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POPULATION STRUCTURE AND ESSENTIAL FISH HABITAT MAPPING OF WESTERN GEORGES BANK COD

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ABSTRACT
The Georges Bank Atlantic cod fishery is managed as a single stock. However, for years fishermen have noted that Atlantic cod (*Gadus morhua*) to the west of the Great South Channel (western Georges Bank, WGB) spawn in late fall/early winter while those to the east (eastern Georges Bank, EGB) spawn in early spring. These spawning grounds are roughly 270 km apart. By using previously developed and new DNA microsatellite markers we found that cod sampled at WGB and EGB belong to genetically different stocks (F_{ST}= 0.0107, P=0.003; R_{ST}= 0.0101, P= 0.004). A second goal of the study was to locate and genetically assign larvae and juveniles originating from the WGB spawning population. We collected and genetically assigned larvae and juveniles to parental populations. Individual assignment tests and F and R statistics showed that larvae on Nantucket Shoals most likely originated from the WGB cod population, while results for juveniles were less conclusive and point to the possibility of origination from an unsampled adult population. Additionally, mapping of the sampled cod demonstrates areas within Nantucket Shoals/Sound that correspond or diverge with areas designated as essential fish habitat (EFH). All of the adult cod sampled correspond to EFH designations. Sampled areas also corresponded to EFH designations for 65% of the sampled cod larvae and 8% of the sampled juvenile cod. The results of this study may help to create a more effective management plan for the cod spawning population at WGB and a more complete life history picture of this heavily exploited fishery.

EXECUTIVE SUMMARY
The Georges Bank Atlantic cod (*Gadus morhua*) fishery covers the area that is known as Georges Bank as well as the waters to the west of the Great South Channel (GSC). For years fishermen have noted that cod to the west of the Great South Channel (western Georges Bank, WGB) spawn in late fall/early winter while those to the east (eastern Georges Bank, EGB) spawn in early spring. These spawning grounds are roughly 270 km apart. However, it was not known whether cod might spawn at multiple times in multiple locations or if these differences in reproductive behavior reflect different spawning populations.

In this study we wanted to determine if the temporal spawning differences constitute two genetically distinct spawning populations, evaluate WGB and Nantucket Shoals as an important habitat for juveniles, determine which stock these juveniles may originate from, and relate the sampled WGB to essential fish habitat (EFH) designations.

Fishermen from the CCCHFA fished western Georges Bank between September 2004 and January 2005 on a weather-permitting basis to collect fin clip samples from reproductively mature (ripe and ripe & running) adult male and female Atlantic cod. Additional blood samples or fin clips of previous years and Eastern Georges Bank were also collected to provide additional samples for comparison analysis. All together 168 spawning cod were sampled from western Georges Bank (WGB) over 3 years (38 in 2002/2003, 45 in 2003/2004, and 85 in 2004/2005 (this study)) and 75 from eastern Georges Bank (EGB) during two years (62 in 2004 and 13 in 2005).

A MATLAB model was used to predict egg and larval trajectories by incorporating the locations and dates of capture of spawning adults during the 2004/2005 western Georges Bank sampling season as well as recorded wind and surface current data in the time between spawning and larval sampling. Sampling for larvae and juveniles occurred in the mean tidal-front entrainment region of western Georges Bank from February –May 2005. Larvae were collected by plankton tow using bongo-net/CTDs with a 505 µm mesh. Tows were made according to standard MARMAP procedures with attached flowmeters. Tows lasted between 15 and 25 minutes at a speed of 2 knots, with an average volume of 362 m3 of water sampled per tow. Juveniles were collected by an otter trawl with a 16 meter circumference, a 7 meter sweep, and 1.25 cm mesh lining. The net was towed between three and six times per site, depending on weather and sampling success. The assignment of larval and juvenile cod was limited by low catch rates and the relatively small genetic difference between the genetically distinct adult populations. Forty-six larvae and 343 juvenile cod were collected and identified.

Five microsatellite markers were used to examine population structure and parental assignment. Genotype and allele frequencies of the microsatellite loci were used to estimate variances in gene frequencies between adult cod populations from Eastern and Western Georges Bank as well as larvae and juveniles. In order to determine the origin of larvae and juveniles, we performed assignment tests in GeneClass2 , using the western and eastern Georges Bank adults as reference populations.

By using previously developed and new DNA microsatellite markers we found that adult cod sampled at WGB and EGB belong to genetically different stocks (F_{ST}= 0.0107, P=0.003; R_{ST}= 0.0101, P= 0.004). The values indicating genetic difference are much higher than in previous studies. However, these differences are mainly based on 2 out of 6 genetic markers. We assigned 6 of the 46 larvae solely to the 2004/2005 western Georges Bank population, none of the larvae solely to eastern Georges Bank. The other 40 larvae were not able to be assigned unequivocally to either reference population. Of the juveniles, 62 of 343 were assigned solely to the 2004/2005 western Georges Bank population, 14 of the juveniles solely to eastern Georges Bank, and 206 of the juveniles to both western and eastern Georges Bank. The remaining 61 juveniles could not be assigned to either reference population. They might originate from an unknown cod stock. Individual assignment tests and population genetic statistics showed that larvae on Nantucket Shoals most likely originated from the WGB cod population, while results for juveniles were less conclusive and point to the possibility of origination from a mixture of adult populations or an unsampled adult population.

Conductivity, temperature and depth recordings for the bongo tows and selected otter trawls were plotted in excel. Tows/trawls with cod samples were compared to tows/trawls with no cod samples to determine if there is any environmental variable that may indicate habitat preference by cod. The range of measured environmental variables appear to have no influence on the likelihood of finding larvae or juvenile cod.

The location and corresponding data of the reproductively mature adults, larvae, and juvenile cod collected by CCCHFA fishermen were mapped in ESRI ArcGIS 9.0 to

create a GIS for the project. Portions of the historical sediment data from the U.S. Geological Survey (Poppe, et al, 2003) were selected for analysis and interpolated into a raster surface. The adult, larvae, and juvenile sample locations were extracted from the interpolated sediment raster to determine the approximate habitat type for each sample. The interpolated GIS sediment surface indicates that the areas where juvenile cod were trawled for, as well as where they were actually collected, are composed of sand and gravel. The majority of the juveniles were collected along the 20 and 30 meter contour lines.

Additionally, mapping of the sampled cod demonstrates areas within Nantucket Shoals/Sound that correspond or diverge with areas designated as essential fish habitat (EFH). All of the adult cod sampled correspond to EFH designations. Sampled areas also corresponded to EFH designations for 65% of the sampled cod larvae and 8% of the sampled juvenile cod. The results of this study may help to create a more effective management plan for the cod spawning population at WGB and a more complete life history picture of this heavily exploited fishery.

Additional in-depth reporting for the project is attached; Emily Weiss developed her masters thesis from this project and it is attached as Appendix B.

PURPOSE

The Georges Bank Atlantic cod fishery covers the area that is known as Georges Bank as well as the waters to the west of the Great South Channel (GSC) (see Figures 1 and 2). Demographic similarities between Atlantic cod (*Gadus morhua*) in the Southern New England management area and the Georges Bank management area are so pronounced that the two areas are considered one stock and referred to as the Georges Bank stock (Serchuk & Cohen, 1997). For years fishermen have noted that cod spawn at different times of the year, despite the geographic closeness of the spawning grounds, approximately 270 km apart. They have observed Cod spawning in the spring to the east of the GSC, and spawning in late fall/early winter to the west of the GSC. However, it was not known whether cod might spawn at multiple times in multiple locations or if these differences in reproductive behavior reflect different spawning populations. In this study we wanted to determine if the temporal spawning differences constitute two genetically distinct spawning populations

For years it has been known that current patterns on Georges Bank act to retain eggs, larvae, pelagic juveniles and recently settled juveniles from spawning adults in the northeast corner. Current patterns and essential fish habitat (EFH) of larvae and juveniles are well-mapped in this region (Figure 3). NMFS bongo tows and otter trawls have also shown that eggs, larvae, and juveniles exist throughout Nantucket Shoals (see Figure 4). Though, the blank space on the map in the vicinity of the Shoals, indicates that much of that area has not been sampled**.** Until now no one has tried to identify the parental populations for these larvae and juveniles. If larvae and juveniles on western Georges Bank and Nantucket Shoals originate from the western Georges Bank population, it is crucial to protect both their habitat and the parent populations.

Circulation patterns on western Georges Bank and on Nantucket Shoals are not very well understood. The current in the shallow areas on Nantucket Shoals is heavily wind-driven and therefore highly unpredictable (Limeburner & Beardsley, 1982). A general clockwise subsurface circulation starting off the elbow of Cape Cod and continuing southwest through Nantucket Shoals is the recorded tidal mixing front for the region (Lough & Manning, 2001). This prevailing current probably entrains eggs, larvae, and juveniles on the shoals (Fogarty & Murawski, 1998). Based on the overall prevailing water flow patterns, we predicted that eggs and larvae from the spawning of Western Georges Bank cod would move southeast towards Nantucket Shoals. This may eventually be the site for juveniles with dispersing larvae being caught in the tidal mixing front surrounding Nantucket Shoals.

The project contributed to an expanded understanding of marine habitats and the productivity of the Georges Bank cod stock. It identified a local and regional need for greater insights into the habitat requirements of life history processes for cod in an area identified by fishermen as critically important and poorly understood. With this priority in mind, the project identified and acquired information about the spawning life history of Western Georges Bank cod, and the connection between these life history processes and habitat components. Better understanding of the Western Georges Bank cod yields insights about recruitment potential and thus suggests ways to maximize productivity through effective management.

This project brought together a novel collaboration of scientists and fishermen to determine, identify, and map Atlantic cod eggs, larvae, and newly settled juveniles resulting from fall spawning events on Western Georges Bank. Poor recruitment is driving a pessimistic long-term forecast for the GB cod stock that will hinder harvest of healthier stocks such as GB yellowtail flounder and GB haddock. Building better understanding of the benthic and pelagic habitats that are critical to egg, larvae, and new juvenile survival is the first step towards unraveling one of the most persistent fisheries management challenges in New England.

Scientific Goals:

- Establish genuine working relationships between fishermen and scientists to collect and analyze egg, larval, juvenile and adult samples from Western Georges Bank cod to track fall spawning event.
- Gather physical data (using bongo nets and otter trawls) to track progeny (eggs larvae, juveniles) of fall spawning Western Georges Bank cod.
- Determine the feasibility of using modern genetic techniques to track progeny (eggs, larvae, juveniles) of fall spawning Western Georges Bank cod.
- Use GIS mapping to describe the geographic location of pelagic and demersal habitats utilized by adult spawning, egg, larvae and juvenile life history stages of Western Georges Bank Cod. Relate this information to EFH priorities.

Social Goals:

- Create supplemental income for fishermen through the participation of fishing businesses in data collection and analysis.

- Continue to build new avenues and mechanisms for commercial fishermen to contribute to science and nurture the strong relationships created through past CCCHFA research coordination. These working relationships are crucial to building and maintaining support for the efforts of government scientists and fisheries managers.
- Overcome communications and perception barriers over data collection and usage between fishermen and government scientists by developing relationships based on trust and mutual understanding.
- CCCHFA strives to meet a new level of cooperative research by actively involving fishermen in research operations, rather than simply chartering their vessels as research platforms.

Objectives:

- Train 30 fishermen in the use of appropriate sampling nets (e.g. bongo nets) to obtain egg and larval samples, and the use of data loggers to record temperature and current.
- Train 30 fishermen in techniques necessary to sample cod for reproductive status, including collection and preparation of tissue samples (ovaries and testes).
- Starting during the reproductive season, fishermen will collect samples; initially eggs and larvae with bongo nets and later juveniles with trawls.
- For all sampling sites, fishermen will record temperature profiles (surface to bottom) and current speed and direction.
- Generate and refine a model for egg/larval dispersal based on the water temperature and current data obtained from the data loggers, and the empirical knowledge of participating fishermen. This work will be a cooperative effort between commercial fishermen and scientists at the Environmental Coastal and Ocean Sciences Program at UMASS Boston.
- Egg, larval and juvenile samples will be analyzed at MBL to determine size and stage of development.
- Adult, larvae and juvenile cod will be analyzed at MBL using micro satellites to track dispersal patterns of the Western George's Bank cod. Genetic identification of the fall spawning Western Georges Bank cod is a prerequisite to identifying the specific habitats utilized by its young, as it will allow us to track the dispersal of the appropriate eggs, larvae and juveniles.
- Produce GIS maps to describe the geographic location of pelagic and demersal habitats utilized by adult spawning, egg, larvae and juvenile life history stages of Western Georges Bank Cod.

APPROACH

Methodology

Adult sample collection:

Fishermen from the CCCHFA fished western Georges Bank between September 2004 and January 2005 on a weather-permitting basis. On board technicians collected the following information from ripe or ripe and running adults: sex, reproductive stage, length, date, location of capture and fin clip. Reproductive stage was identified by milt

and eggs. Ripe and running females' eggs were released from the vent when applying a small amount of pressure to the belly. Ripe was characterized by eggs in ovaries being at least 50% hydrated. Ripe and running males spouted milt from their vents with minimal or no pressure to the belly. Ripe males' testes were engorged with milt and released milt when cut.

Additional blood samples or fin clips of previous years and Eastern Georges Bank were also collected according to a similar methodology, using hand line or trawl net. Western Georges Bank was sampled September 2002 to January 2003 and September 2003 to December 2003 and blood samples of ripe or ripe and running cod fish were collected. Eastern Georges Bank was sampled March-April 2004 and April 2005 by the National Marine Fisheries Service. Scientists collected fin clips (approximately 3 cm in length), and the sex, reproductive stage, length, date, and location of capture of each fish was recorded. Eastern and Western Georges Bank distinctions are illustrated in figure 1.

During the spring of 2005, CCCHFA fishermen collected additional tissue samples of cod on Eastern Georges Bank. Fin clips were taken regardless of reproductive status. Sex, reproductive stage, length, date, and location of capture were recorded.

Larvae and juvenile sample collection:
We used a model created on MATLAB (James Manning, NOAA) to predict the best locations to perform larval sampling. The MATLAB model predicted egg and larval trajectories by incorporating the locations and dates of capture of spawning adults during the 2004/2005 western Georges Bank sampling season as well as recorded wind and surface current data in the time between spawning and larval sampling.

Due to abnormally strong wind conditions caused by a nor'easter in late January our model predicted a southeastern trajectory that would have put the larvae hundreds of miles farther down the coast than Nantucket Shoals. Due to the impossibility of sampling the model's projection, we instead sampled in the mean tidal-front entrainment region of western Georges Bank laid out in Lough & Manning (2001). Larvae and juveniles were collected from this area based on a grid (see Figure 5), in which we predicted a clockwise movement of larvae spawned in the fall from northeast to southwest of western Georges Bank.

Therefore, sampling for larvae began in the northeast corner of the grid at the end of February, 2005 and moved in a clockwise direction through the middle of April onto Nantucket Shoals. Severe weather conditions made sampling before February impossible. Sampling should have occurred farther south, but federal permitting did not allow us to tow the bongo nets in the Lightship Closed Area. Each portion of the approximately 75 x 100 km grid was sampled at least three to seven times on the date that it was visited. Individual sites were approximately 20 x 28 km. Each tow lasted between 15 and 25 minutes, with the boat traveling at 2 knots uptide when possible and keeping the nets at least 4 meters from the bottom. Larvae were collected by plankton tow using bongo-net/CTDs with a 505 µm mesh. Tows were made according to standard MARMAP procedures (Jossi & Marak, 1983) and (Pogsay & Marak, 1980), with

attached flowmeters. The average volume of water sampled per tow was 362 m^3. The contents of each tow were rinsed, sieved and placed in 99% ethanol-filled jars. Ethanol was changed every few days between the date of capture and the sorting procedure.

Sampling for juveniles began in early April and continued through early May, 2005, following the same grid as the larvae. Each grid site was trawled between three and six times depending on sampling success and weather conditions. If the first date of larval sampling was successful at a grid site, the site was visited at least once more. Trawls within a site were spaced out over at least three different areas (see Figure 5). Juveniles were collected by an otter trawl with a 16 meter circumference, a 7 meter sweep, and 1.25 cm mesh lining. The net was towed between three and six times per site, depending on weather and sampling success. After the net was hauled in, any fish that was cod-like (having three dorsal fins) was placed in a container of 99% ethanol. Ethanol was changed every few days between the date of capture and the sorting procedure.

Larvae and juveniles were sorted in the laboratory using a compartmentalized plexiglass tray and forceps. All gadid-like larvae were first pulled from the pooled larvae and put aside for individual identification as cod larvae. Cod larvae were identified by comparing them to pictures and morphological features (Fahay, 1983) and (Auditore et al., 1994). Identification was later confirmed by Elizabeth Broughton (NOAA). All larvae and juveniles were measured and assigned an individual ID, with records kept of their date and location of collection. Individuals were then stored in 99% ethanol for later DNA extraction.

Conductivity, temperature and depth recordings for the bongo tows and selected otter trawls were plotted in excel. Tows/trawls with cod samples were compared to tows/trawls with no cod samples to determine if there is any environmental variable that may indicate habitat preference by cod.

DNA extraction, amplification, and visualization:
Genomic DNA was extracted from blood samples or fin clips using a 10% Chelex® (Bio-Rad) solution (Walsh et al., 1991) following the instructions of the manufacturer.

In order to examine population structure and parental assignment we used five microsatellite loci—Gmd897 (see attached thesis for primer development), Gmo132 (Brooker et al., 1994), Gmo8, Gmo19, and Gmo34 (Miller et al., 2000). Using an Eppendorf Mastercycler Gradient thermal cycler, we performed polymerase chain reactions (PCR) of all loci. Final concentrations of reagents for 25 µL PCR reactions follow: 1 µL supernatant from Chelex® DNA extraction, 2.5 µL 10× RedTaq™ PCR Reaction Buffer (Sigma), 2.5 µL 200 µM each dNTP (Promega), 0.5 µL 10 µM forward primer (labeled with a D3 or D4 WellRED dye), 0.5 µL 10 µM unlabeled reverse primer (Sigma), and 0.25 units of Red Taq DNA polymerase (Sigma). PCR conditions follow (Lage et al., 2004): initial 5 min. at 95°C , 30 cycles of denaturing at 95°C for 1 minute, annealing at 50°C (Gmo8, Gmo19, and Gmo34), 57°C (Gmo1), and 55°C (Gmd897) for 1 min 30 s, and extending at 72°C for 10 min.

Fluorescent labeled microsatellite PCR products were pooled when possible and visualized on a CEQ™ 2000XL DNA Analysis System (Beckman Coulter, Inc., Fullerton, CA) and analyzed using CEQ™ 8000 Series Genetic Analysis System Software. Most individual samples were run at least two times and each run scored blindly multiple times to ensure consistent and accurate scoring.

Data analyses:

Genotype and allele frequencies of the microsatellite loci were used to estimate variances in gene frequencies between adult cod populations from Eastern and Western Georges Bank as well as larvae and juveniles. Within subpopulations deviations from Hardy-Weinberg equilibrium were estimated by F_{IS}-values (f-values) calculated with FSTAT version 2.9 updated from Goudet (Goudet, 1995) based on Weir & Cockerham (Weir & Cockerham, 1984). To determine the degree of genetic divergence between cod populations F_{ST}-values (Weir & Cockerham, 1984) were calculated using FSTAT version 2.9 (Goudet, 1995). Significant differences in gene frequencies were tested by permutation tests advocated by (Goudet et al., 1996). When F_{IS}-values indicated that random mating could not be assumed within populations, statistical significance for F_{ST}-values was estimated using an exact G-test by randomizing genotypes among samples, for details, see Goudet et al. (Goudet et al., 1996). To reduce the likelihood of type I errors, a Bonferroni correction (Sokal & Rohlf, 1995) was applied. Population structure based on a stepwise mutation model, R_{ST}-values and associated P-values, were calculated using ARLEQUIN 2.0 (Schneider et al., 2000).

In order to determine the origin of larvae and juveniles, we performed assignment tests in GeneClass2 (Piry et al., 2004) (http://www.montpellier.inra.fr/CBGP/softwares/index.htm), using the western and eastern Georges Bank adults as reference populations. We used only 2004/2005 western Georges Bank and eastern Georges Bank as reference populations for the larvae and in a first run for the juveniles as the larvae and the majority of the juveniles were too young to originate from spawning events prior to 2004/2005. In a second analysis of the juveniles, we examined young-of-year juveniles using the 2002/2003 Georges Bank adults and the eastern Georges Bank adults as the reference populations, as these juveniles were too large to have been parented by the 2004/2005 western Georges Bank adults. We employed a Bayesian model (Rannala & Mountain, 1997a) with assignment probabilities again computed based on a Monte-Carlo resampling method (Paetkau et al., 2004). A total of 1000 individuals were simulated with a threshold of 0.01. If individuals did not meet the threshold, they were not assigned to either population. We also ran analyses with the Bayesian model without assignment probabilities, maintaining an assignment threshold of 0.01, for the purposes of raw assignment. This means that larvae and juveniles were assigned to the reference population of highest likelihood even if Monte-Carlo resampling would not have allowed assignment to any of the reference populations.

Geographic Information Systems (GIS) Mapping:

The location and corresponding data of the reproductively mature adults, larvae, and juvenile cod collected by CCCHFA fishermen were mapped in ESRI ArcGIS 9.0 to create a GIS for the project. Shapefiles of fishing effort were created from the latitude

and longitude coordinates collected during the study; if Loran-C TD's were collected, they were converted to latitude/longitude using the U.S. Coast Guard's Positioning Aid, version 2.1a (USCG, 2004). Corresponding data tables of information specific to fishing effort and individual fish sampled were joined to the shapefiles. Maps were designed to illustrate effort and collected samples.

Portions of the historical sediment data from the U.S. Geological Survey (Poppe, et al, 2003) were selected for analysis based on correspondence with the project's sampling effort. The following datasets were included in the GIS: Dec41_GOM (Poppe, 2003a), NOSGOM (Poppe, 2003b), Reid 82 (Poppe, 2003c), Smithsonian (Poppe, 2003d), USGS ECSTDB (Paskevich and Poppe, 2003), and Wigley 65 (Poppe, 2003e). These point shapefiles were merged into a single shapefile, standardized for sediment descriptions, and interpolated into a continuous raster surface. The interpolation was ultimately completed through ordinary kriging, with a spherical semivariogram model, variable search radius type of 12 points, and output cell size of 0.01999. The adult, larvae, and juvenile sample locations were extracted from the interpolated sediment raster to determine the approximate habitat type for each sample.

Essential fish habitat (EFH) data for Atlantic cod were retrieved from the New England Fisheries Management Council (NMFS, 2005b). Once the data were added to the GIS, the EFH for each life history stage of the cod was overlaid with the location of collected samples. This process allowed for the identification of where the project's sampling coincided with or expanded upon current EFH priorities.

Project Management

Individuals	Organization	Scope of Involvement
Melissa Sanderson	CCCHFA	Project Coordinator
Paul Parker	CCCHFA	Principle Investigator
Dr. Gabrielle Gerlach	MBL	Lead scientist
Emily Weiss	MBL	Genetic analysis, final statistical analysis, and write up
Tom Rudolph	CCCHFA	Counsel: scientist/fishermen relationships, implementation of study
Eric Brazer	CCCHFA	Intern: developed and tested bongo protocol
Elisabeth Broughton	NEFSC	Counsel: Larvae identification and bongo protocol; bongo equipment loan
James Manning	NMFS	Data modeler: particle tracking model in MATLAB for larvae
Dave Mountain	NMFS	Physical oceanographic consultant
Don Clark	DFO Canada	Provided outgroup of Canadian cod fin clips
Peter Chase	NMFS	Reproductive staging and fin clip organization
Michael Bennie	MBL	Intern student: DNA analysis
Loretta O'Brien	NMFS	Consulted on current NMFS regulations concerning cod.
Ron Borjeson	F/V Angenette	Juvenile Sampling
Tom Szado	F/V Arlie X	Adult Sampling

David Nadeau	F/V Bad Seed	Larvae Sampling
Jan Margeson	F/V Growing Old	Larvae Sampling
Mike Russo	F/V Gulf Venture	Adult Sampling
Mike Abdow	F/V Magic	Adult Sampling
Glen LeGeyt	F/V Miss Morgan	Adult Sampling
Bruce Kaminiski	F/V Never Enough	Adult Sampling
Ted Ligenza	F/V Riena Marie	Adult Sampling
Peter Taylor	F/V Seahound	Adult Sampling
Tom Traina	F/V Sue-Z	Adult Sampling
Alfred Yuknavich	F/V Surf Breaker	Adult Sampling
Eric Hesse	F/V Tenacious	Adult Sampling
Tom Barker	F/V Tuna Eclipse	Adult Sampling
Albert Nardini	CCCHFA	Industry Technician
Charlie Pitts	CCCHFA	Industry Technician
Chip Foster	CCCHFA	Industry Technician
Ethan Estey	CCCHFA	Industry Technician
John Kenneway	CCCHFA	Industry Technician
Ray Kane	CCCHFA	Industry Technician
Ronnie Braun	CCCHFA	Industry Technician

ACCOMPLISHMENTS & FINDINGS

All together 168 spawning cod were sampled from western Georges Bank (WGB) over 3 years (38 in 2002/2003, 45 in 2003/2004, and 85 in 2004/2005) and 75 from eastern Georges Bank (EGB) during two years (62 in 2004 and 13 in 2005). We exceeded the proposed sampling schedule and included adult reproductive cod from WGB from 3 years and EGB 2 years (instead of the proposed 1 year sampling). This allowed for evaluating temporal stability of the population structure of cod at WGB. We used genetic markers that have been applied in other population genetic studies on cod at the East coast of the US to be able to compare data sets. We could find small but statistically significant genetic differences between WGB and EGB cod. By using previously developed and new DNA microsatellite markers we found that cod at WGB and EGB belong to genetically different stocks (F_{ST}= 0.0107, P=0.003; R_{ST}= 0.0101, P= 0.004). The values indicating genetic difference are much higher than in previous studies. However, these differences are mainly based on 2 out of 6 genetic markers.

The assignment of larval and juvenile cod was limited by low catch rates and the relatively small genetic difference between adult populations. Forty-six larvae and 343 juvenile cod were collected and identified at the MBL in collaboration with Elisabeth Broughton (NOOA/NMFS). Most juveniles landed were under 60 mm in length (average length= 43 mm; median length= 39 mm) (shown in green in Figure 6), indicating that they were from a recent spawning event. The smaller juveniles were most frequently caught in clusters of 6-14, with occasional yields of up to 145 fish in one trawl. Juveniles in the 89-91 mm size class were most likely smaller individuals from the prior year's

spawning event; only two individuals fell into this category. The sixteen juveniles in the >100 mm category were actually young-of-year (products of the prior year's spawning event). Larvae and juveniles were sometimes caught within the same site. This occurred most frequently at site 2 (see Figure 5).

A further goal of the study was to evaluate WGB and Nantucket Shoals as an important habitat for juveniles and from which stock these juveniles may originate. Only a part of the larvae could be assigned unequivocally to the WGB cod stock. We assigned 6 of the 46 larvae solely to the 2004/2005 western Georges Bank population, none of the larvae solely to eastern Georges Bank. The other 40 larvae were not able to be assigned unequivocally to either reference population. Of the juveniles, 62 of 343 were assigned solely to the 2004/2005 western Georges Bank population, 14 of the juveniles solely to eastern Georges Bank, and 206 of the juveniles to both western and eastern Georges Bank. The remaining 61 juveniles could not be assigned to either reference population. They might originate from an unknown cod stock. Individual assignment tests and population genetic statistics showed that larvae on Nantucket Shoals most likely originated from the WGB cod population (see Figure 7), while results for juveniles were less conclusive and point to the possibility of origination from a mixture of adult populations or an unsampled adult population (see Figure 8).

The interpolated GIS sediment surface indicates that the areas where juvenile cod were trawled for, as well as where they were actually collected, are composed of sand and gravel. The juveniles' parent population assignment appears to have no correlation with sediment type. Both Western and Eastern Georges Bank juveniles were found in both sand and gravel (Figure 9). The majority of the juveniles were collected along the 20 and 30 meter contour lines.

The measured environmental variables appear to have no impact on the likelihood of finding larvae or juvenile cod. Measured variables for trawls and tows where juveniles and larvae were caught are described in Table 1 and 2, respectively. Measured salinity for larvae range from 28.70 to 29.62 psu; for juveniles the range was 29.85 to 30.11 psu. Measured temperature for larvae range from 36.79 to 43.27 °F; for juveniles the range was 41.99 to 43.26 °F. Average depth for juveniles was 32.06 m and 26.97 m for larvae.

Documents provided by the National Marine Fisheries Service (2005a) indicate the range of temperatures, depths, and salinities commonly associated with essential fish habitat for cod as larvae and juveniles. Larvae are generally found in depths of 30-70 meters, sea surface temperatures below 50°F, and salinities of 32-33%. Our larvae were caught at much shallower depths, lower salinities, and within the temperature range (Table 2). Juveniles are generally found in depths of 25-75 meters, salinity ranges of 30-35%, and water temperatures below 68°F. Our juveniles were collected within the depth and temperatures ranges, and at salinities just below the reported range (Table 1).

JUVENILES	**Minimum**	**Maximum**	**Average**
Depth (m)	22.50	42.50	32.06

Temperature (°F)	41.99	43.26	42.55
Salinity (psu)	29.85	30.11	29.96

Table 1. Measured environmental variables collected by CTD attached to otter trawl while towing for juvenile cod on 4/18/2005.

LARVAE	**Minimum**	**Maximum**	**Average**
Depth (m)	8.70	47.0	26.97
Temperature (°F)	36.79	43.27	39.55
Salinity (psu)	28.70	29.62	29.05

Table 2. Measured environmental variables collected by CTD attached to all bongo nets while towing for larval cod.

The assessed location of Atlantic cod at critical life history stages (spawning adults, larvae, juveniles) overlaps with some of the previously identified essential fish habitat areas. The map in Figure 10 shows that all reproductively mature cod were caught within areas already designated as adult EFH. The majority of the larvae collected were also caught within areas already designated as larvae EFH (65%); however, there are areas on the map (Figure 11) in Nantucket Sound and south-east of Nantucket Island where we found larvae that are not included in the current EFH designation. A small portion of the juveniles caught (8%) were collected within areas designated as juvenile EFH (Figure 12).

Significant Problems

Severe weather problems delayed the sampling schedule of larvae and juveniles. The late sampling schedule may explain the small numbers of cod and larvae collected. Additionally, the severe winter storms in New England may have also blown significant portions of the larvae and juvenile population far to the south and out of sampling range. The MATLAB modeling of the larvae drift confirms that the currents and wind would support the transport of larvae below the mid-Atlantic states. The severe weather also compressed the sampling time frame; instead of distributing the sampling evenly over the time period, intense sampling occurred when we had windows of safe weather.

As protocol was developed for larvae identification, it was determined that cod eggs are visibly indistinguishable from other gadoid species. Consequently, the collection and analysis of eggs was removed from the study.

Genetic differences between adult cod populations were much higher than reported from former studies on cod on a comparable geographic scale, but nevertheless small. We developed new genetic markers to improve the resolution of the existing genetic differences. However, due to financial constraints, we were not able to use more than one of these markers in the current study.

Suggested Additional Work

1) Newly developed markers should be applied to improve the resolution of the existing genetic differences.
2) More populations of cod should be sampled to evaluate different population dynamics and existence of further stocks at the east coast of the US and Canada.
3) Sampling should be repeated for consecutive years to create a time series of data to indicate where and when larvae, juveniles, and adults use particular habitats.
4) Sediment samples should be collected in areas sampled for fish. Other characteristic information, such as structural profile and invertebrate populations should be collected.

EVALUATION

Note: For all goals/objectives, we did not sample or analyze cod eggs; upon learning that cod eggs are visibly indistinguishable from other gadoids, it was decided that the egg portion of the study would be cancelled due to the difficulty of separating them from pollock or haddock eggs.

Scientific Goals:

- *Establish genuine working relationships between fishermen and scientists to collect and analyze egg, larval, juvenile and adult samples from Western Georges Bank cod to track fall spawning event.*

The collaboration of scientist of the MBL, NOOA/NMFS, CCCHFA and 21 local fishermen was successful. Meetings among the participants yielded discussions and decisions regarding protocols, sampling schedules and locations, models, and analysis. While there were some lively discussions and disagreements, in the end everyone came to a consensus.

The results of this project will be published in a peer reviewed scientific journal. Our data are very exciting and show for the first time that Georges Bank cod consists of two genetically different spawning populations.

- *Gather physical data (using bongo nets) to track progeny (eggs larvae, juveniles) of fall spawning Western Georges Bank cod.*

Bongo nets were successfully deployed in the mean tidal-front entrainment region of western Georges Bank in an attempt to collect larval cod. Ninety-six 15-25 minute tows were made throughout 16 sites from early February to mid April 2005. Sampling would have begun earlier, but severe weather conditions made sampling before February impossible. Because cod eggs can not be distinguished from eggs of other gadid-like species, we focused on collecting larvae. While our data models predicted that the larvae would be as far south as Florida, the impracticability of sampling in southern states led us to focus our tow deployment on a random sampling of the Western Georges Bank/Nantucket Shoal area hypothesized for larvae drifting. From these tows, 46 larvae

cod were collected. Juveniles were collected by an otter trawl with a 16 meter circumference, a 7 meter sweep, and 1.25 cm mesh lining. A total of 60 tows across nine sites yielded 357 juvenile cod, ranging in size from 3-180mm in length.

- *Determine the feasibility of using modern genetic techniques to track progeny (eggs, larvae, juveniles) of fall spawning Western Georges Bank cod.*

Genetic techniques are the only feasible method way to determine the origin of larvae and juvenile cod. Because genetic differences between adult cod populations were small only a part of the larvae and juveniles could be assigned unequivocally to the parental population. 13% of the collected larvae were assigned to a parent population. 21% of the collected juveniles were assigned to a parent population. The application of new genetic markers created at MBL would improve the assignment of progeny substantially. However, new funding is required for this approach.

- *Use GIS mapping to describe the geographic location of pelagic and demersal habitats utilized by adult spawning, egg, larvae and juvenile life history stages of Western Georges Bank Cod. Relate this information to EFH priorities.*

Maps illustrating the locations of where spawning adult, larvae, and juvenile samples were caught and their genetic relationship were successfully created in a geographic information system. The adjustment to our modeling objective resulted in a lack of mappable information on pelagic habitats. Instead, CTD data were used to calculate correlations between water temperature/salinity and larvae presence. Historic sediment data were successfully used to interpolate benthic habitat maps. Further sampling of habitat at actual location of cod presence would be useful in the future for increasing the accuracy of the analysis. The GIS of sampled life history stages was successfully overlaid with EFH designations (NMFS, 2005b) and analyzed to identify areas that coincide or supplement current determinations.

Social Goals:

- *Create supplemental income for fishermen through the participation of fishing businesses in data collection and analysis.*

The involvement of the local fishing community in this project was very successful. A total of 21 fishermen participated in the project; 14 of the 21 participants were small, local fishing businesses. A total of $119,050 was direct compensation to the fishing industry for participation in the project.

- *Continue to build new avenues and mechanisms for commercial fishermen to contribute to science and nurture the strong relationships created through past CCCHFA research coordination. These working relationships are crucial to building and maintaining support for the efforts of government scientists and fisheries managers.*

The commercial fishermen participating in the project strengthened their commitment to working with scientists on cooperative research projects. Of the 21 participants, 3 were new additions to our cooperative research team, thus increasing the impact of cooperative research in the region. The fishermen have voiced the desire to continue this work for multiple years, having understood the need for a time series of data in order to truly have meaningful results for management actions.

- *Overcome communications and perception barriers over data collection and usage between fishermen and government scientists by developing relationships based on trust and mutual understanding.*

This project was a good example of building trust and understanding between the fishermen and scientists. The merging of scientific sampling regiments and fishermen's expertise led to discussions that resulted in increasing mutual respect and innovative protocol that combined the scientist's random sampling grids with flexible sampling within the grids, to make use of the fishermen's knowledge.

- *CCCHFA strives to meet a new level of cooperative research by actively involving fishermen in research operations, rather than simply chartering their vessels as research platforms.*

Participating fishermen helped to develop protocol and were trained in the use of sampling techniques and data collection. Additionally, the technicians onboard the vessels assisting in data collection were members of the fishing industry. Not only were the vessels used as research platforms, but the fishermen conducted the science themselves once they were trained by the scientists.

Objectives:

- *Train 30 fishermen in the use of appropriate sampling nets (e.g. bongo nets) to obtain egg and larval samples, and the use of data loggers to record temperature and current.*

Six fishermen were trained in the use of bongo nets to obtain larval samples. This number was significantly lower than the original objective due to changes in participating vessels. Originally, we planned on using the hook and line fleet to tow the bongo nets; once we actually had a bongo net and understood deployment requirements, it was determined that hook and line vessels could not tow the nets. Instead, we recruited a scallop vessel and a quahog vessel that had the hardware required for towing the net (an A-frame/winch and wire). These two vessels, their crew, and industry technicians comprised the six trainees. In addition to learning how to deploy bongo nets and process the resulting plankton samples, they were also trained in deploying the flowmeter and CTD.

- *Train 30 fishermen in techniques necessary to sample cod for reproductive status, including collection and preparation of tissue samples (ovaries and testes).*

A total of twenty-seven fishermen were trained in adult spawning cod sampling. Of these, 22 were the captain and crew of 11 fishing businesses. Six were fishermen that were trained as industry technicians to assist in the sampling. The training involved protocol and image recognition in the office and hands on practice while fishing. The fishermen were able to identify the five stages of gonad development for each sex, determine the appropriate stage for sampling, collect a non-contaminated tissue sample, and preserve it in ethanol.

- *Starting during the reproductive season, fishermen will collect samples; initially eggs and larvae with bongo nets and later juveniles with trawls.*

Larvae and juvenile samples were successfully collected by the fishermen as described in the goal evaluation section. However, sampling began later than desired due to severe weather that prevented any on the water activity.

- *For all sampling sites, fishermen will record temperature profiles (surface to bottom) and current speed and direction.*

During larvae and juvenile sampling, a temperature profile was recorded by attaching a CTD to the tow/trawl apparatus. The CTD measured conductivity, temperature, and depth. Additionally, current direction was recorded on all datasheets during the tows. The purpose of collecting this current data was for generating the egg/larval dispersal models. Since our modelers ended up using pre-existing models to determine larval dispersal, our collected current data was not needed. Ultimately, we chose to not collect the current speed, since it required purchasing additional equipment and the data would not be used.

- *Generate and refine a model for egg/larval dispersal based on the water temperature and current data obtained from the data loggers, and the empirical knowledge of participating fishermen. This work will be a cooperative effort between commercial fishermen and scientists at the Environmental Coastal and Ocean Sciences Program at UMASS Boston.*

We changed this part of the project because the development of a new elaborate model would have been beyond the financial scope of the project. Instead we worked together with Dave Mountain and Jim Manning from NOOA/NMFS in Woods Hole who developed a model to predict larval dispersal based on released surface drifters. Before fishermen were about to go out to bongo for larvae and using trawl nets to catch juveniles we consulted the computer program to make predictions about sampling locations with high probability of catching success. However, the heavy storms at the beginning of the year 2005 made predictions very difficult. Based on surface drifter-models, larvae that had remained in the upper level of the water column were supposed to have drifted to Florida. According to Elizabeth Broughton, our sampling success was rather low compared to other years. These heavy storms and the resulting displacement might be the reason why we couldn't find many larvae.

- *Egg, larval and juvenile samples will be analyzed at MBL to determine size and stage of development.*

Forty-six larvae and 343 juvenile cod were collected and identified at the MBL by project staff and MBL interns. Analysis occurred in collaboration with Elisabeth Broughton (NOOA/NMFS). In addition to training the MBL staff in identification and sorting techniques, Elisabeth confirmed the positive identification of cod larvae and juveniles. Stage of development was determined by length, in comparison to prior research on cod development.

- *Adult, larvae and juvenile cod will be analyzed at MBL using micro satellites to track dispersal patterns of the Western George's Bank cod. Genetic identification of the fall spawning Western Georges Bank cod is a prerequisite to identifying the specific habitats utilized by its young, as it will allow us to track the dispersal of the appropriate eggs, larvae and juveniles.*

All together 168 spawning cod were sampled from western Georges Bank (WGB) over 3 years (38 in 2002/2003, 45 in 2003/2004, and 85 in 2004/2005) and 75 from eastern Georges Bank (EGB) during two years (62 in 2004 and 13 in 2005). We exceeded the proposed sampling schedule and included adult reproductive cod from Western Georges Bank from 3 years and WGB 2 years (instead of the proposed 1 year sampling). This allowed for evaluating temporal stability of the population structure of cod at WGB. We developed a set of new, much more informative markers and used one of them in our studies. This marker showed genetic differences that were up to 4 times higher than the old markers. Unfortunately - because of financial constraints – we could not test and use the other markers. The assignment of larval and juvenile cod was limited by low catch rates and the relatively small genetic difference between adult populations.

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- APPENDIX A: FIGURES -

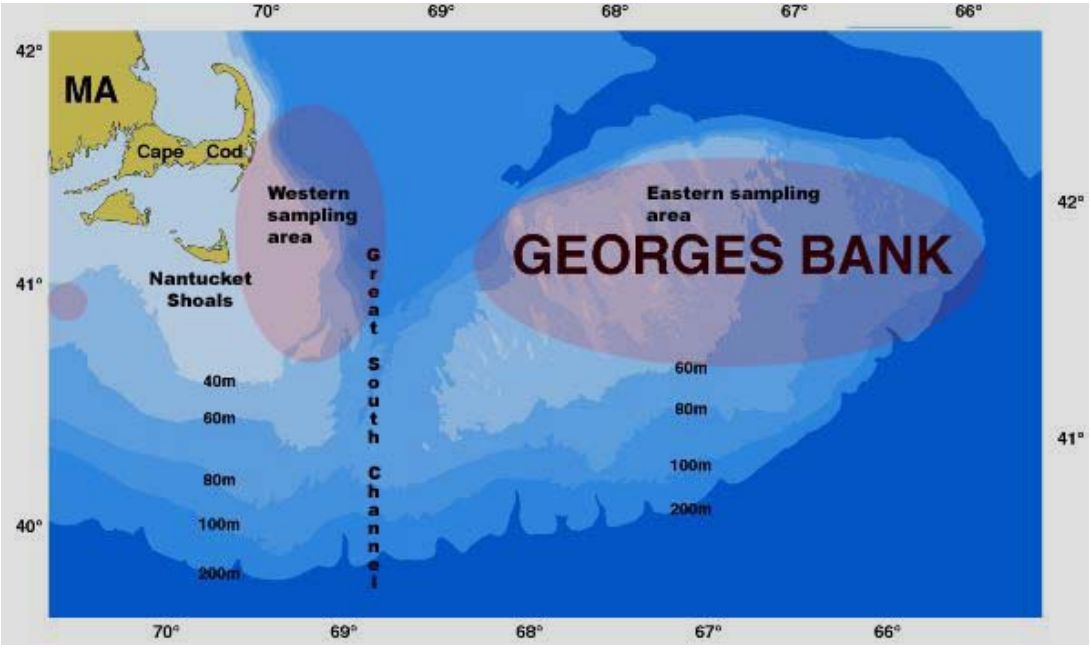

Figure 1. Map of adult sampling areas. Western and eastern Georges Bank sampling regions are shaded in pink.

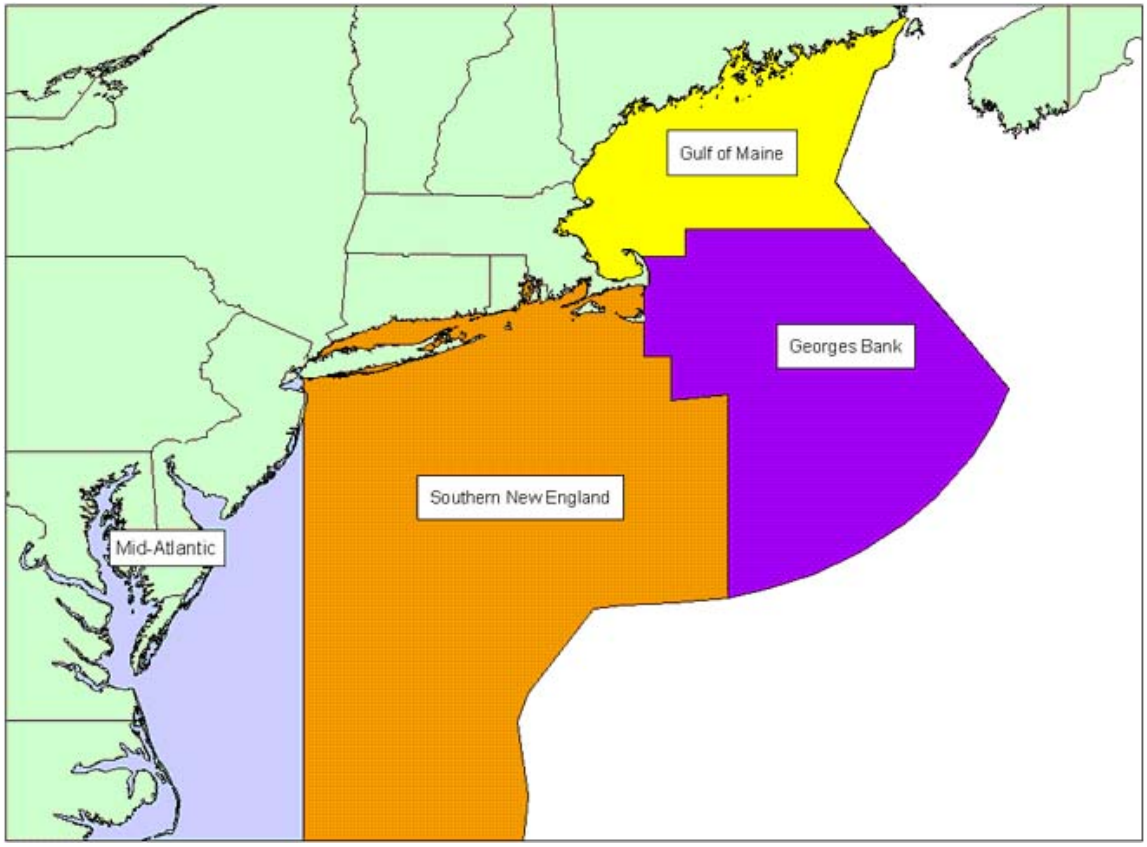

Figure 2. NOAA management areas. http://www.nero.noaa.gov/nero/fishermen/

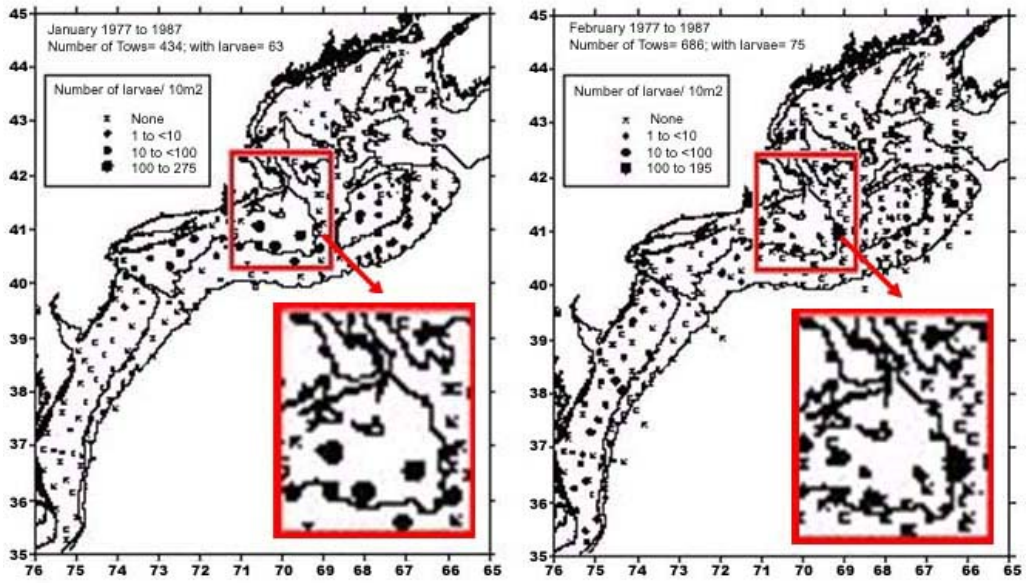

Figure 3. Map of NMFS larvae tows from 1977-1987 during January (left) and February (right). Red squares indicate the western Georges Bank and Nantucket Shoals region. Blank space on the maps indicates that the region was not towed. Dot size correlates to number of larvae found at a site; x indicates that a site was towed but no larvae were found (Fahay et al., 1999). [1]

[1] Used with permission of the author.

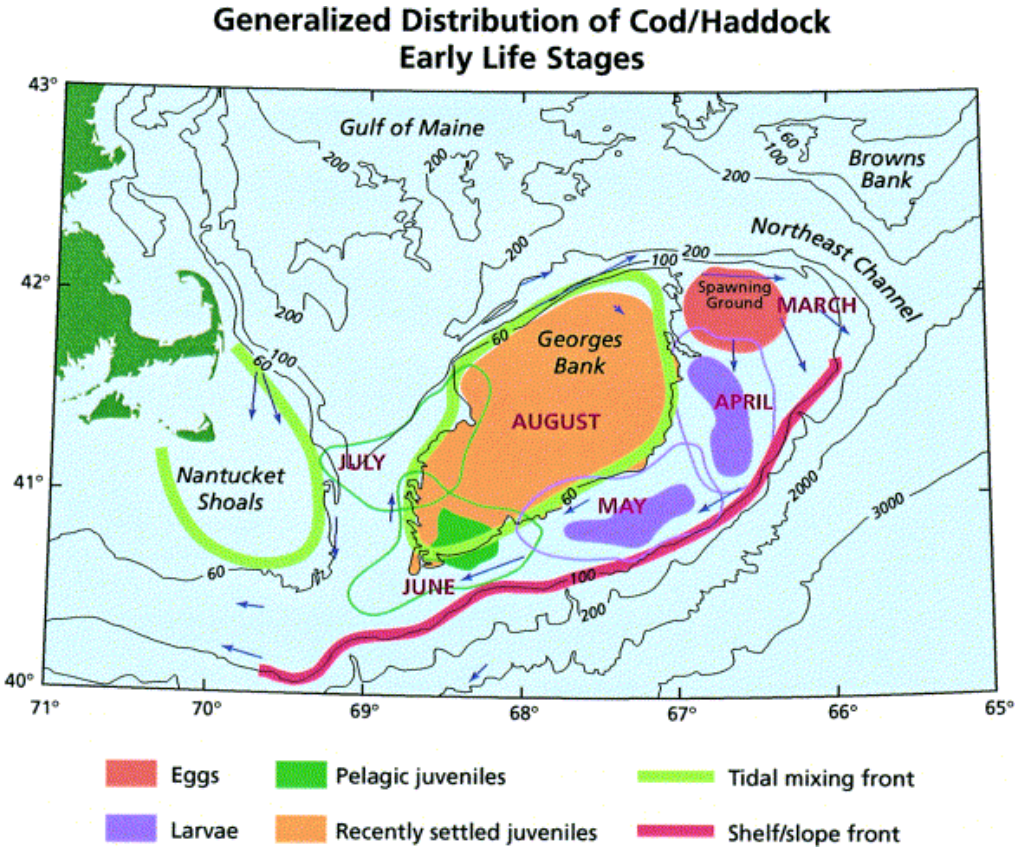

Figure 4. Map of generalized distribution of cod/haddock eggs, larvae, and pelagic juveniles during their first 3–4 months of life in the clockwise circulation over Georges Bank. Arrows represent the direction and relative speed of mean subsurface flow. (Lough & Manning, 2001)[2]

[2] Used with permission of the authors.

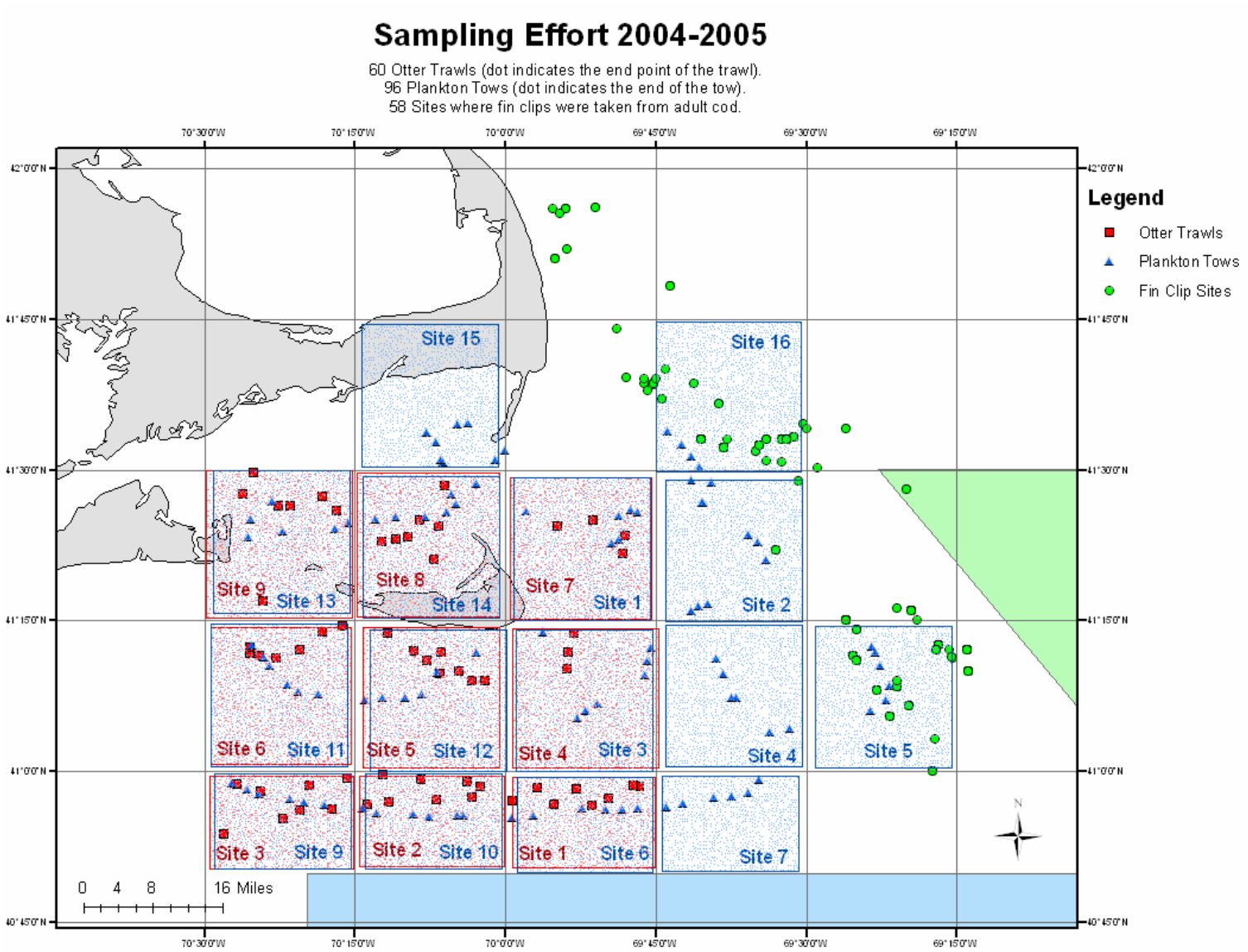

Figure 5. Map of adult, larvae and juvenile sampling areas. Plankton tows and otter trawls are indicated by blue triangles and red squares, respectively. Each marker indicates the end of a tow or trawl. Adult fin clip sample locations are indicated by green circles. The sampling grids are denoted by blue and red squares; blue indicating the larvae grid and red indicating the juvenile grid.

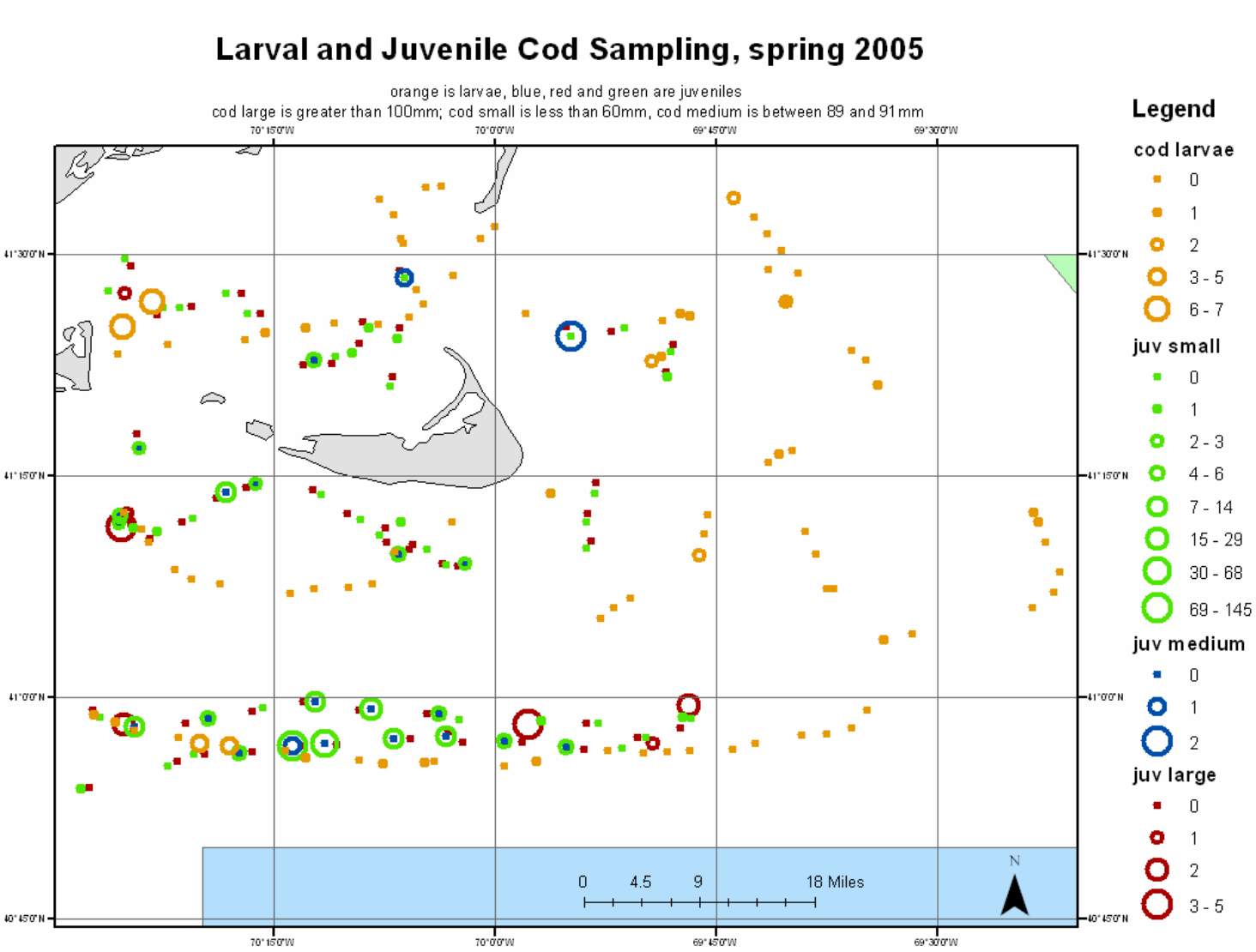

Figure 6. Map of larvae and juvenile sampling results. Each site is represented by a box on the grid. See Figure 5 for corresponding site numbers. Juveniles of different size categories are represented in red, blue and green. Larvae are represented in orange. The number of larvae or juveniles captured per tow/trawl is represented by bubble size.

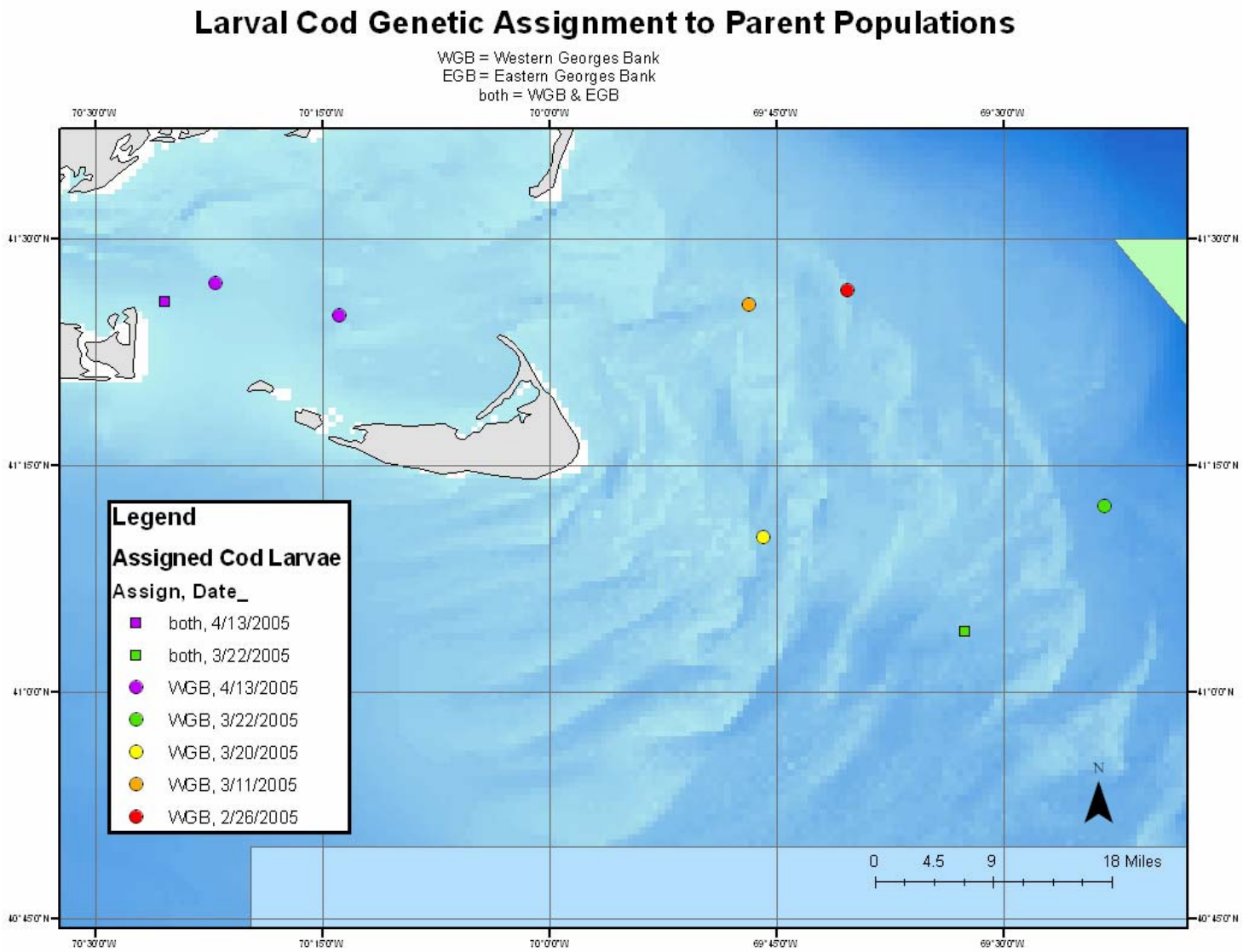

Figure 7. Map of larval genetic assignments. Each marker is a single cod larvae that was successfully assigned back to a parent population, either Western Georges Bank (WGB), Eastern Georges Bank (EGB) or both populations. The colors indicate date of sampling.

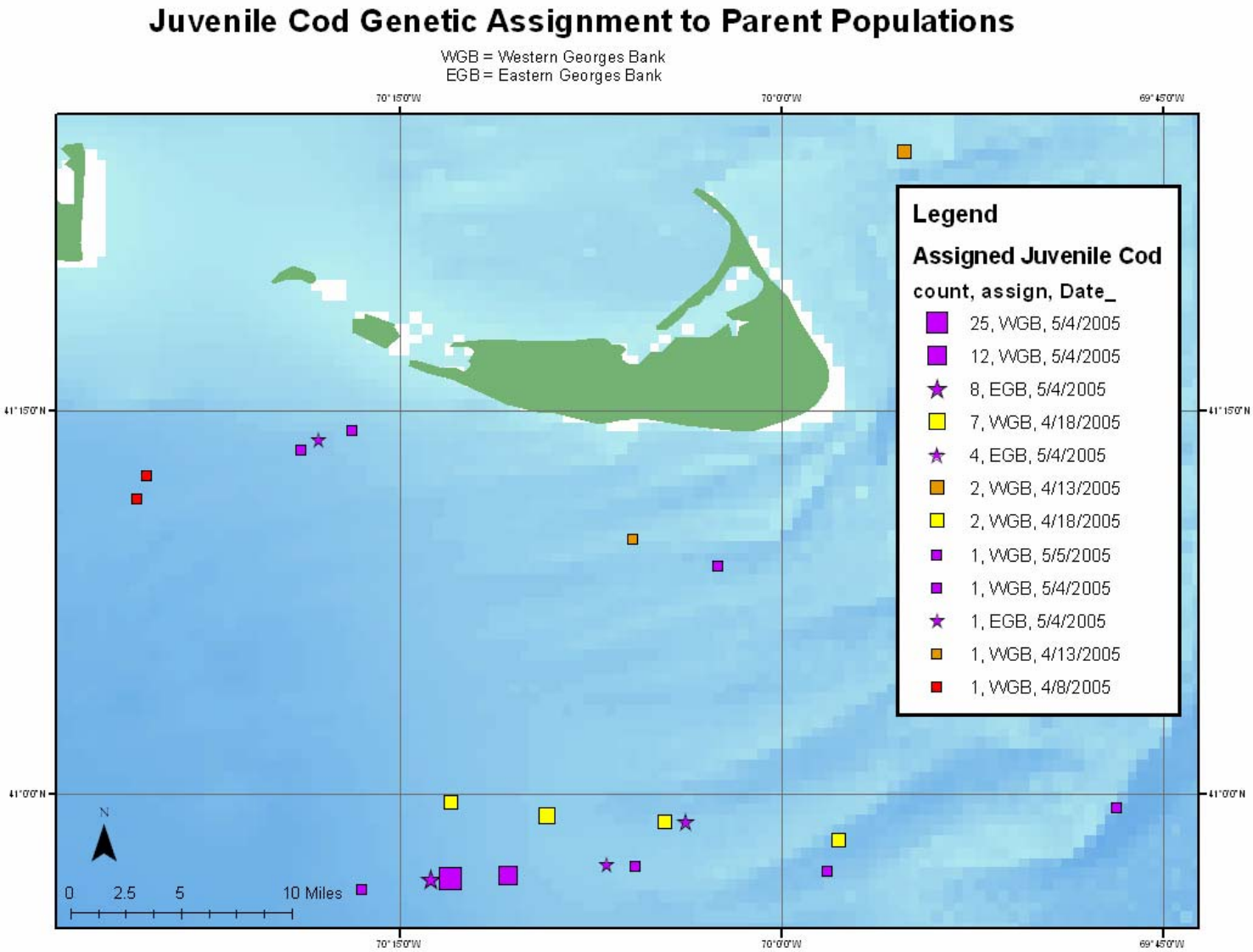

Figure 8. Map of juvenile genetic assignments. Each marker is a trawl that collected cod juveniles that were successfully assigned back to a parent population, either Western Georges Bank (WGB, square markers), Eastern Georges Bank (EGB, star markers). The colors indicate date of sampling and the size of the marker indicates the quantity of juveniles collected and assigned.

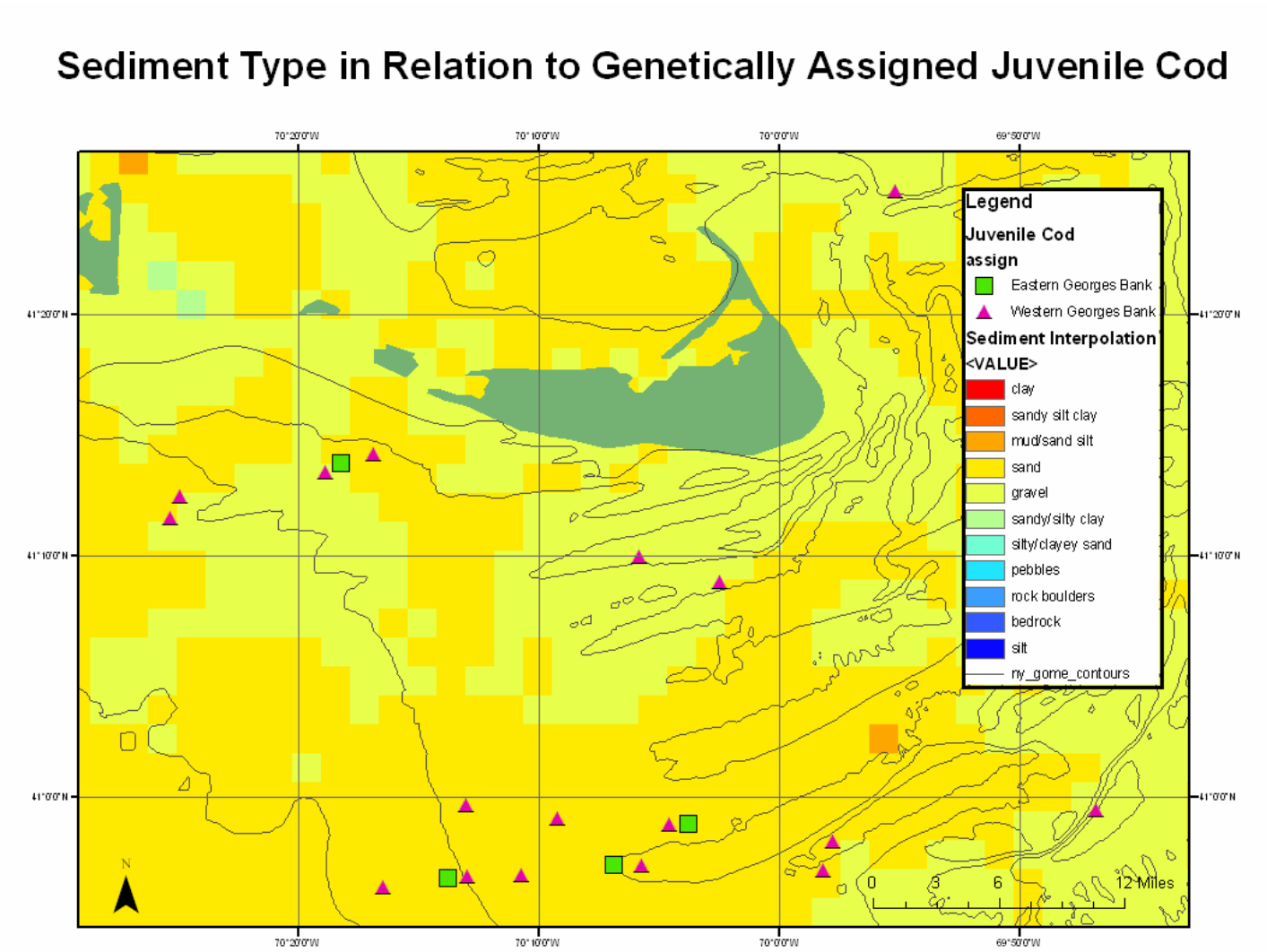

Figure 9. Map of Interpolated Sediment Types and Genetically Identified Cod Juveniles. Each marker indicates a trawl location that resulted in a juvenile that was genetically assigned to a parent population. Juveniles from Eastern Georges Bank are indicated by the green squares; juveniles from Western Georges Bank are indicated by the pink triangles. The grey lines are 10 meter contours. All samples were collected in a sediment habitat composed of sand or gravel.

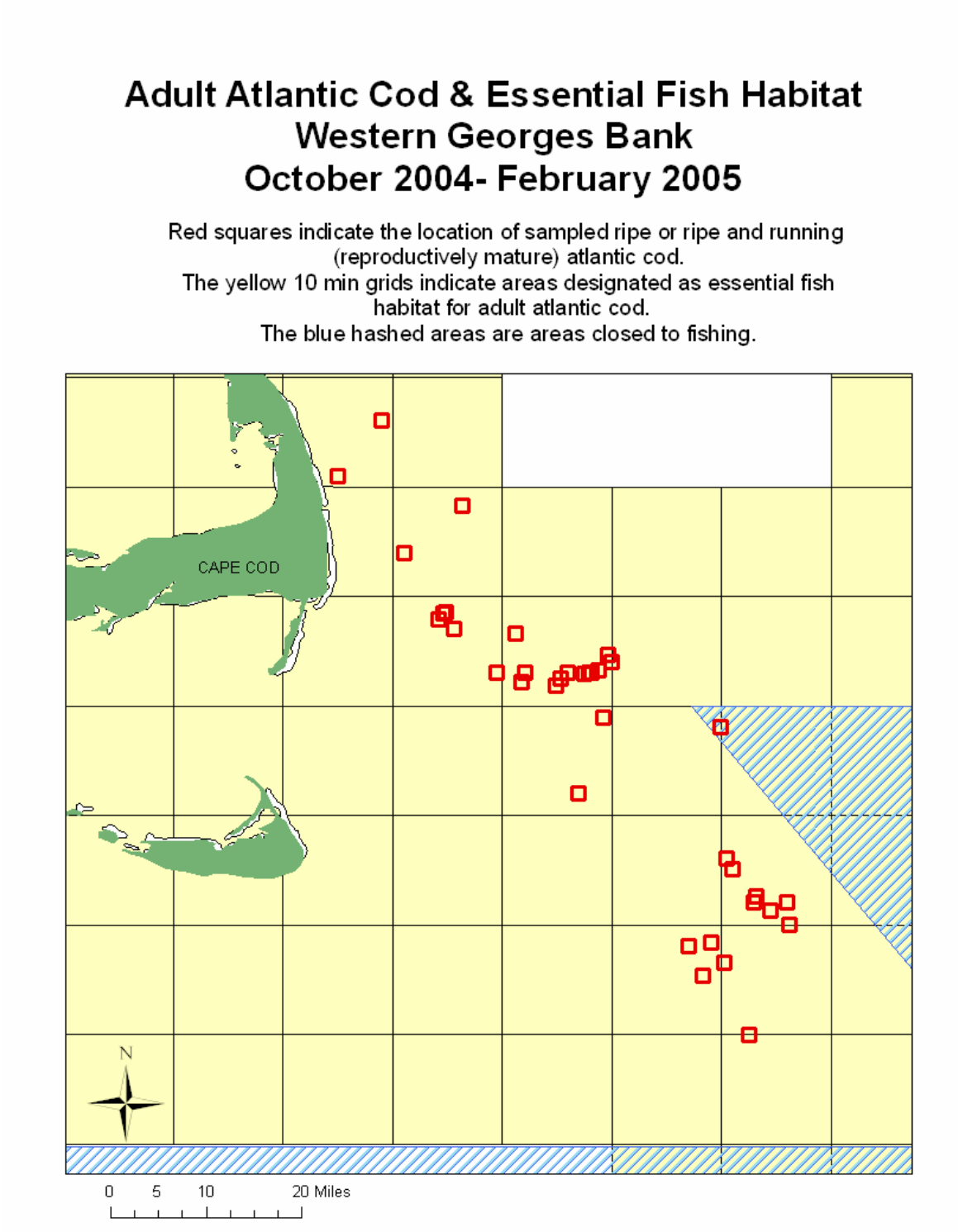

Figure 10. Map of Reproductively Mature Atlantic Cod Samples and Essential Fish Habitat. Each marker indicates a jig location that resulted in an adult that was ripe or ripe and running. The yellow squares are classified as essential fish habitat, according to the New England Fisheries Management Council.

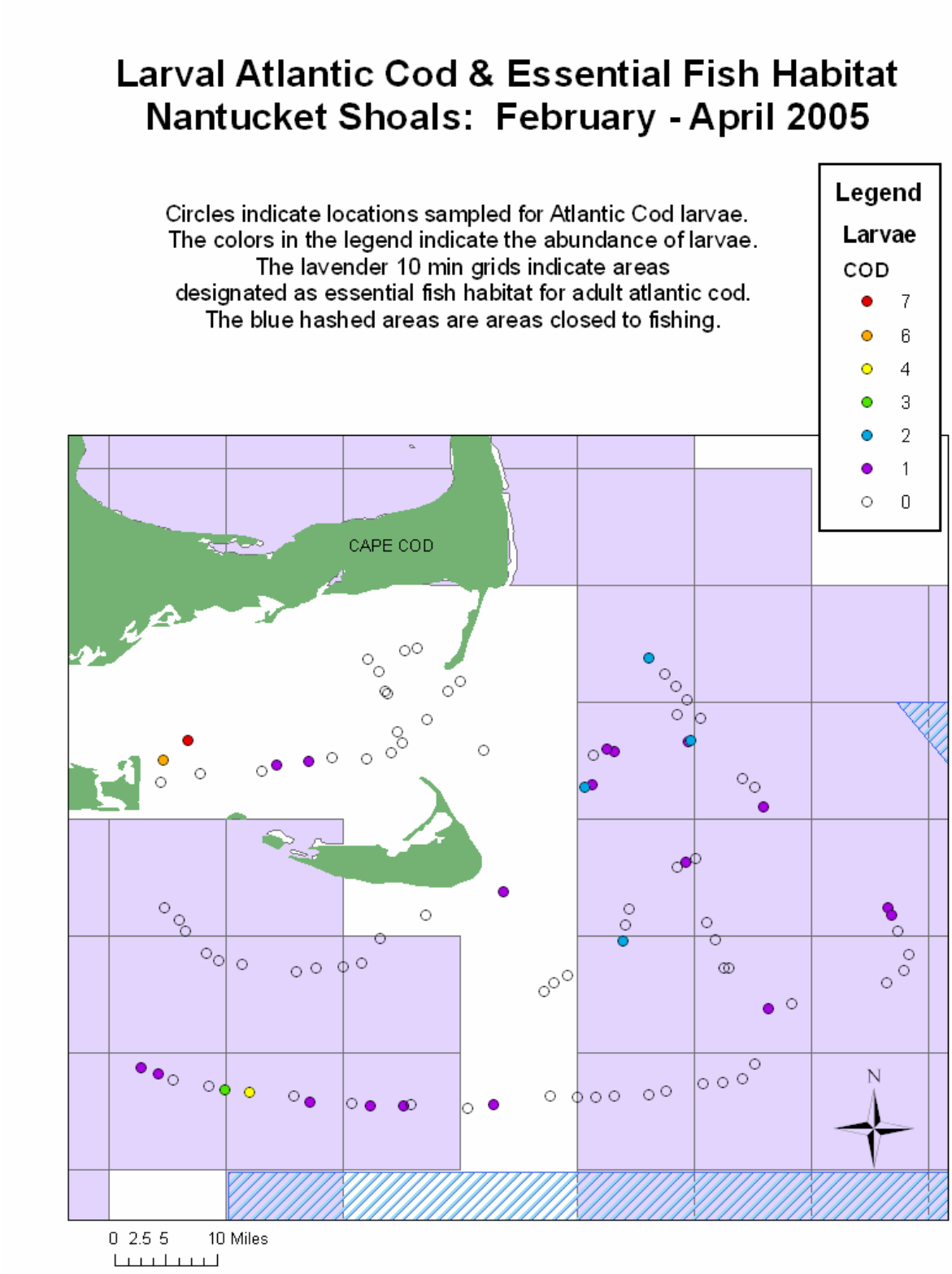

Figure 11. Map of Larval Atlantic Cod Samples and Essential Fish Habitat. Each marker indicates a bongo tow location; colored circles indicate the quantity of larvae collected. The lavender squares are classified as essential fish habitat, according to the New England Fisheries Management Council.

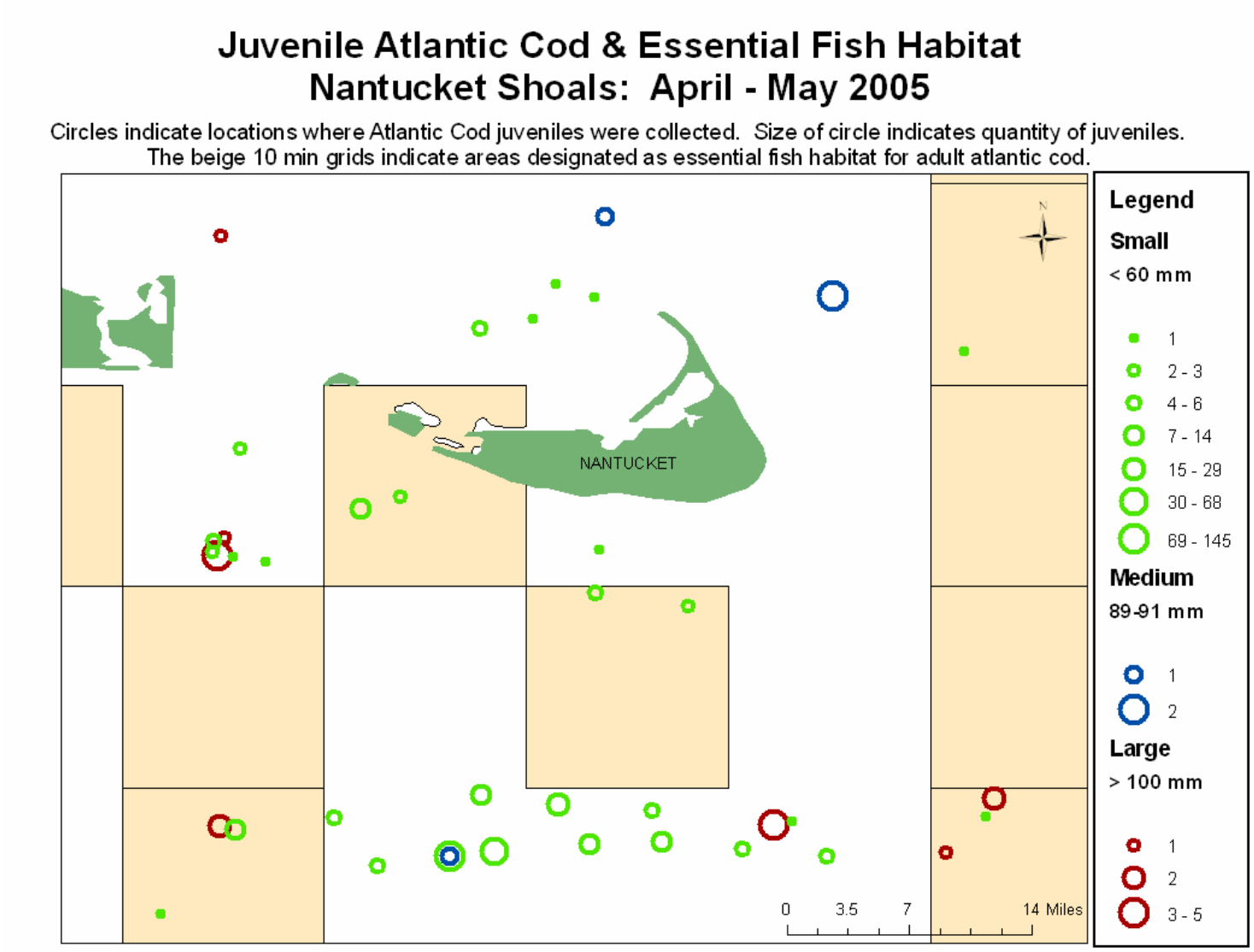

Figure 12. Map of Juvenile Atlantic Cod Samples and Essential Fish Habitat. Each marker indicates a benthic trawl location where juveniles were collected. The colors indicate the size of the juvenile while the size of the circles indicate the quantity collected. Small juveniles are less than 60mm in length (green), medium juveniles are between 89 and 91mm in length (blue), large juveniles are greater than 100mm in length (red). The beige squares are classified as essential fish habitat, according to the New England Fisheries Management Council.