9
165	New Haven	30	0-2	4/28/04	B	9.6	25.9

Table 3.(cont.)

Sample	Location	Station	Strata	Date	Replicate	Temp. (C)	Salinity (ppt)
166	New Haven	39	0-2	4/28/04	A	8.8	24.6
167	New Haven	39	0-2	4/28/04	B	8.8	24.6
168	New Haven	5	2-4	4/29/04	A	8.8	25.1
169	New Haven	5	2-4	4/29/04	B	8.8	25.1
170	New Haven	9	4-6	4/29/04	A	8.7	25.4
171	New Haven	9	4-6	4/29/04	B	8.7	25.4
172	New Haven	17	4-6	4/29/04	A	9	25.6
173	New Haven	17	4-6	4/29/04	B	9	25.6
174	Milford	12	2-4	4/29/04	A	9	26.1
175	Milford	12	2-4	4/29/04	B	9	26.1
176	Milford	19	2-4	4/29/04	A	8.5	26.2
177	Milford	19	2-4	4/26/04	B	8.5	26.2
178	New Haven	25	4-6	4/29/04	A	8.3	25
179	New Haven	25	4-6	4/29/04	B	8.3	25
180	Milford	21	4-6	4/29/04	A	8.2	26.3
181	Milford	21	4-6	4/29/04	B	8.2	26.3
182	Milford	1	4-6	4/29/04	A	9.6	26.5
183	Milford	1	4-6	4/29/04	B	9.6	26.5
184	Milford	2	4-6	4/29/04	A	9.4	24.4
185	Milford	2	4-6	4/29/04	B	9.4	24.4

Table 4. Winter flounder eggs classified by developmental stage.







Site	Station	Dead	Morula	Blastula	Gastrula	Early Embryo	Tail-bud	Tail-free	Late Embryo	Hatched	Totals
Milford	1	1	0	0	0	0	0	0	0	0	1
Milford	2	0	1	0	1	0	0	0	0	0	2
Milford	14	9	0	1	0	1	0	4	0	0	15
Milford	20	0	4	0	2	0	0	0	0	0	6
Milford	22	3	0	0	1	1	0	0	0	0	5
Milford	26	3	1	0	2	1	0	0	0	0	7
New Haven	1	2	3	0	0	0	11	1	7	0	24
New Haven	26	0	2	0	0	0	0	0	0	0	2
New Haven	27	0	0	0	0	1	0	0	0	0	1
New Haven	29	6	0	0	0	0	0	0	0	0	6
New Haven	30	0	0	0	4	1	0	0	0	0	5
New Haven	34	1	0	1	0	0	2	0	0	1	5
New Haven	36	1	24	0	0	7	1	0	1	0	34
New Haven	37	0	3	0	0	0	0	0	2	1	6
New Haven	39	2	0	0	15	0	0	0	0	0	17
New Haven	41	0	9	0	0	1	0	0	2	0	12
New Haven	44	2	0	0	0	1	0	0	0	1	4
New Haven	45	1	3	0	0	1	0	1	0	4	10
New Haven	55	0	1	0	0	0	0	0	0	0	1
New Haven	63	0	0	0	0	0	1	0	0	0	1
Totals		31	51	2	25	15	15	6	12	7	164

Table 5. Results of Fisher's Exact Test examining distribution of eggs and stations on hard and soft sediments. More stations on soft bottom than would be expected by chance had eggs in Milford but not in New Haven.

	Fisher's Exact			
	Milford		New Haven	
	Hard	Soft	Hard	Soft
No eggs	9	3	8	14
Eggs	0	6	8	6
	p=0.009		p=0.307	

Milford Harbor

Sampling Strata

-  1 meter
-  2 meters
-  4 meters
-  6 meters
-  Sites
-  Video

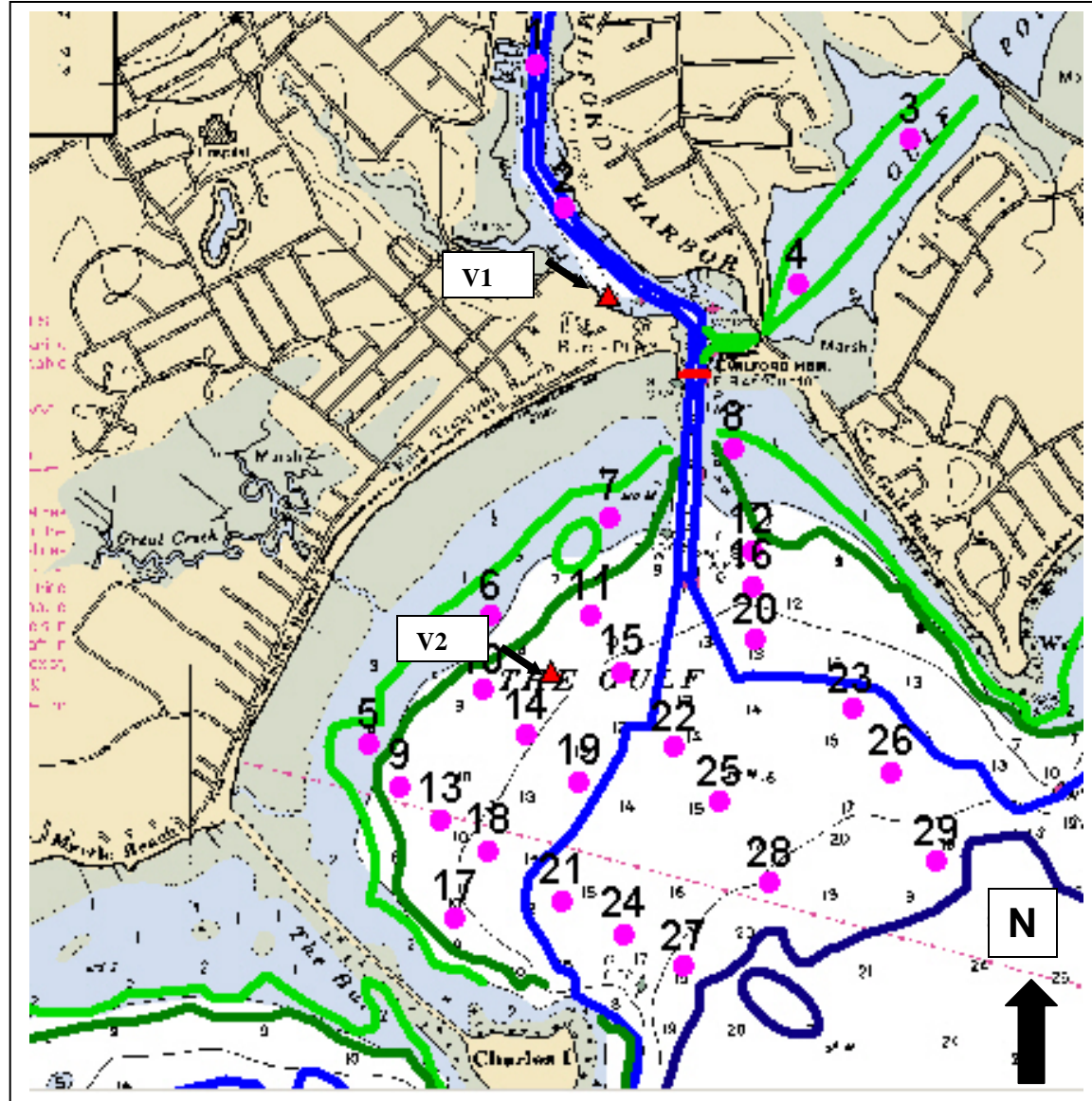








Figure 1. Sampling sites in Milford Harbor. Red triangles indicate where video images of the bottom were collected.

New Haven Harbor Inner Stations

Sampling Strata

-  1 meter
-  2 meters
-  4 meters
-  6 meters
-  Sites
-  Video

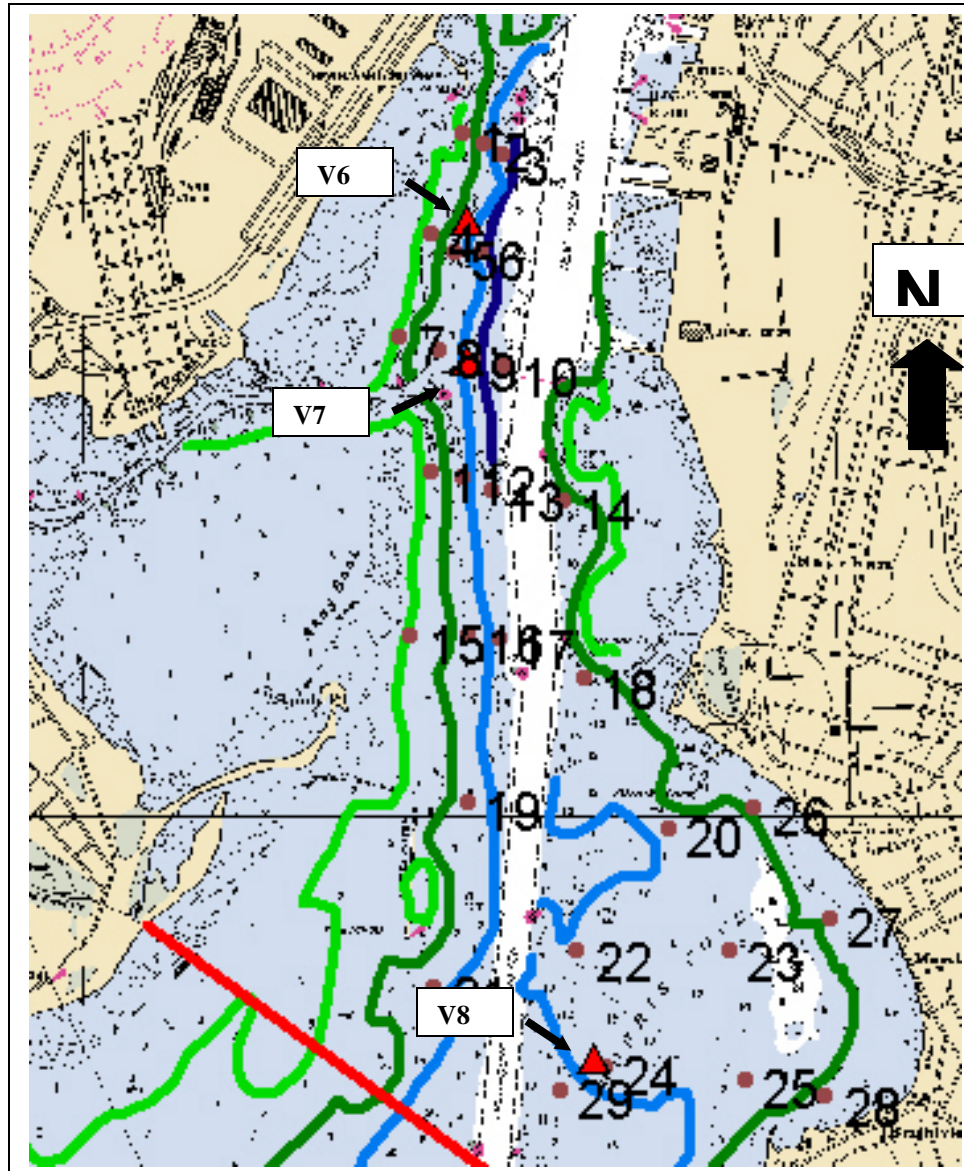


Figure 2. Inner harbor stations in New Haven. Red triangles indicate where video images of the bottom were collected.

New Haven Harbor Outer Stations

Sampling Strata

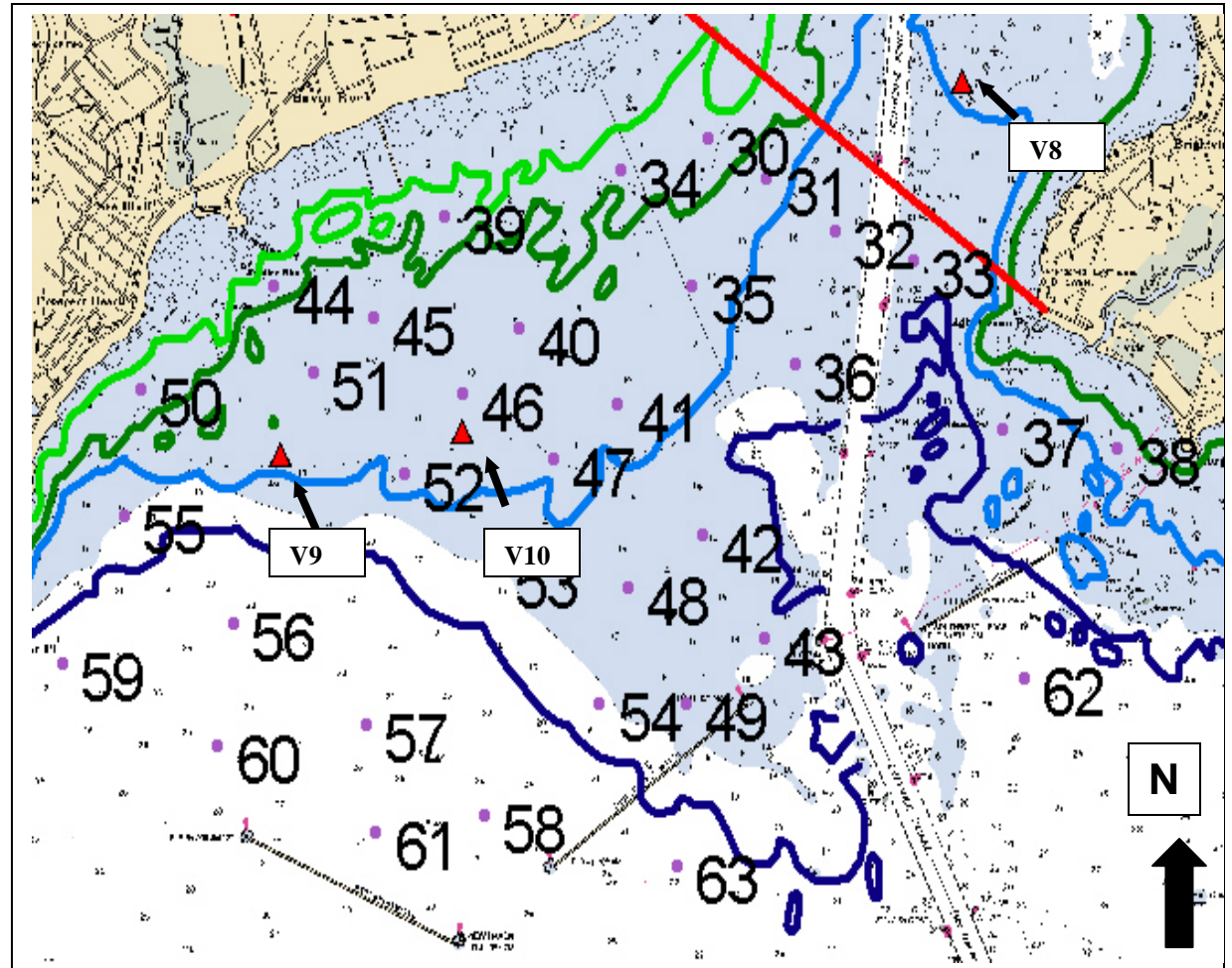
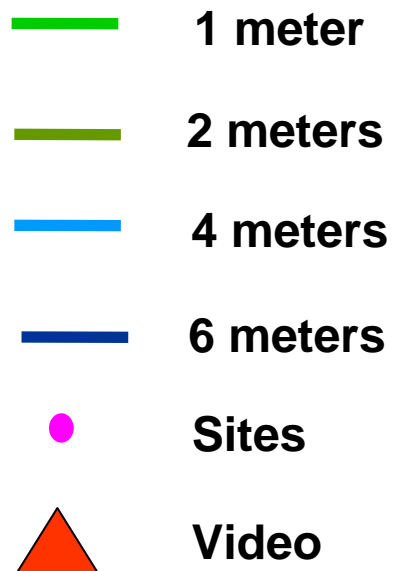


Figure 3. Outer harbor stations in New Haven. Red triangles indicate where video images of the bottom were collected.

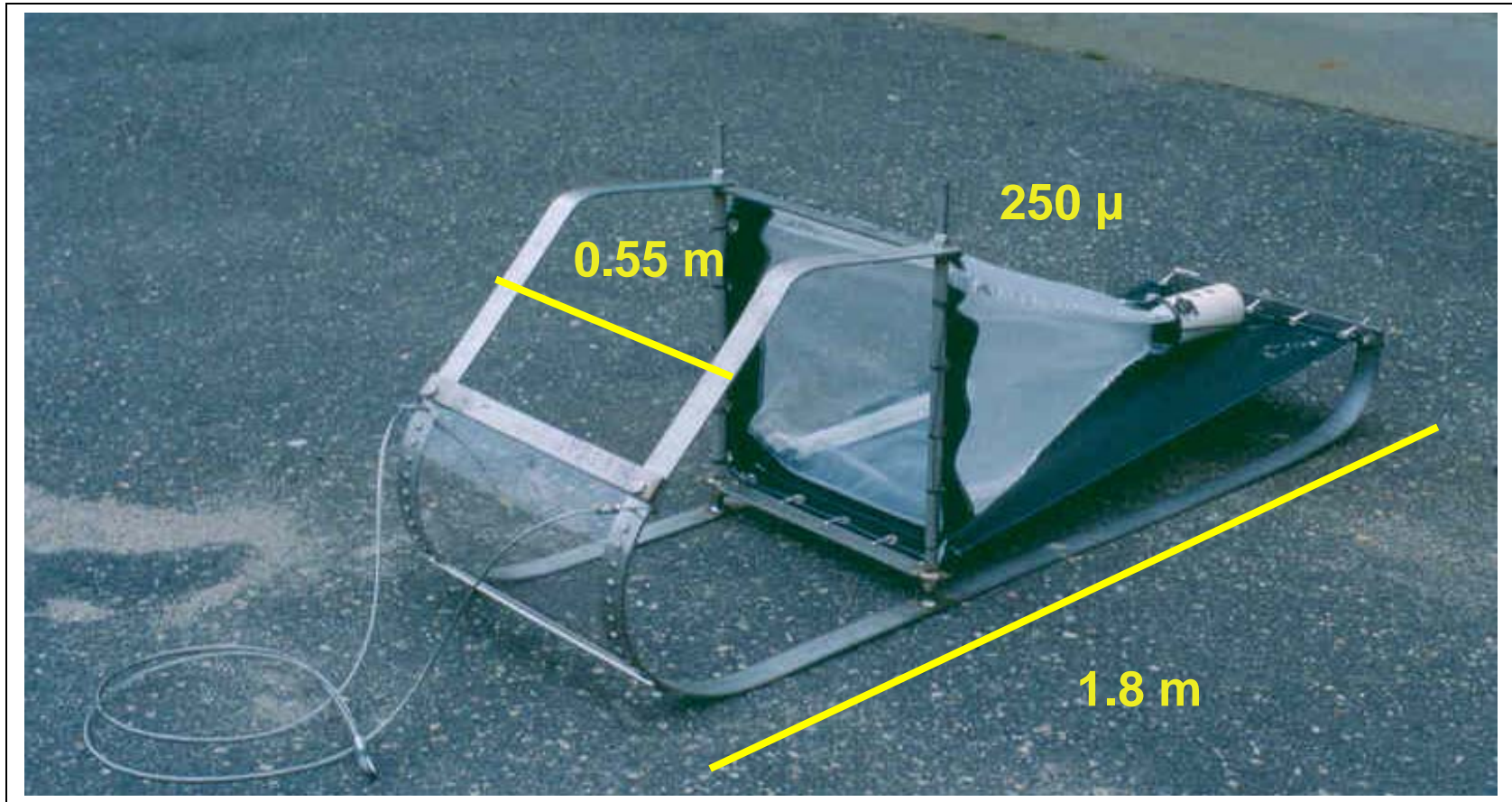


Figure 4. The benthic sled used to collect winter flounder eggs.

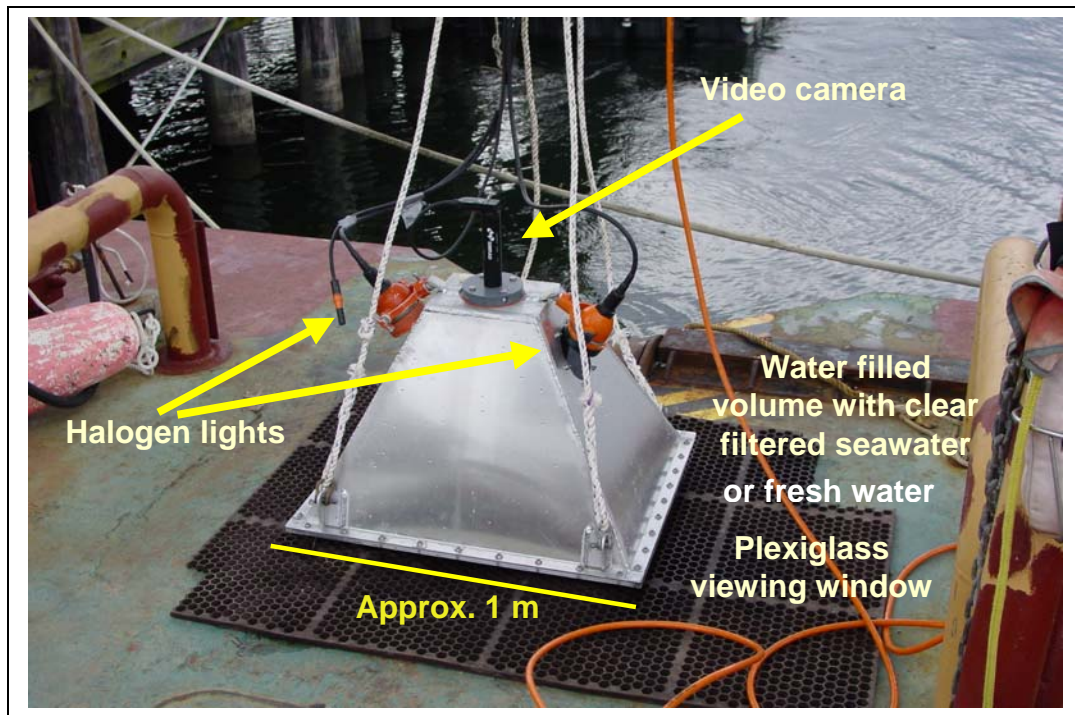


Figure 5. Drop camera

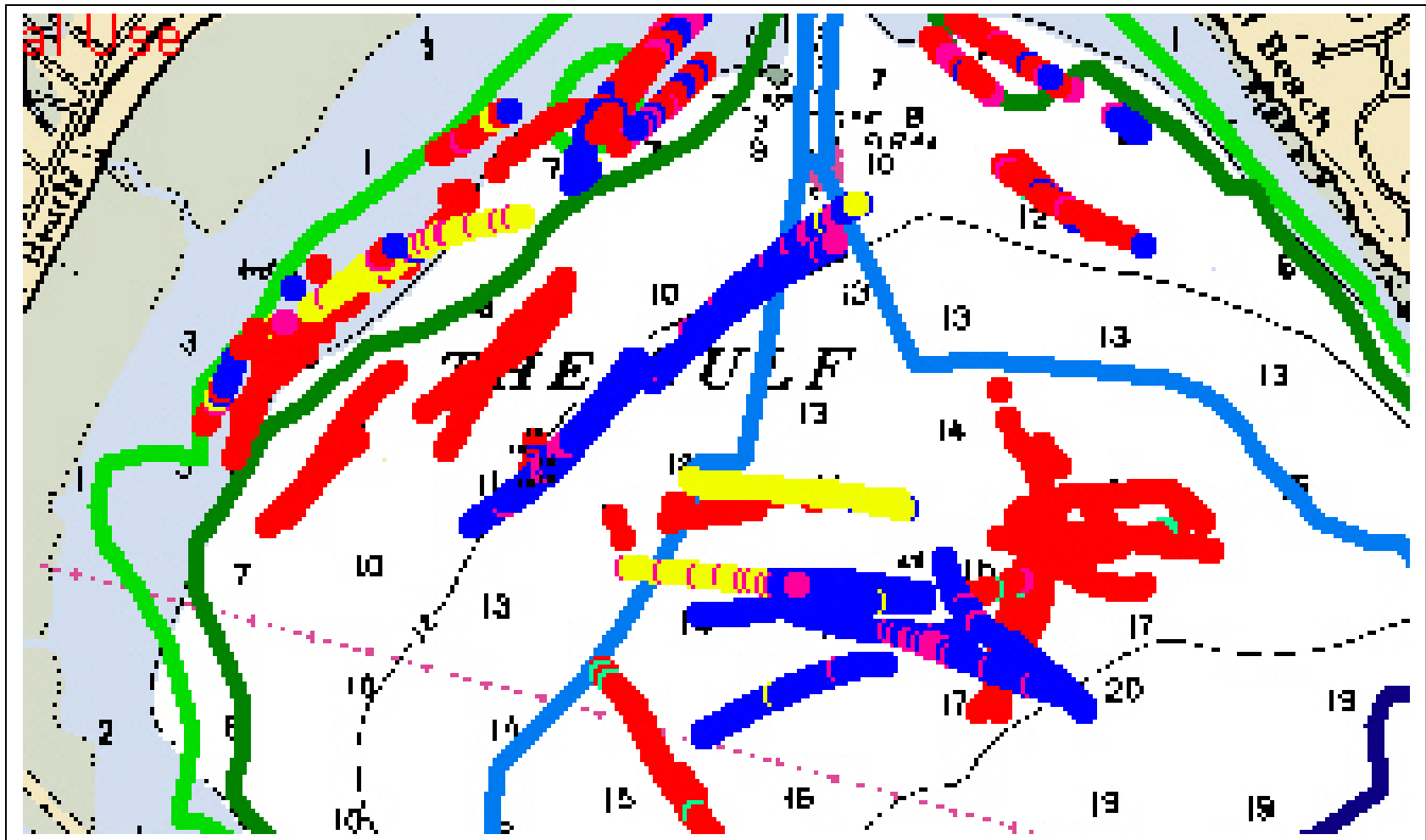


Figure 6. Acoustic classification of benthic sled runs. Different colors represent different acoustic signatures. Tracks that overlap gave different signals on different occasions indicating inconsistency of the results.

Milford Harbor

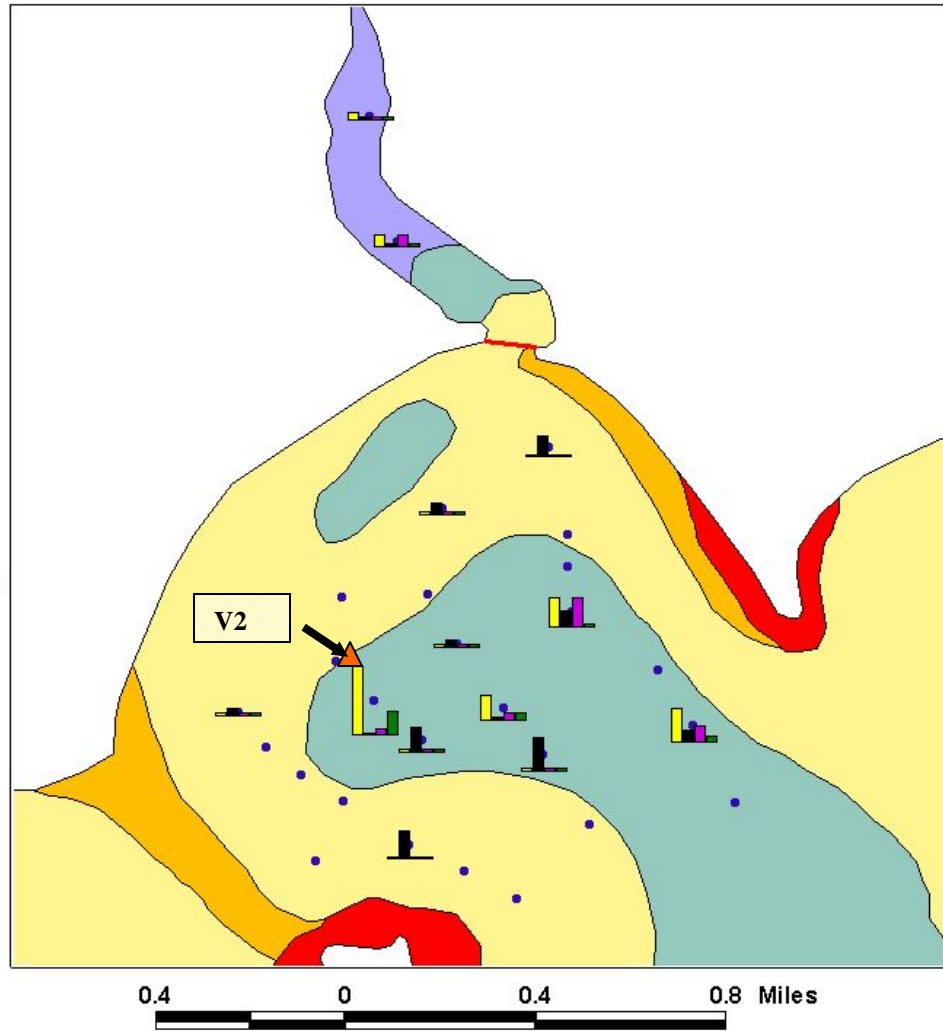


Figure 7. Spatial distribution of eggs and larvae and in Milford Harbor. Eggs are further broken down by stage of development. Group 1 embryos are those up to and including the gastrula stage (morula, blastula or gastrula). Group 2 includes eggs beyond the gastrula stage (early embryo, tail bud, tail free, late embryo or hatched). The red triangle indicates the location of V2, one of the video ground truth stations.



Figure 8. Still frame from the ground truth video showing evidence of soft sediments (mud snails and tracks). Location is shown in Figure 7.

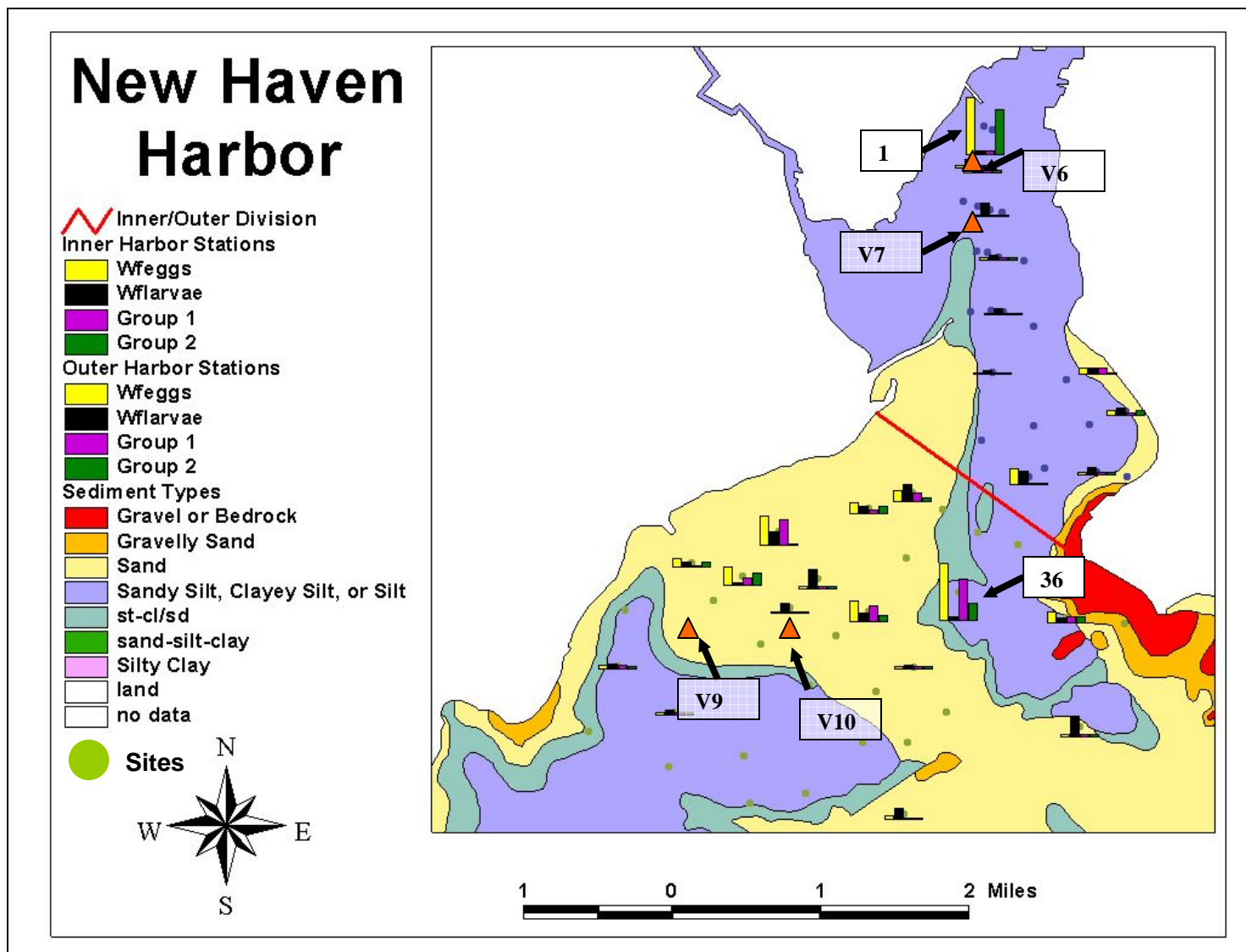


Figure 9. Spatial distribution of eggs and larvae and in New Haven Harbor. Eggs are further broken down by stage of development. Group 1 embryos are those up to and including the gastrula stage (morula, blastula or gastrula). Group 2 includes eggs beyond the gastrula stage (early embryo, tail bud, tail free, late embryo or hatched). Stations 1 and 36 where the largest number of eggs were caught, are indicated by the arrows and the white boxes. Red triangles indicate video ground-truth sites (V6,V7,V9, V10).

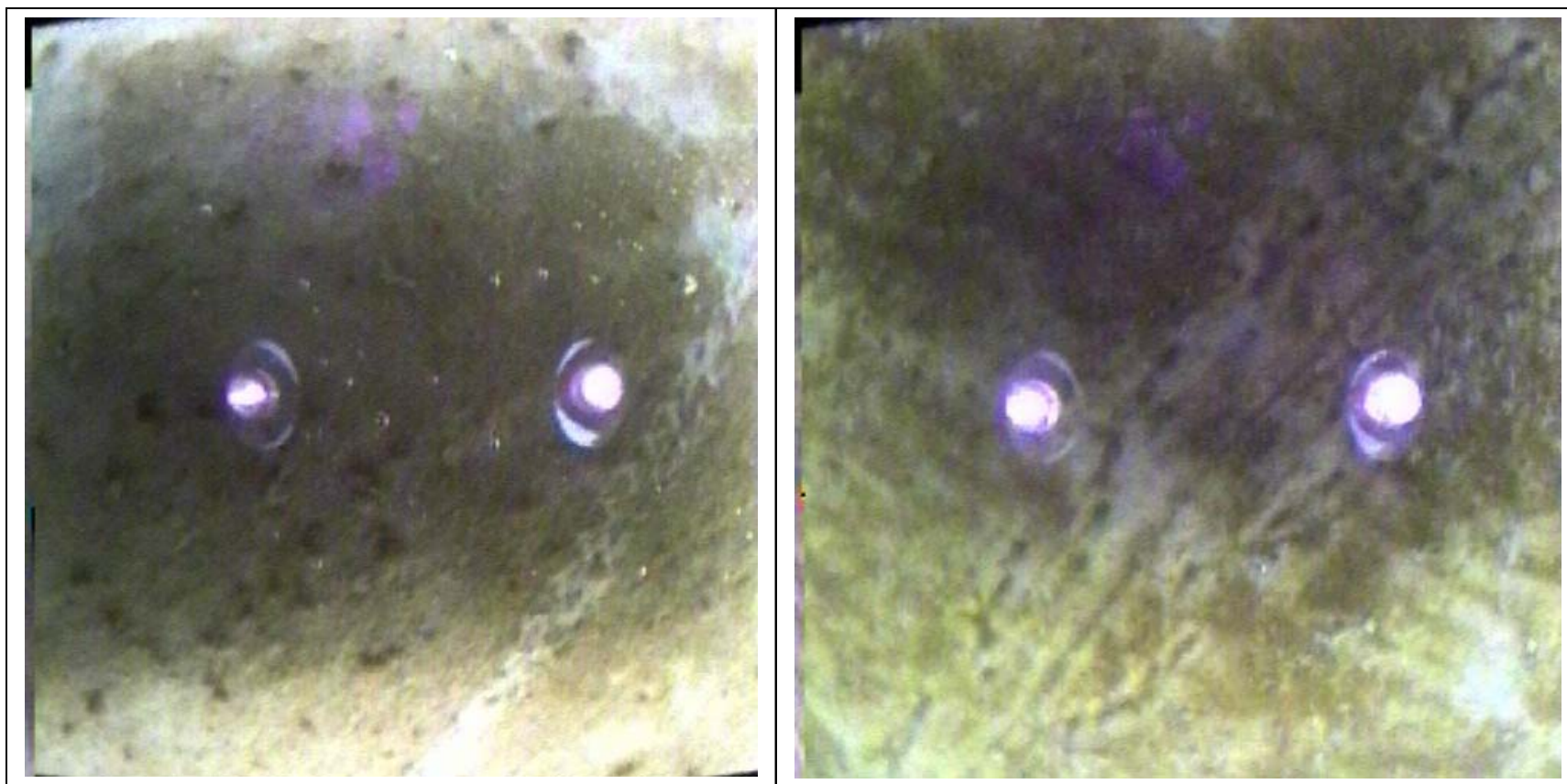


Figure 10. Video station V6 (left) and V7 (right) confirm the presence of soft sediments in the Long Wharf area. See Figure 9. for locations

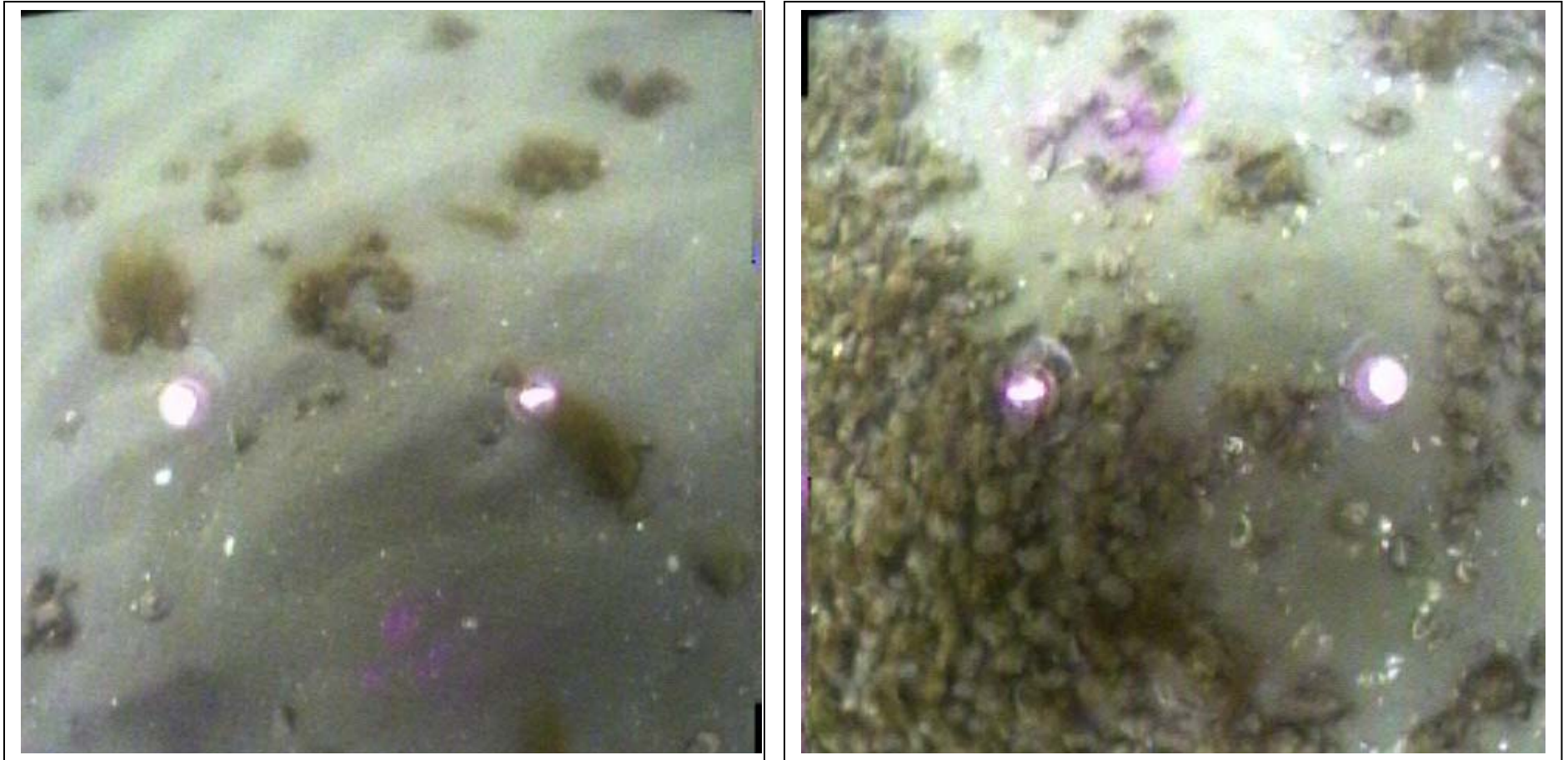


Figure 11. Video stations V9 (left) and V10 (right). Ripple marks at V9 are evidence of higher current velocities. A higher percentage of the bottom at V10 is covered with shell. See Figure 9 for locations.

Temporal Distribution of Egg and Larval Collections

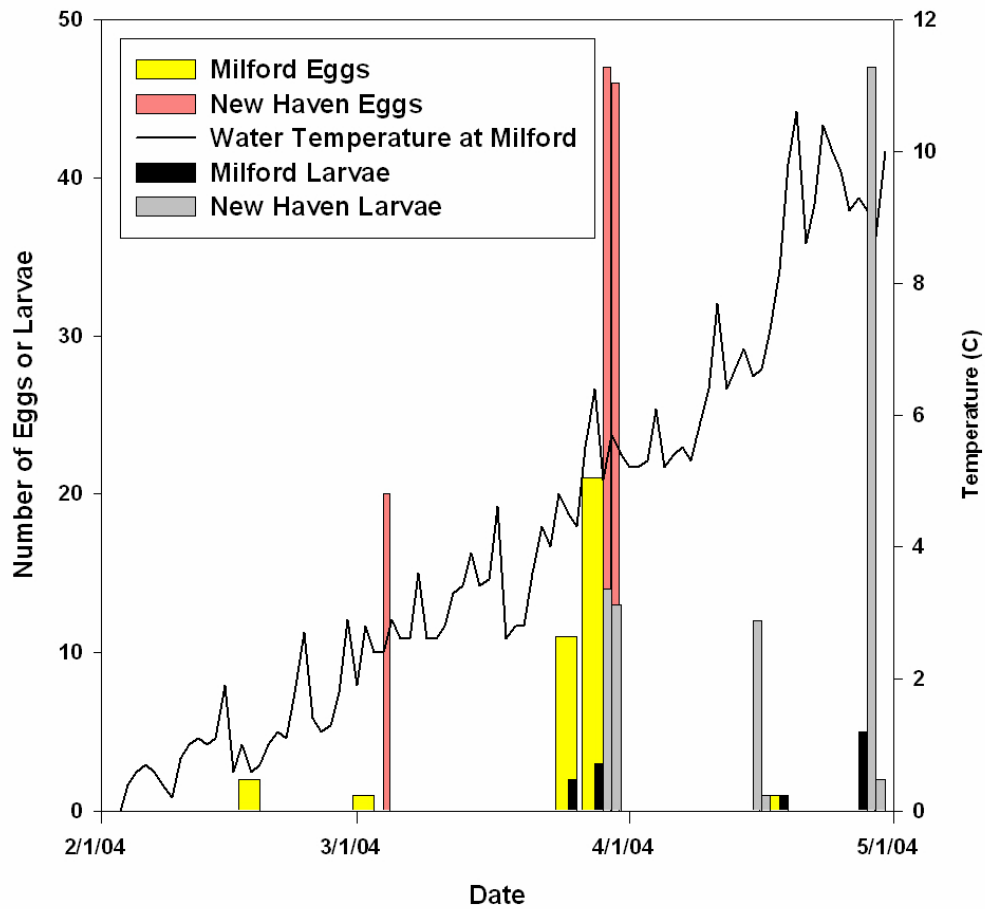


Figure 12. Temporal distribution of winter flounder eggs and larvae in Milford and New Haven Harbors. Black line represents daily water temperatures taken at the Milford Laboratory during this period.

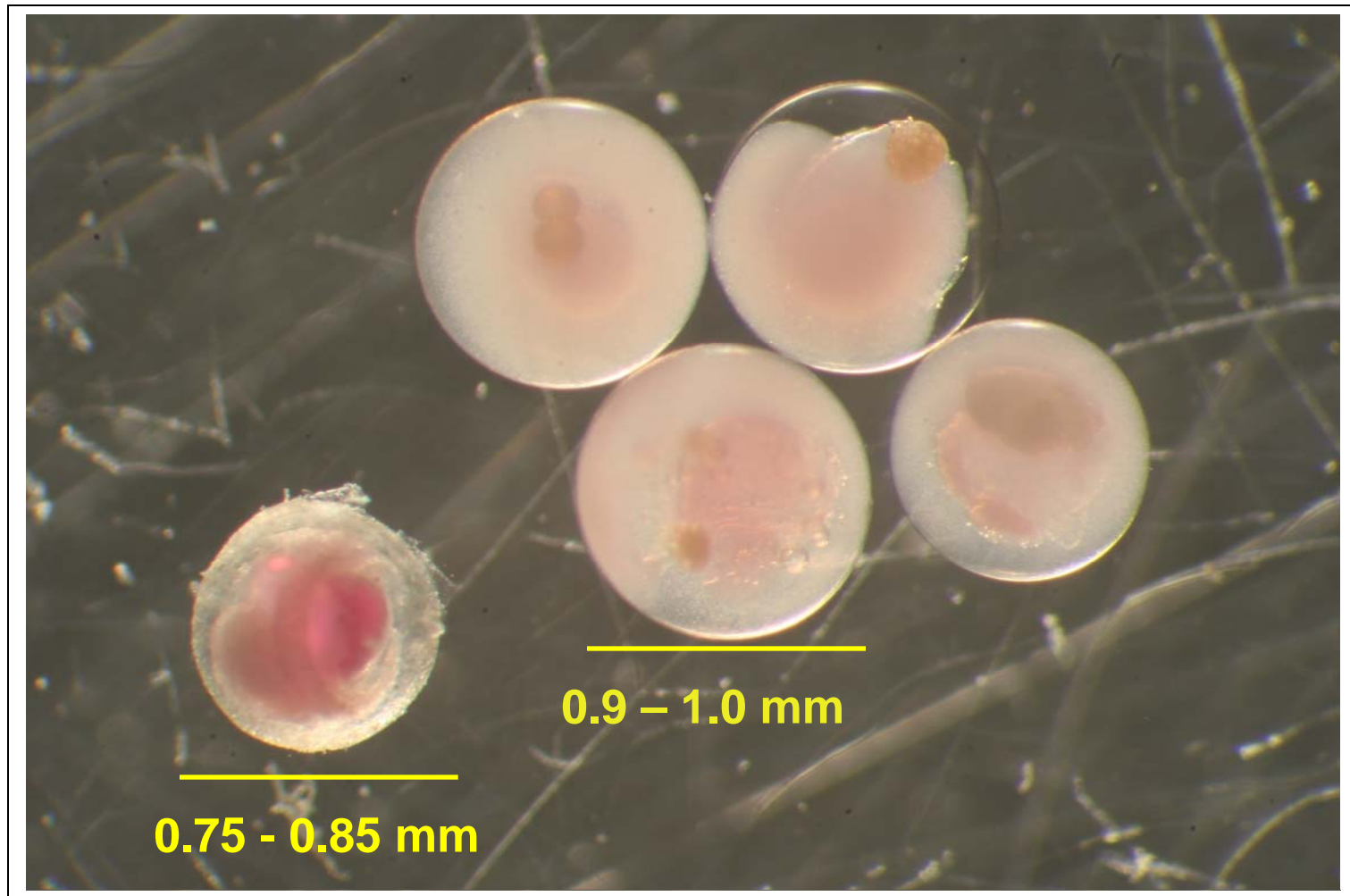


Figure 13. Eggs collected during the present study, stained with rose Bengal. In the lower left is a winter flounder egg (late embryo stage) showing the typical textured surface. The other eggs are four-beard rockling eggs (*Enchelyopus cimbrius*)

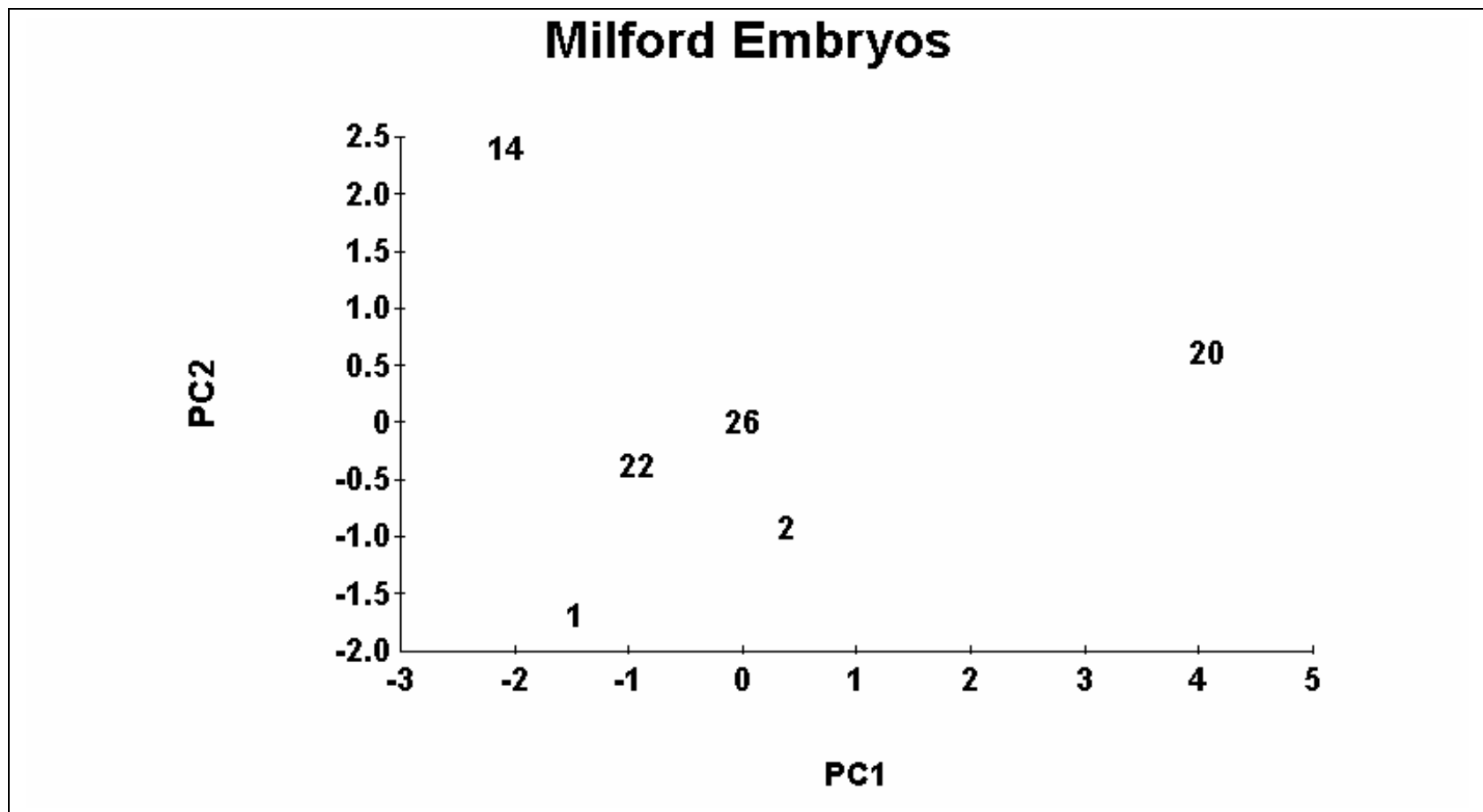


Figure 14. Plot of sampling stations in Milford based on their complement of Group 1 (PC1) vs Group 2 (PC2) embryos

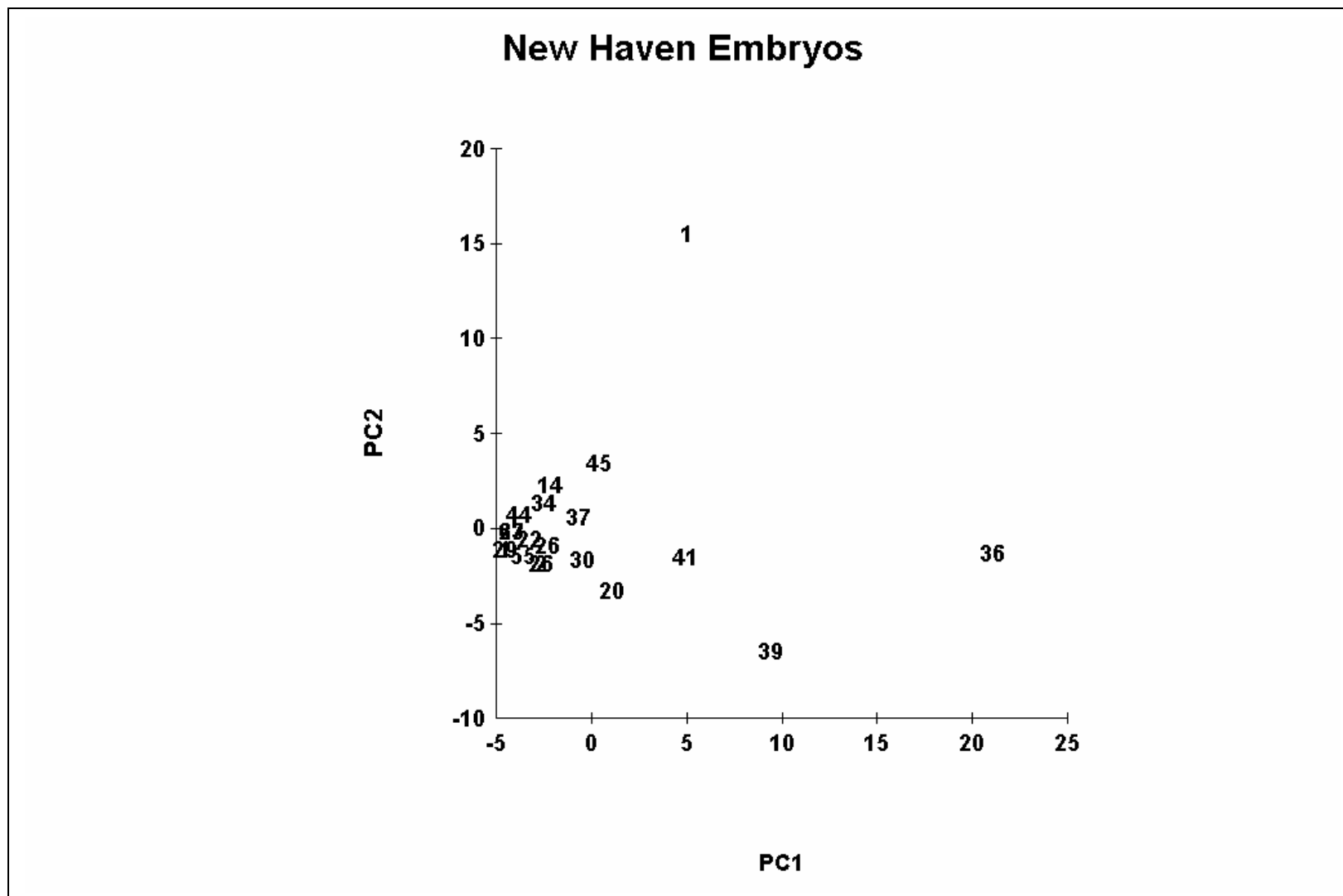


Figure 15. Plot of sampling stations in New Haven based on their complement of Group 1 (PC1) vs. Group 2 (PC2) embryos

Distribution of Stations, Eggs and Larvae Among Sediment Types

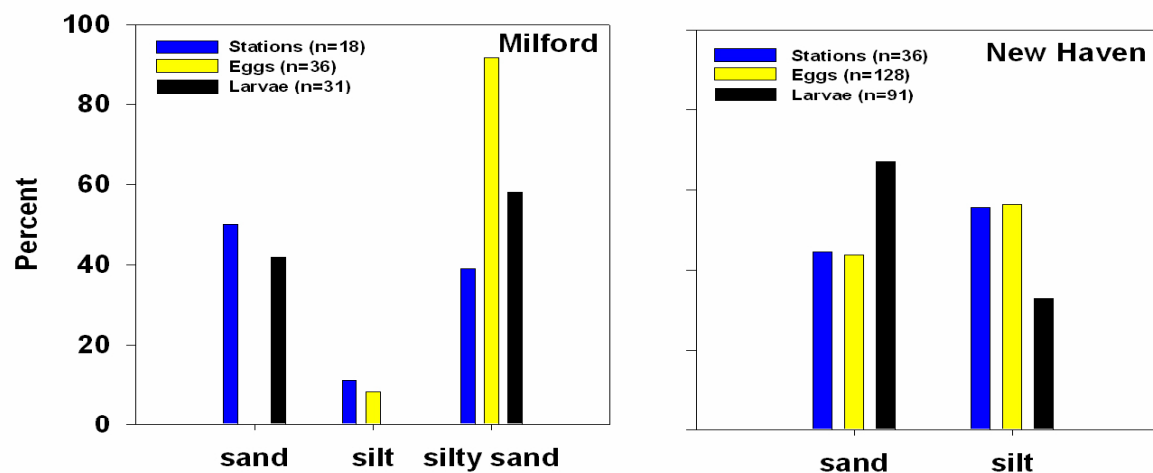
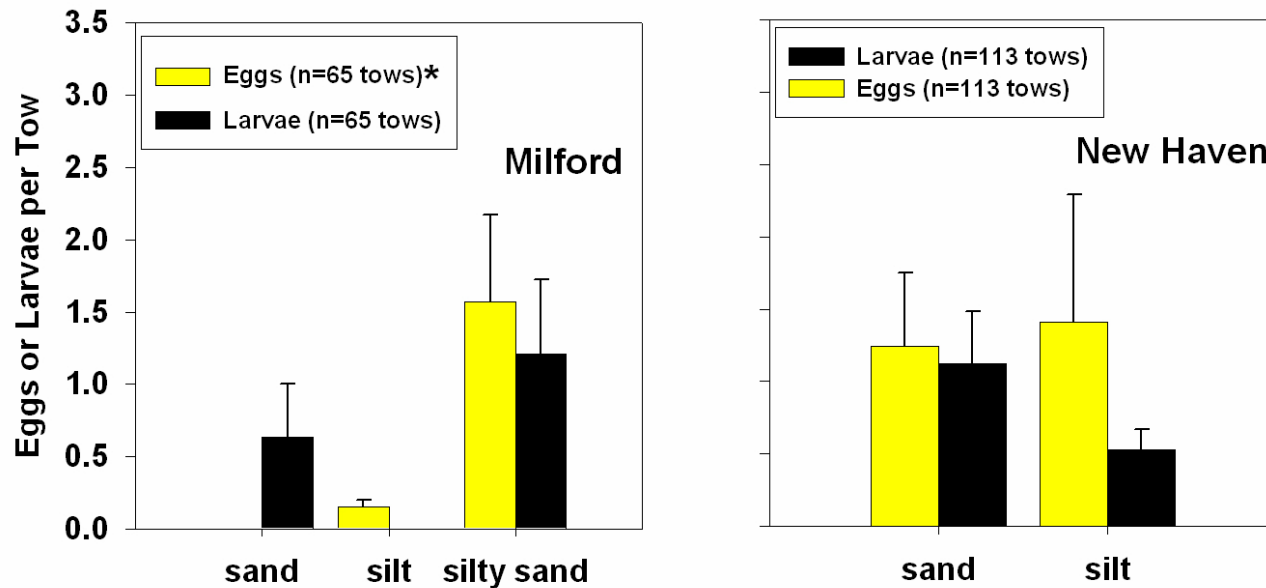


Figure 16. Distribution of stations, eggs, and larvae among sediment types shown as a percentage of the total.

Catch of Eggs and Larvae Among Sediment Types



* K-W test shows a significant difference in catches of eggs among sediment types ($p=0.017$). Combining the softer sediments results in significant difference with sand ($p=0.0045$).

Figure 17. Number of eggs or larvae per tow caught on various substrates in Milford or New Haven Harbors. The catch of eggs in Milford is significantly different among the sediment types.

Distribution of Stations, Eggs and Larvae Among Depth Strata

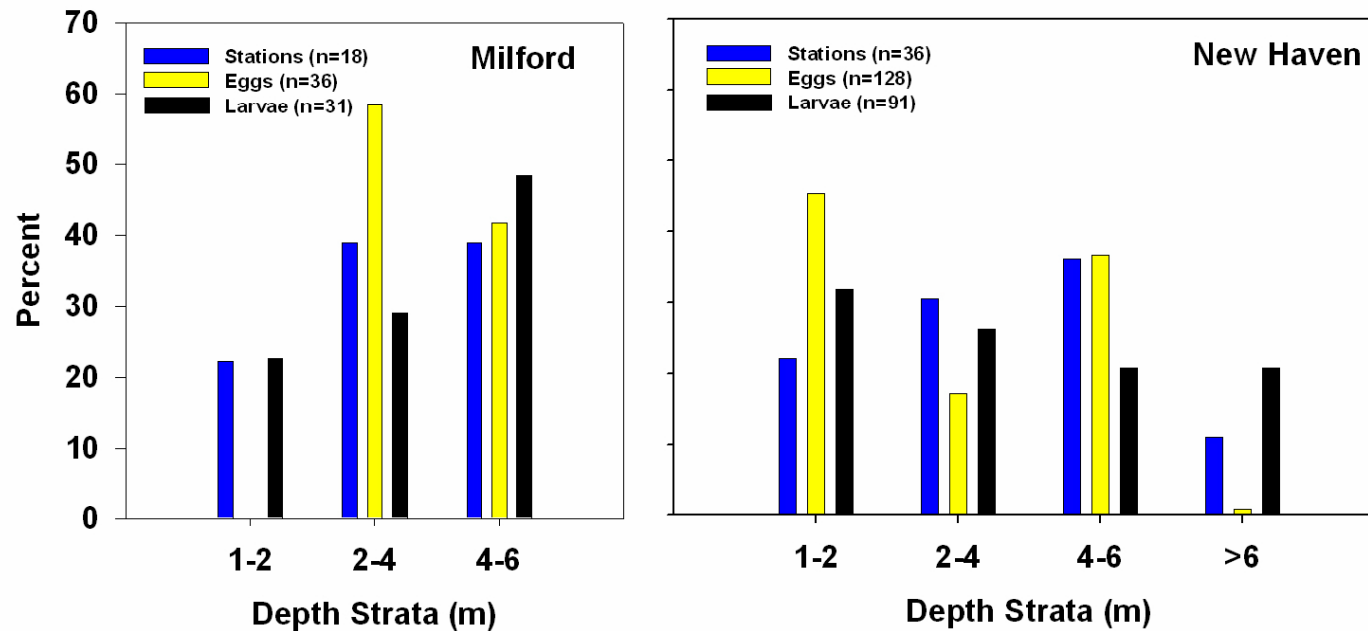


Figure 18. Distribution of stations, eggs and larvae among depth strata expressed as a percent of the total. There were no significant differences in number of eggs or larvae among depth strata ($p>0.05$)

Catch of Eggs and Larvae Among Depth Strata

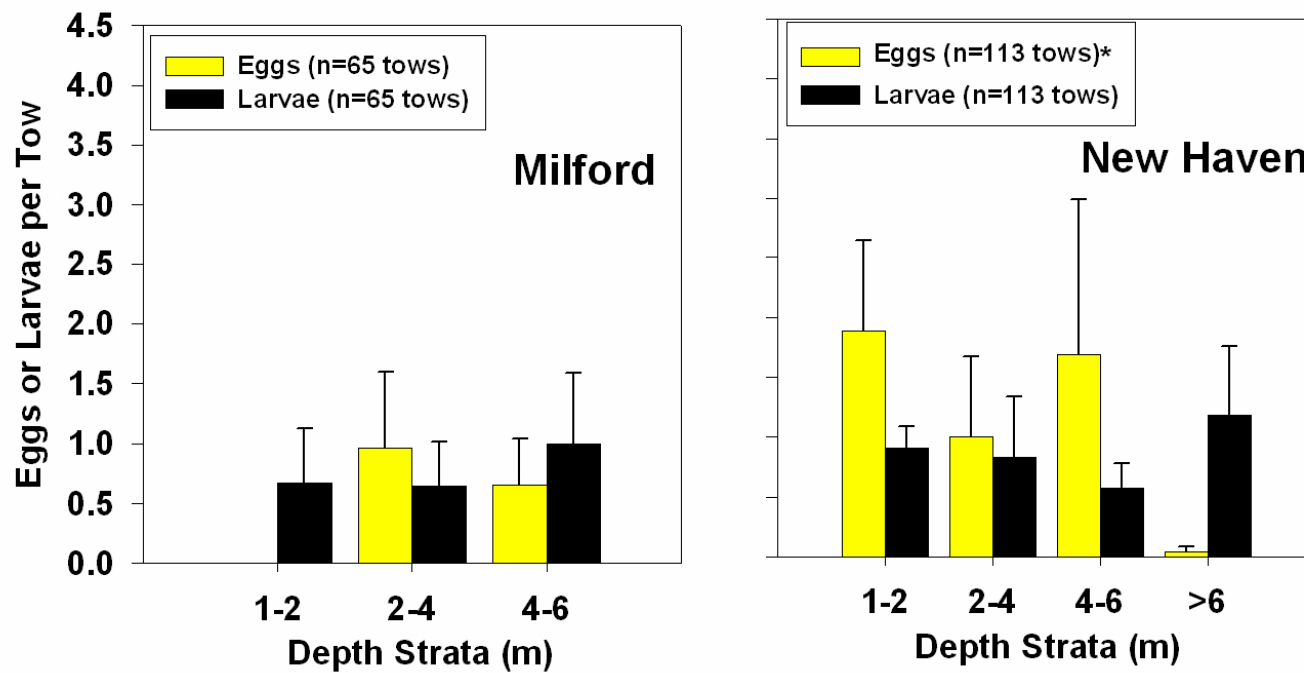


Figure 19. Number of eggs or larvae per tow caught in different depth strata in Milford or New Haven Harbors. The catch of eggs among depth strata in New Haven is significantly different ($p=0.017$).

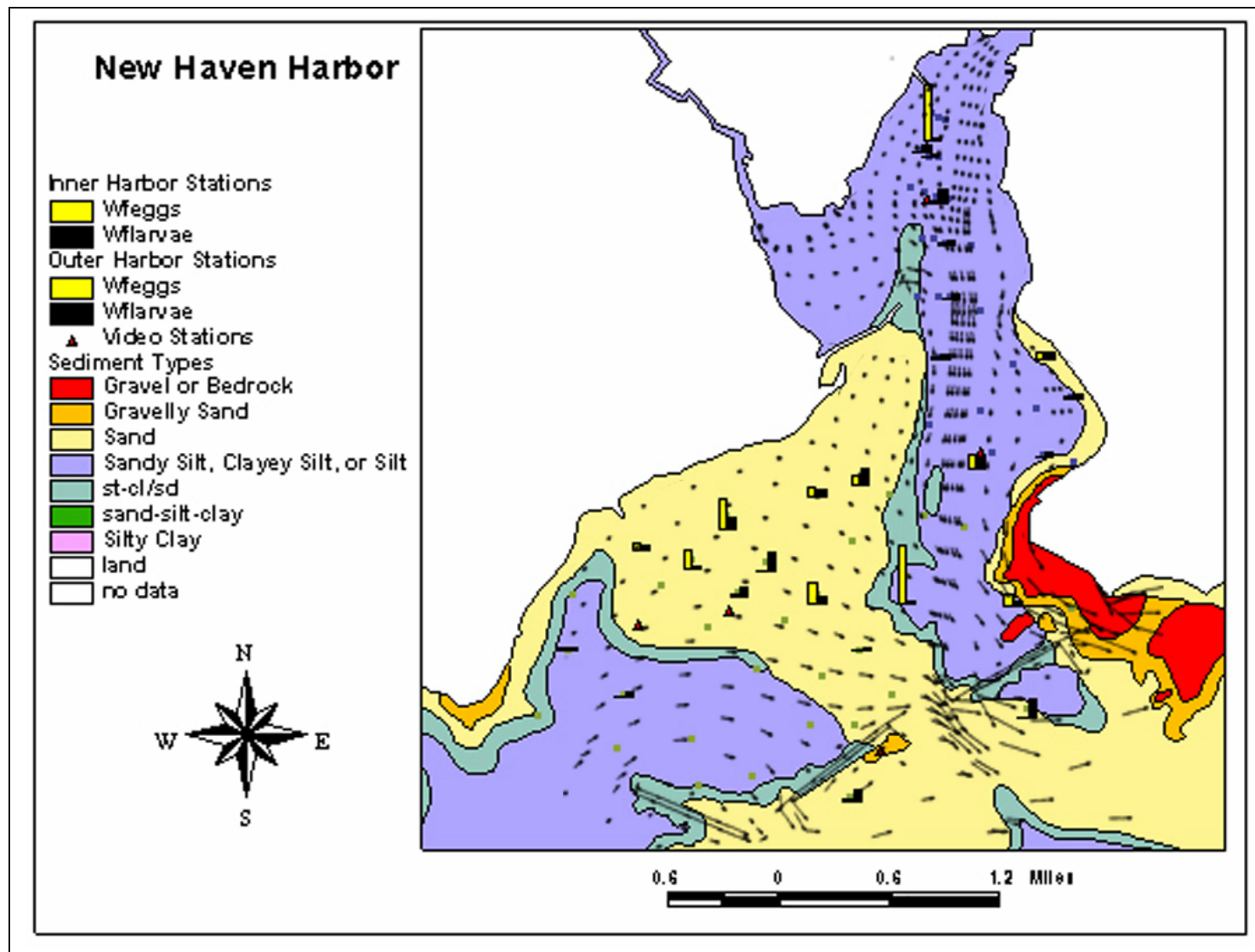


Figure 20. Current speed predictions for New Haven Harbor on full ebb tide from Richards (1988). Maximum current speeds recorded in the harbor were at the eastern end of the east breakwater (2 FPS or 1.2 knots). Current in areas where eggs were found appeared to be 0.6 knots or less.

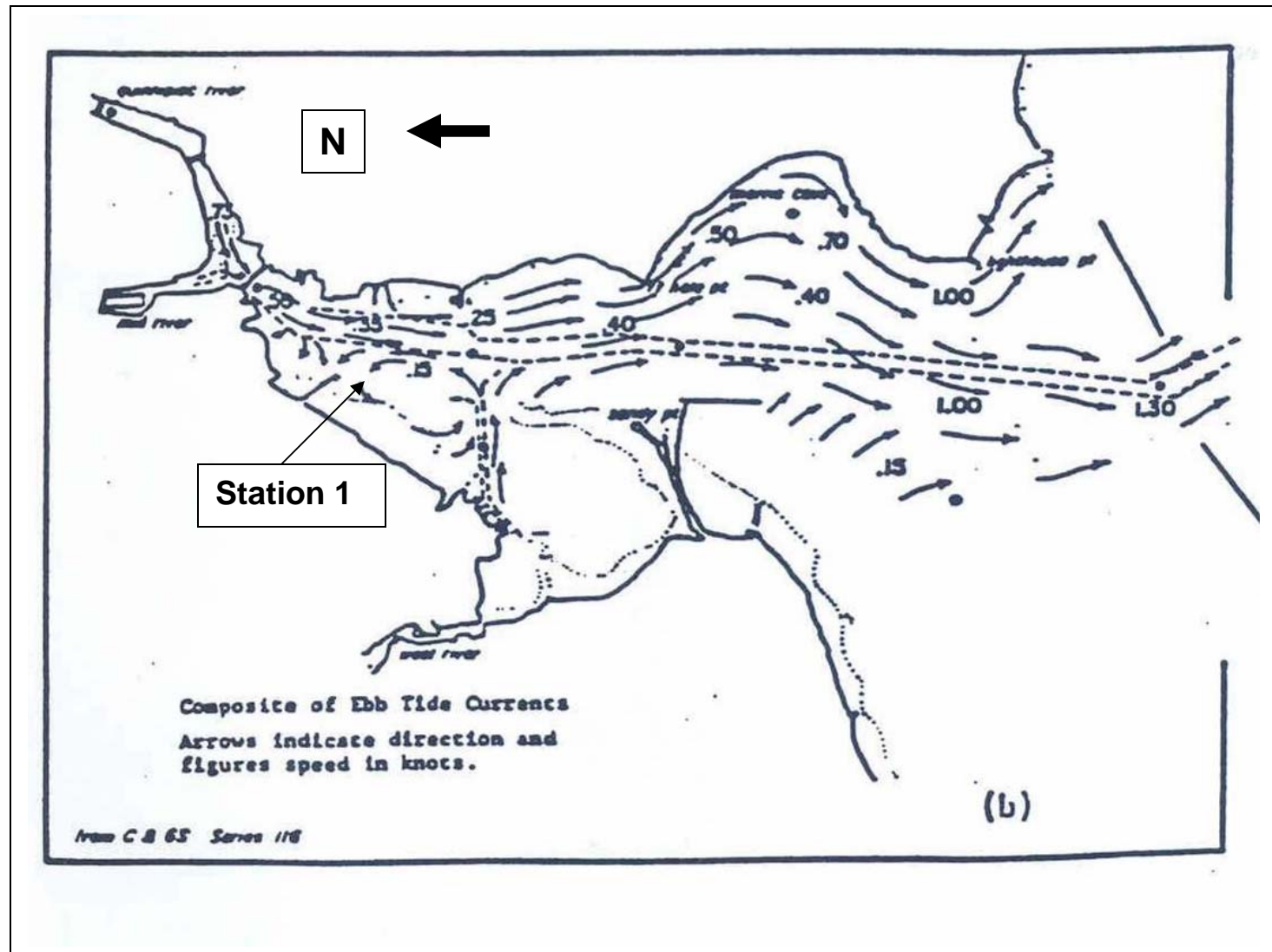


Figure 21. Current patterns and speeds in New Haven Harbor from McCusker and Bosworth (1977) which shows low current speeds and a counter clockwise pattern near Station 1. We collected 24 eggs at this station..

Milford Harbor

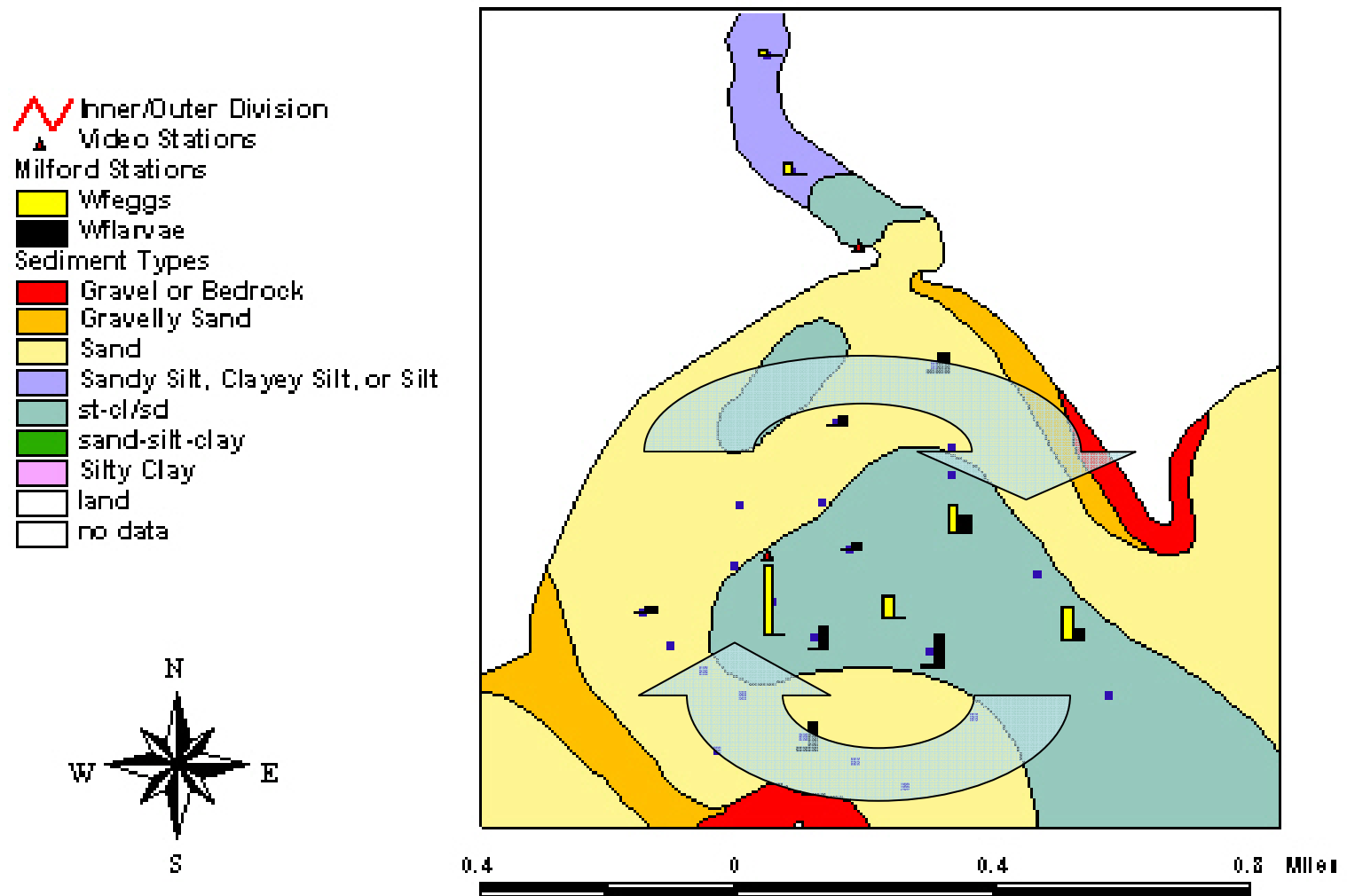


Figure 22. Clockwise circulation in the outer harbor as proposed by Ludwig (pers. com.)