

**Research on the biology of sand lance in Massachusetts Bay and Stellwagen Bank
Final technical report to the Northeast Consortium**

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ABSTRACT

Sand lances are a key link in the complex food webs of Stellwagen Bank and Massachusetts Bay. The purpose of this work was to learn more about the ecology of two species, *Ammodytes dubius* and *A. americanus*, through development of efficient sampling gear and effective husbandry techniques, and through comparisons of diets. The work produced a proven means of systematically capturing live sand lance for study. Methods of transporting sand lance after capture and husbandry techniques were refined and culminated in the exhibition of live sand lance at the New England Aquarium. Diet analyses revealed that *A. americanus* and small *A. dubius* eat similar prey items. Large *A. dubius* eat larger prey items, including predatory Hyperiid amphipods. Additional outcomes were achieved: morphological measurements to discern between the two species, an understanding of their prey capture mechanism, novel observations of sand lance behavior in the wild and in captivity, and new insight into fungal pathogens that proliferate in captivity. This work has led to a long-term collaboration among Boston University, our two research/fishing vessel captains, the Stellwagen Bank National Marine Sanctuary, the Whale Center, and the New England Aquarium to further explore questions related to the ecology of upwelling zones on Stellwagen Bank/Massachusetts Bay. Additionally, a second important outcome of this work has been the decision by COMPASS in collaboration with the Massachusetts Ocean Partnership to declare Stellwagen Bank-Mass Bay to be Pilot Zone 1 of a forage fishes modeling experiment in the development of tools to support ecosystem-based management.

FINAL TECHNICAL REPORT TO THE NORTHEAST CONSORTIUM: RESEARCH ON THE BIOLOGY OF SAND LANCE IN MASSACHUSETTS BAY AND STELLWAGEN BANK

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1. INTRODUCTION

For several years, Boston University, The Massachusetts Fishermen's Partnership, The New England Aquarium and MIT, in collaboration with fishing vessels from Rockport and Provincetown, have been engaged in the study of two small silvery fishes that school in hordes in the shallow waters of Massachusetts Bay and the Gulf of Maine. They are known as sand lances or sand eels, and locally comprise two species in the genus *Ammodytes*. Sand lances abound in nearshore waters over much of the world, usually feeding on zooplankton by day and retiring to sandy habitats to rest and refuge from predators by night. Our two local species are *Ammodytes americanus*, found inshore, and *A. dubius*, dominant offshore. That is a good part of what we knew about our local sand lances prior to this study. Support to launch this study came from a Northeast Consortium cooperative fishery research grant, and currently continues with modest support from The Stellwagen Bank National Marine Sanctuary, Boston University, The New England Aquarium, and most important the fishing boat Captains Bill Lee and Phil Michaud themselves.

Stellwagen Bank is a plateau blanketed by shallow waters and bordered by a steep drop into the deeper waters of Stellwagen and Wilkinson Basins. When moving water encounters the wall made by the bank, it creates upwelling of deep nutrient rich waters to the surface, culminating in plankton blooms in the photic zone. This explosion of productivity flows through all of the trophic levels from phytoplankton to top predators such as bluefin tuna, cod, sea birds and marine mammals. Sand lances are a key and often overlooked link in this productive food. Sand lances are an integral part of a complex food web and function in roles other than that of prey. They are thought to compete for available food with gadoids and herring and also feed on their eggs and larvae (Hendrickson 1979, Rankine and Morrison 1988). In turn, adult gadoids, mackerel, and herring (Bowman et al. 1984, Fogarty et al. 1991) are predators of sand lance. Studies have shown that when numbers of predators such as herring or cod decrease due to over fishing sand lance abundance tends to increase (Bowman et al. 1984, Fogarty et al. 1991, Furness 1982, Hempel 1978a, 1978b, Licciardello et al. 1985, Payne et al. 1990, Sherman et al. 1981, Winters 1983) and consequently they may become a more abundant food source for other predators (Robards et al. 1999). Sand lance abundance influences the numbers and locations of predators, having been shown to affect seabird reproductive success (Robards et al. 1999), humpback whale distributions in the Gulf of Maine (Payne et al. 1986, Wienrich et al. 1997), and to negatively correlate with right whale abundance. It is thought that sand lance may compete with right whales for their prey (Kenney et al. 1986, Payne et al. 1990). Many important piscivorous marine animals feed heavily on sand lance including humpback whales and other

cetaceans, gadoids, flatfishes, salmonids, bluefin tuna, herring, cephalopods, seals and many seabirds (see Robards et al. 1999 for a summary). Sand lances have been called the "quintessential forage fish" and possibly "the most important taxon of forage fish in the Northern Hemisphere" (Springer and Speckman 1997). Sand lances act as a conduit of energy from the primary producers all the way to the highest trophic levels. They are a high quality forage fishes, more rewarding in energy density compared to small gadoids and herring of the same size. They are especially rewarding prey for summer-foraging predators (Robards et al. 1999) such as humpback whales, which migrate to the Stellwagen Bank region in the summer and may feed on them when they reach their peak energy density before spawning. The sand lances are also the preferred prey of many nesting seabirds including the endangered roseate tern.

Historically, the distinction between the two species of sand lance in the Gulf of Maine has been unclear. It has been argued there may only be a single species (Jensen 1941, 1944). Nizinski et al. (1990) defined each species' diagnostic meristics and concluded that there are indeed two species in the western North Atlantic. *A. americanus* tend to be found in shallow coastal waters from Delaware to Labrador. *A. dubius* is the offshore species typically found in deeper water ranging from North Carolina to Greenland. This distinction in habitat suggests that the two species, long thought to be one, may fill unique niches in the Gulf of Maine food web.

Sand lances inhabit areas with sandy substratum in which they burrow for refuge. Preferred habitat is loose sand with little silt or mud in which they can easily burrow, in which bottom currents sufficiently aerate interstitial water in the sand (Auster & Stewart 1986, Robards et al. 1999). They are most common above 50 meters depth, but range to 100 meters, and one specimen of *A. dubius* was collected from a depth of 400 meters (Robards et al. 1999). Sand lance larvae feed on phytoplankton and increasingly more and larger zooplankton as they grow (Auster & Stewart 1986, Monteleone & Peterson 1986, Robards et al. 1999). Zooplankton makes up the majority of the adult sand lance diet, being shown to comprise 80% of gut contents by volume (Garrison & Link 2000). More specifically, copepods are the primary prey item for sand lance, found to comprise 41.4% of the adult sand lance diet by weight in *A. americanus* (Meyer et al. 1979). Other prey include epibenthic invertebrates, gammarid amphipods, harpacticoid copepods, and larval fish (Robards et al., 1999) such as herring (Rankine and Morrison 1988). It is unknown if these prey are only rare occurrences or a regular part of the sand lance diet. Epibenthic invertebrates are more prevalent in gut contents during the fall and winter likely because pelagic prey species are less available (Robards et al. 1999).

It behooves us to understand the ecology of sand lance due to the importance of this fish to the abundance and location of many valued species, and to the stability and diversity of the Stellwagen ecosystem. The purpose of the work described in this report was to learn more about the ecology of the two species, *A. dubius* and *A. americanus*, through development of efficient sampling gear, development of effective husbandry techniques for keeping sand lances alive for laboratory study, and comparisons of feeding habits and behavior based on gut contents and stable isotope analyses.

2. PROJECT GOALS and OBJECTIVES

The overarching goal of this project was to profile the ecological niches of the two species, *A. dubius* and *A. americanus*, through field observations and laboratory analyses.

The objectives for this project were three-fold:

- to develop gear that could capture sand lance alive and uninjured as well as measure abundance in a quantitative and replicable manner;
- to test the hypothesis that *A. dubius* and *A. americanus* can be considered as a single taxonomic unit for management purposes, as they fill identical ecological niches; and,
- to develop husbandry methods that could keep sand lance alive in transport and captivity for long-term behavioral studies.

3. PARTICIPANTS

*Les Kaufman, Principal Investigator and professor at Boston University (BU)

*Capt. Bill Lee, captain of the F/V *Ocean Reporter* and co-PI

*Capt. Phil Michaud, captain of the F/V *Susan C III*

*Cliff Goudey, professor at Massachusetts Institute of Technology (gear technology)

* David Bergeron and Olivia Free, Massachusetts Fishermen's Partnership

Bill Murphy, NEAQ research scientist and aquarist

Briana Brown, graduate student at BU

Kathryn Kovitvongsa, graduate student at BU

Andrew Fogel, undergraduate student at BU

Clare Hansen, undergraduate student at BU

Justin Scace, lab manager, BU Marine Program

* indicates participants who played key roles in project design and implementation

4. METHODS

Sampling gear development

Gear development, trials and modifications were carried out by Captain Bill Lee of Rockport, MA on his vessel, the *RV Ocean Reporter* and by Captain Phil Michaud of Provincetown, MA on his vessel, the *F/V Susan III*. Potential sand lance hotspots were searched for using an aerial survey of Cape Cod Bay, Stellwagen Bank, Provincetown coastal areas, and Massachusetts North Shore coastal areas. Additionally, these areas were scanned with underwater video cameras. Three locations were found to harbor sand lance in abundance: the northwest corner of Stellwagen Bank (Figure 1), inside the Stellwagen Bank National Marine Sanctuary, Provincetown Harbor, and Race Point (Figure 2), in Provincetown, Massachusetts. Three types of gear were tested to catch sand lance in these locations: minnow traps, a six foot square drop frame net (Figure 3), and a modified shrimp beam trawl. The minnow traps were tested in two locations, Rockport Harbor and Provincetown Harbor. In Rockport, a vertical array of unbaited minnow traps was tested. Two traps were on the seafloor, with a trap at 10 foot intervals up to the surface, for a total of 11 traps. In Provincetown, a horizontal array was tested. Traps were arranged in a lobster-trawl fashion on the seafloor for a five-hour soak interval. The drop frame net was designed by Cliff Goudey to catch schools of sand lance and was tested in the northwest corner of Stellwagen Bank. The shrimp beam trawl was modified by Capt. Lee. The beam was shortened from 17 feet to 7 feet wide. The net is lined with 2 mm mesh. The cod end is also lined with 2mm mesh. The net mouth is 27 inches high; it is reduced at a 2:1 ratio to the cod end. The trawl was further modified after initial trials, as will be detailed in the "Sampling gear development" sub-section of the "Results".

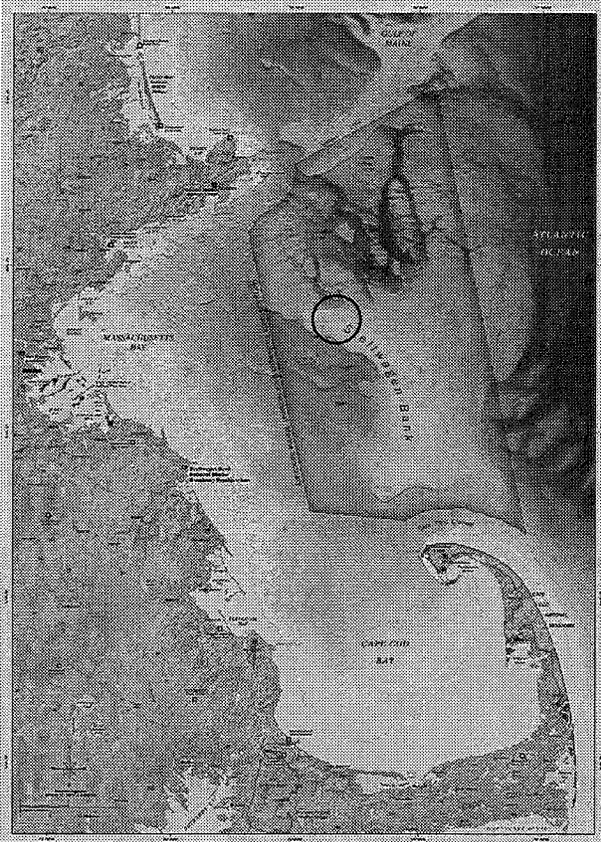


Figure 1. Stellwagen Bank National Marine Sanctuary is outlined in red. The northwest corner of Stellwagen Bank is circled in black.

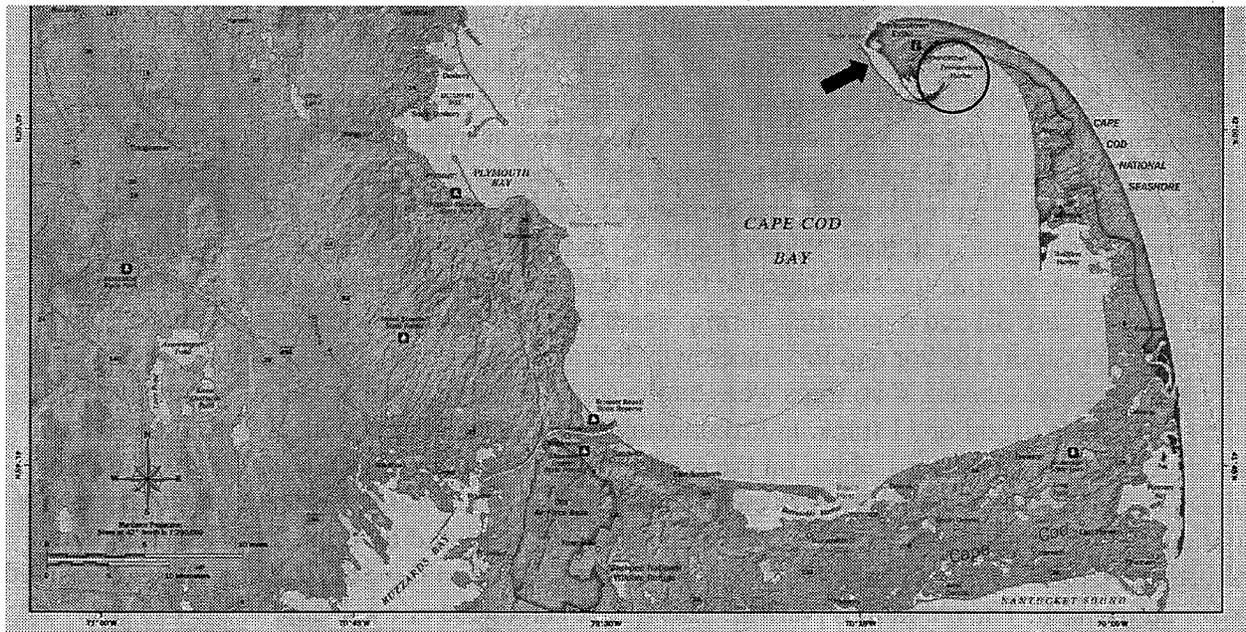


Figure 2. Provincetown Harbor is circled in black. The black arrow is pointing to Race Point. (Figure deleted from this version to meet email server limitations).

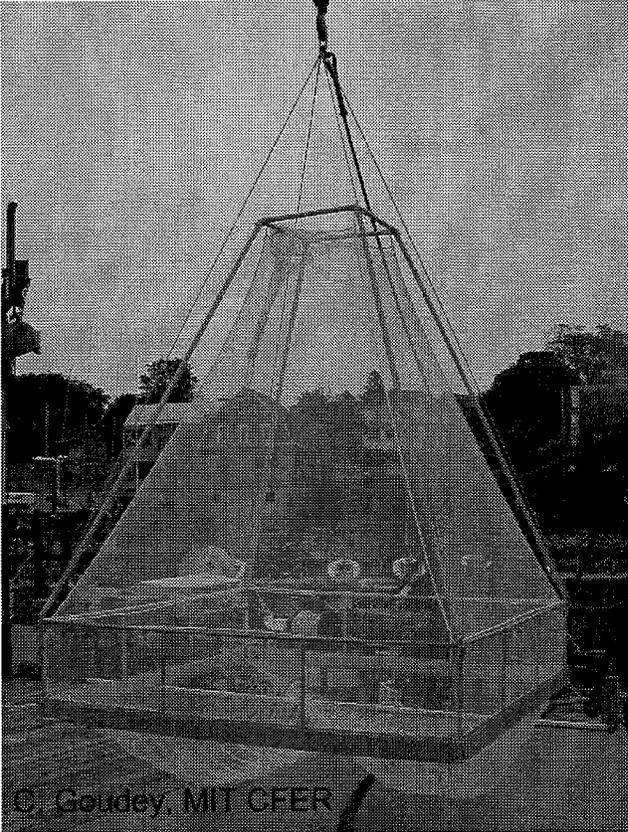
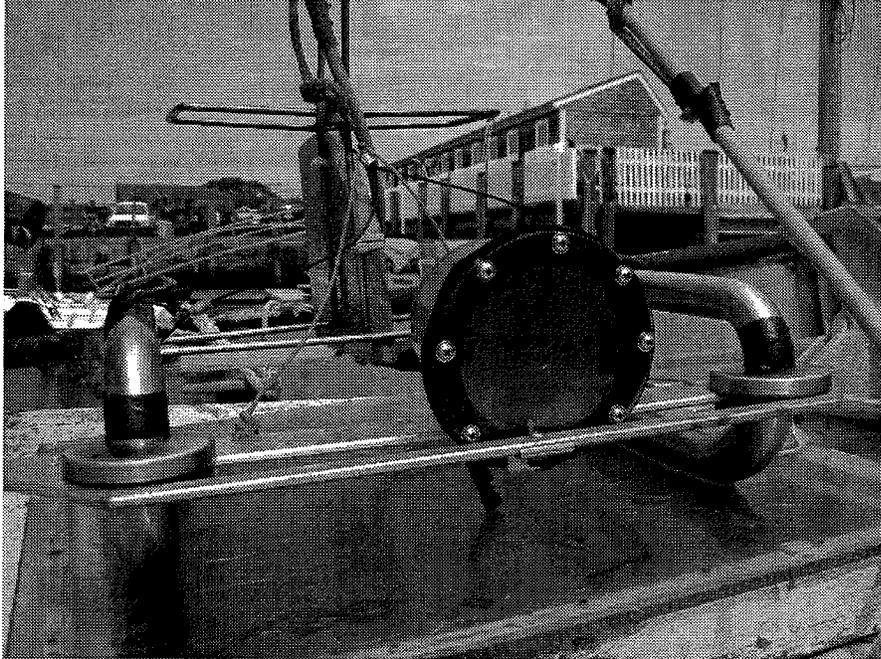


Figure 3. This is the 6 ft square drop net designed by C. Goudey for use in sampling schools of sand lance.

Field sampling

Sand lance habitats of sandy bottoms with sand waves in each sampling area (northwest corner of Stellwagen Bank, Provincetown Harbor, and Race Point) were located using a Hummingbird 1197c GPS Fishing System. An underwater video camera (designed by Bill Lee; Figure 4) with feed to the surface was used to groundtruth the side-scan sonar results. Once suitable habitat and sand lance were located with the camera, the modified shrimp beam trawl was deployed. Video cameras were attached to the trawl to show the net mouth and forward of the net during towing. This system was put into place to monitor catch numbers per tow and to ensure that sand lance were not being damaged by the net. The net was towed at speeds between 1.7 and 2.4 knots. Individuals that were uninjured by the catch process were kept for rearing and laboratory study (see *Husbandry* section of Methods). Catch mortalities were frozen on ice immediately following catch sorting. Frozen samples were transported to BU and stored at -30°C or preserved by fixing in 37% formalin (diluted with seawater) followed by a series of ethanol baths (10%, 30%, 50%), with a final storage in 70% ethanol solution.

Figure 4. Underwater camera in housing designed by Bill Lee.



Husbandry

Following a sampling tow, live sand lances were housed in a cooler with sea water and an air source. They were transported to the New England Aquarium and the marine laboratory at Boston University (BU). At the BU marine laboratory, approximately 150 individuals were housed in a 50 gallon salt water aquarium. Salinity was maintained at 1.0235 ± 0.015 ppt. Water temperature was kept at 16°C in a cold-storage room. At the New England Aquarium, larger numbers of sand lances were housed in a circular 1500 gallon tank. Two large trays, eight to ten inches deep and filled with filter sand, were on the bottom of the tank. The trays were used to provide cover for the sand lances to swim into and behave normally. The water was chilled to 55 F and had a constant trickle of new water to turn the water over, thus water changes weren't necessary. Sand lances were fed live adult brine shrimp initially to get them actively feeding and then they were switched to a diet of frozen zooplankton that was thawed out and fed them this several times a day. The bottom of the tank was cleaned on a regular basis to keep the water quality at optimal levels and to remove any uneaten food and fecal matter. The aquarium system had a Rapid sand filter, a UV sterilizer, and a heat exchanger. Additionally, there was a pump attached to the tank with an under gravel filter.

Morphometrics

Two independent studies were done by students to compare morphological characteristics of *A. dubius* and *A. americanus*. Sand lances used for the first study were obtained frozen from beam trawl surveys conducted in September 2005 near Provincetown, MA. The frozen sand lance were either thawed and refrigerated or subjected to an alcohol series for preservation (as described in the *Field sampling* section of Methods). Total length, standard length, fork length, head length, eye diameter, distance from snout to first dorsal ray, and distance from snout to anus were measured on each of 141 individuals. For comparative analysis, measurements were converted into ratios of standard length. The original measurements were also used to determine

caudal fin length and create ratios of eye length over head length, length from the snout to the first dorsal ray over the snout to the anus, head length over the distance from the snout to the first dorsal ray, and head length over length from the snout to the anus. The effectiveness of external characters as proxies for differentiating between species were then examined using the accepted meristics established in previous studies as a basis for separation. This was done by identifying 49 individuals as either *A. dubius* or *A. americanus* using counts of lateral plicae (distinctive rows of oblique folds in skin). Plicae extending from the area above the pectoral fin base to the caudal peduncle were counted. The number of plicae on each side of each fish was determined and the average of the two values calculated. *A. americanus* has between 106 and 126 plicae while *A. dubius* has between 124 and 147 (Nizinski et al 1990). We categorized individuals in our sample set as *A. americanus* if the average plicae count was below 125 and *A. dubius* if the average plicae count was 125 or above. The statistical program JMP 5.1.1 was used to perform a discriminant analysis to determine which of the external aspects could be used most reliably to assign individuals to either *A. americanus* or *A. dubius*.

The second study utilized preserved specimens caught and preserved during field sampling in October 2007, in the northwest corner of Stellwagen Bank and Provincetown Harbor. All specimens caught on Stellwagen Bank are of the species *A. dubius* and all specimens caught in Provincetown Harbor are assumed to be of the species *A. americanus*, due to their location in shallow, inshore waters and their plicae counts. Measures of total length, standard length, fork length, eye diameter, head length, snout length, distance from snout to first dorsal ray, and distance from snout to anus were taken. Counts were taken of plicae on the right side of the body, dorsal fin and anal fin rays, left side pectoral fin rays, and gill rakers on the first arch of the right side. The statistical program JMP 5.1.1 was used to perform a discriminant analysis to determine which of the external aspects could be used most reliably to assign individuals to either *A. americanus* or *A. dubius*.

Feeding behavior

Feeding behavior was described from underwater video obtained during field sampling and from laboratory observations including hide-speed video analysis of wild-caught *Ammodytes spp.* Under-water video was taken by cameras on the trawl net as described above. Laboratory videos of feeding were taken with a Fastec Imaging TroubleShooter high-speed camera. The camera captured video at a rate of 250 frames per second (fps) at a screen resolution of 640 x 480 pixels. Lighting for filming was provided by six 100 watt incandescent lamps surrounding the filming tank. The filming tank was a 25 gallon salt water tank with conditions maintained as described in the *Husbandry* methods. Digital video sequences were analyzed using ImageJ software (<http://rsb.ino.nih.gov/ij/>). Sequences were analyzed if they captured the prey and the attack, and showed the sand lance individual in a plane perpendicular to the camera lens.

The filming tank contained a movable tank divider, which was used to constrain available feeding area. A range of three to 12 individuals were placed in the feeding tank during video trials. Frozen krill were used as the target prey. Prior to trials, krill were defrosted in a sample of water from the filming tank. The krill were added to the filming tank by pipette, three to 20 at one time. Following consumption of all prey items or the loss of feeding behavior by the fish, remaining prey items were allowed to sink to the tank floor. More krill were then added to the

tank so that feeding would resume. When feeding ceased to take place at all, fish were assessed to be sated or uninterested in the prey and filming was suspended until the following day.

Multiple criteria were measured for each complete filmed sequence: head length, total length, head length with maximum jaw protrusion, maximum mouth opening, size of prey, distance from the prey when mouth began to open, and the angles between the head and body before, during, and after the attack.

Diet composition

Individual sand lances from the three sampling seasons (2006, 2007 and 2008) were sub-sampled at random to represent the range of sizes represented in each catch. Standard length and muscle tissue were collected for each selected individual. Muscle tissues and invertebrate samples were dried at 60°C for 48 hours and ground using a mortar and pestle in preparation for stable isotope analyses. Samples were then processed by the Boston University Stable isotope laboratory according to their protocol (<http://www.bu.edu/sil/quality.htm>).

5. DATA

Morphometrics

Table 1a. Data from the first morphometrics study. SL=standard length, TL=total length, Eye=eye diameter, HL=head length, CFL=caudal fin length, Sn-DR=snout to first dorsal ray, Sn-A=snout to anus, E/H=eye to head length ratio, H/SL=head length to standard length ratio, D/SL=snout to first dorsal ray measure ratio with standard length, A/SL=snout to anus length to standard length ratio, H/D=head length to snout to first dorsal ray length ratio, H/A= head to anus length to standard length ratio.

ID	Plicae	Measures							Ratios					
		SL (mm)	TL (mm)	Eye (mm)	HL (mm)	CFL (mm)	Sn-DR (mm)	Sn-A (mm)	E/H	H/SL	D/SL	A/SL	H/D	H/A
102		119	129	3	25	10	34	77	0.12	0.21	0.29	0.65	0.74	0.32
103		117	128	3.5	24	11	33	77	0.15	0.21	0.28	0.66	0.73	0.31
104		115	125	3.5	25	10	33	75	0.14	0.22	0.29	0.65	0.76	0.33
106		114	123	4	23	9	30	75	0.17	0.20	0.26	0.66	0.77	0.31
108		115	124	3	25	9	33	74	0.12	0.22	0.29	0.64	0.76	0.34
109		115	125	4	25	10	31	74	0.16	0.22	0.27	0.64	0.81	0.34
110		119	128	4	25	9	30	75	0.16	0.21	0.25	0.63	0.83	0.33
112		118	125	3	27	7	33	76	0.11	0.23	0.28	0.64	0.82	0.36
203		108	115	3	22	7	33	70	0.14	0.20	0.31	0.65	0.67	0.31
204		115	125	4	25	10	33	73	0.16	0.22	0.29	0.63	0.76	0.34
205		110	119	3	24	9	32	70	0.13	0.22	0.29	0.64	0.75	0.34
207		116	125	3.5	24	9	32	77	0.15	0.21	0.28	0.66	0.75	0.31
211		110	120	3	23	10	29	70	0.13	0.21	0.26	0.64	0.79	0.33
215		108	118	3	22	10	30	71	0.14	0.20	0.28	0.66	0.73	0.31
216		119	129	3	25	10	32	74	0.12	0.21	0.27	0.62	0.78	0.34
217		114	125	3	21	11	34	75	0.14	0.18	0.30	0.66	0.62	0.28
219		117	128	3	25	11	32	74	0.12	0.21	0.27	0.63	0.78	0.34
220		110	119	3	24	9	30	71	0.13	0.22	0.27	0.65	0.80	0.34
221		119	129	3	24	10	33	76	0.13	0.20	0.28	0.64	0.73	0.32
222		105	114	4	23	9	31	65	0.17	0.22	0.30	0.62	0.74	0.35

223		112	121	3	25	9	29	70	0.12	0.22	0.26	0.63	0.86	0.36
225		117	125	3	24	8	33	75	0.13	0.21	0.28	0.64	0.73	0.32
226		117	125	3	25	8	34	76	0.12	0.21	0.29	0.65	0.74	0.33
227		118	126	3	24	8	32	73	0.13	0.20	0.27	0.62	0.75	0.33
228		111	121	3	22	10	31	73	0.14	0.20	0.28	0.66	0.71	0.30
229		110	121	3	23	11	31	71	0.13	0.21	0.28	0.65	0.74	0.32
230	123	113	123	3	25	10	28	71	0.12	0.22	0.25	0.63	0.89	0.35
301		107	117	3	21	10	27	70	0.14	0.20	0.25	0.65	0.78	0.30
303		104	113	3	23	9	31	69	0.13	0.22	0.30	0.66	0.74	0.33
304		119	128	4	25	9	33	77	0.16	0.21	0.28	0.65	0.76	0.32
305		116	126	3	25	10	32	75	0.12	0.22	0.28	0.65	0.78	0.33
306		109	117	3	24	8	30	70	0.13	0.22	0.28	0.64	0.80	0.34
309		107	117	3	23	10	30	68	0.13	0.21	0.28	0.64	0.77	0.34
310		115	126	3	25	11	32	77	0.12	0.22	0.28	0.67	0.78	0.32
311		113	123	3	23	10	31	75	0.13	0.20	0.27	0.66	0.74	0.31
315		106	116	3	23	10	30	70	0.13	0.22	0.28	0.66	0.77	0.33
316		112	123	3.5	25	11	32	71	0.14	0.22	0.29	0.63	0.78	0.35
402		104	113	3	23	9	32	68	0.13	0.22	0.31	0.65	0.72	0.34
405	129.5	116	125	3.5	25	9	32	75	0.14	0.22	0.28	0.65	0.78	0.33
406		109	118	3	24	9	30	70	0.13	0.22	0.28	0.64	0.80	0.34
502		119	128	3	25	9	30	75	0.12	0.21	0.25	0.63	0.83	0.33
505		116	125	3.5	25	9	32	75	0.14	0.22	0.28	0.65	0.78	0.33
507	135	117	126	3	24	9	31	72	0.13	0.21	0.26	0.62	0.77	0.33
508		115	125	3	24	10	30	75	0.13	0.21	0.26	0.65	0.80	0.32
510		110	118	3	23	8	30	70	0.13	0.21	0.27	0.64	0.77	0.33
512	118.5	111	120	3.5	24	9	32	71	0.15	0.22	0.29	0.64	0.75	0.34
514		112	121	3	23	9	31	71	0.13	0.21	0.28	0.63	0.74	0.32
519		118	130	3.5	25	12	31	76	0.14	0.21	0.26	0.64	0.81	0.33
520		119	130	3	25	11	32	75	0.12	0.21	0.27	0.63	0.78	0.33
521		109	118	3	23	9	30	70	0.13	0.21	0.28	0.64	0.77	0.33
523		114	124	3	25	10	31	74	0.12	0.22	0.27	0.65	0.81	0.34
524		115	124	3	24	9	32	75	0.13	0.21	0.28	0.65	0.75	0.32
525		118	123	3	25	5	32	76	0.12	0.21	0.27	0.64	0.78	0.33
528		117	127	3	24	10	32	75	0.13	0.21	0.27	0.64	0.75	0.32
529	134.5	115	125	3.5	24	10	33	71	0.15	0.21	0.29	0.62	0.73	0.34
531	126	119	127	4	25	8	35	76	0.16	0.21	0.29	0.64	0.71	0.33
532	121	115	125	3	23	10	32	74	0.13	0.20	0.28	0.64	0.72	0.31
534		116	127	3.5	26	11	32	76	0.13	0.22	0.28	0.66	0.81	0.34
536	129.5	119	128	3.5	24	9	32	75	0.15	0.20	0.27	0.63	0.75	0.32
538		113	122	3	24	9	32	71	0.13	0.21	0.28	0.63	0.75	0.34
539		110	120	3.5	24	10	32	73	0.15	0.22	0.29	0.66	0.75	0.33
541		119	128	4	25	9	33	79	0.16	0.21	0.28	0.66	0.76	0.32
542		114	124	3	25	10	32	74	0.12	0.22	0.28	0.65	0.78	0.34
544		118	128	4	25	10	33	76	0.16	0.21	0.28	0.64	0.76	0.33
545		118	127	3.5	25	9	32	77	0.14	0.21	0.27	0.65	0.78	0.32
546	132.5	105	114	3	23	9	30	69	0.13	0.22	0.29	0.66	0.77	0.33
547	128.5	104	113	3	22	9	29	37	0.14	0.21	0.28	0.36	0.76	0.59
548		118	127	3.5	25	9	32	76	0.14	0.21	0.27	0.64	0.78	0.33

549		102	111	3	22	9	29	64	0.14	0.22	0.28	0.63	0.76	0.34
550	125	107	116	3.5	22	9	31	69	0.16	0.21	0.29	0.64	0.71	0.32
552		117	126	3	24	9	34	78	0.13	0.21	0.29	0.67	0.71	0.31
555		115	125	4	25	10	31	75	0.16	0.22	0.27	0.65	0.81	0.33
557		112	121	3.5	23	9	32	72	0.15	0.21	0.29	0.64	0.72	0.32
603	122.5	113	122	3	23	9	31	74	0.13	0.20	0.27	0.65	0.74	0.31
604		111	120	3.5	23	9	30	63	0.15	0.21	0.27	0.57	0.77	0.37
607		118	128	3.5	24	10	33	76	0.15	0.20	0.28	0.64	0.73	0.32
614		104	113	3.5	22	9	29	69	0.16	0.21	0.28	0.66	0.76	0.32
616	133	112	122	3.5	24	10	32	71	0.15	0.21	0.29	0.63	0.75	0.34
618		118	128	3.5	23	10	31	77	0.15	0.19	0.26	0.65	0.74	0.30
619	134.5	119	128	3.5	25	9	33	78	0.14	0.21	0.28	0.66	0.76	0.32
620		116	125	3	23	9	31	74	0.13	0.20	0.27	0.64	0.74	0.31
101		168	182	4	33	14	45	110	0.12	0.20	0.27	0.65	0.73	0.30
105		121	129	4	26	8	32	79	0.15	0.21	0.26	0.65	0.81	0.33
107		130	140	4	27	10	33	83	0.15	0.21	0.25	0.64	0.82	0.33
111		122	134	4	26	12	36	80	0.15	0.21	0.30	0.66	0.72	0.33
201		121	132	4	25	11	31	79	0.16	0.21	0.26	0.65	0.81	0.32
202		134	145	4	25	11	36	85	0.16	0.19	0.27	0.63	0.69	0.29
206		120	133	4	26	13	33	80	0.15	0.22	0.28	0.67	0.79	0.33
208		134	145	3	28	11	36	84	0.11	0.21	0.27	0.63	0.78	0.33
209		127	139	3	27	12	36	81	0.11	0.21	0.28	0.64	0.75	0.33
210	129	127	137	4	27	10	34	80	0.15	0.21	0.27	0.63	0.79	0.34
212	119	125	136	3.5	26	11	34	78	0.13	0.21	0.27	0.62	0.76	0.33
213		146	159	4	27	13	36	95	0.15	0.18	0.25	0.65	0.75	0.28
214		133	145	4	30	12	38	90	0.13	0.23	0.29	0.68	0.79	0.33
218		126	136	3	26	10	34	80	0.12	0.21	0.27	0.63	0.76	0.33
224	138.5	120	130	3.5	25	10	35	79	0.14	0.21	0.29	0.66	0.71	0.32
302		121	134	3.5	24	13	34	81	0.15	0.20	0.28	0.67	0.71	0.30
307		124	135	3.5	27	11	36	81	0.13	0.22	0.29	0.65	0.75	0.33
308		126	136	4	24	10	34	82	0.17	0.19	0.27	0.65	0.71	0.29
312		122	133	3	26	11	32	80	0.12	0.21	0.26	0.66	0.81	0.33
313	126	133	145	3.5	28	12	38	87	0.13	0.21	0.29	0.65	0.74	0.32
314		121	132	3	27	11	35	79	0.11	0.22	0.29	0.65	0.77	0.34
401		130	141	4	27	11	36	80	0.15	0.21	0.28	0.62	0.75	0.34
403	125	189	199	4.5	37	10	50	119	0.12	0.20	0.26	0.63	0.74	0.31
404	116	142	155	4	28	13	39	92	0.14	0.20	0.27	0.65	0.72	0.30
501	123	124	133	4	26	9	34	81	0.15	0.21	0.27	0.65	0.76	0.32
503	132.5	123	132	3.5	26	9	34	80	0.13	0.21	0.28	0.65	0.76	0.33
504		125	135	3	26	10	35	80	0.12	0.21	0.28	0.64	0.74	0.33
506		122	132	4	27	10	33	78	0.15	0.22	0.27	0.64	0.82	0.35
509		124	135	3	25	11	32	81	0.12	0.20	0.26	0.65	0.78	0.31
511		127	136	3.5	26	9	35	79	0.13	0.20	0.28	0.62	0.74	0.33
513		122	131	3	26	9	34	76	0.12	0.21	0.28	0.62	0.76	0.34
515	120.5	131	144	4	26	13	35	83	0.15	0.20	0.27	0.63	0.74	0.31
516	132	121	132	4	26	11	33	78	0.15	0.21	0.27	0.64	0.79	0.33
517	124	137	148	3	26	11	37	88	0.12	0.19	0.27	0.64	0.70	0.30
518		124	133	3	26	9	35	77	0.12	0.21	0.28	0.62	0.74	0.34

522		127	136	3	26	9	34	79	0.12	0.20	0.27	0.62	0.76	0.33
526	127.5	134	144	4	28	10	38	85	0.14	0.21	0.28	0.63	0.74	0.33
527	137.5	132	141	4	25	9	33	81	0.16	0.19	0.25	0.61	0.76	0.31
530		123	135	3.5	26	12	32	81	0.13	0.21	0.26	0.66	0.81	0.32
533		121	132	3.5	26	11	34	78	0.13	0.21	0.28	0.64	0.76	0.33
535		126	136	4	27	10	35	82	0.15	0.21	0.28	0.65	0.77	0.33
537		122	133	3	26	11	35	77	0.12	0.21	0.29	0.63	0.74	0.34
540		123	132	3	26	9	35	79	0.12	0.21	0.28	0.64	0.74	0.33
543		127	137	3.5	27	10	31	82	0.13	0.21	0.24	0.65	0.87	0.33
551	133	132	144	3.5	26	12	38	86	0.13	0.20	0.29	0.65	0.68	0.30
553		132	144	3	27	12	36	85	0.11	0.20	0.27	0.64	0.75	0.32
554	109	131	142	4	27	11	32	84	0.15	0.21	0.24	0.64	0.84	0.32
556	128.5	120	131	3.5	26	11	34	78	0.13	0.22	0.28	0.65	0.76	0.33
601		170	182	5	34	12	46	104	0.15	0.20	0.27	0.61	0.74	0.33
602	131.5	130	141	4	27	11	36	81	0.15	0.21	0.28	0.62	0.75	0.33
605	140.5	134	145	4	27	11	37	86	0.15	0.20	0.28	0.64	0.73	0.31
606	114	147	159	4	28	12	38	93	0.14	0.19	0.26	0.63	0.74	0.30
608		132	143	3.5	27	11	36	87	0.13	0.20	0.27	0.66	0.75	0.31
609	133	176	192	4.5	35	16	48	114	0.13	0.20	0.27	0.65	0.73	0.31
610	123	153	166	4	30	13	40	95	0.13	0.20	0.26	0.62	0.75	0.32
611		170	185	4.5	39	15	46	112	0.12	0.23	0.27	0.66	0.85	0.35
612	118	139	150	4	28	11	38	90	0.14	0.20	0.27	0.65	0.74	0.31
613		133	143	3.5	26	10	35	84	0.13	0.20	0.26	0.63	0.74	0.31
615		123	133	3.5	25	10	33	79	0.14	0.20	0.27	0.64	0.76	0.32
617	120.5	142	154	4	27	12	38	91	0.15	0.19	0.27	0.64	0.71	0.30

Table 1b. Data from the second morphometrics study. TL= total length, SL= standard length, FL= fork length, HL= head length, SnL=snout length, Sn-DR= snout to first dorsal ray, Sn-A=snout to anus, Eye=eye diameter, BD=body depth, plicae=count of plicae, GR=count of gill rakers on first arch of the right side of the body, PFR=pectoral fin ray count on the left side of the body, DFR=dorsal fin ray count, AFR=anal fin ray count. Individuals with the letters NWC in their codes were taken from the northwest corner of Stellwagen Bank, and were assumed to be *A. dubius* due to location offshore and large size. Individuals with PTH in their codes were taken from Provincetown Harbor and were assumed to be *A. americanus* due to their inshore location.

Fish ID	TL (cm)	SL (cm)	FL (cm)	HL (mm)	SnL (mm)	Sn-DR (cm)	Sn-A (cm)	Eye (mm)	BD (mm)	Plicae	GR	PFR	DFR	AFR
07-NWCM1	19.60	18.00	19.10	35.00	12.00	5.10	11.40	4.00	17.00	128	42	12	59	28
07-NWCM2	18.75	17.00	18.25	35.00	12.00	4.75	11.00	4.00	18.00	130	36	11	52	27
07-NWCM3	17.70	16.30	17.30	33.00	11.00	4.20	10.10	4.00	16.00	130	30	13	60	30
07-NWCM4	19.00	17.50	18.60	35.00	11.00	4.60	10.50	4.00	16.00	129	38	14	48	26
07-NWCM5	17.90	16.50	17.40	33.00	11.00	4.50	9.90	4.00	14.00	131	33	12	51	30
07-NWCM6	17.00	15.50	16.50	34.00	11.00	4.40	10.20	4.00	17.00	131	28	11	54	26
07-NWCM7	18.10	16.70	17.30	34.00	12.00	4.50	10.80	5.00	15.00	129	35	12	59	29
07-NWCM8	18.20	16.40	17.20	33.00	12.00	4.30	10.80	4.00	15.00	126	27	11	47	31
07-NWCM9	18.70	17.30	18.20	35.00	12.00	5.10	10.80	4.50	17.00	145	39	14	57	29
07-NWCM10	18.10	16.80	17.70	30.00	11.00	4.50	10.70	4.50	16.00	136	54	11	61	16

07-NWCM11	18.30	17.00	17.90	31.00	11.00	4.40	10.30	4.50	17.00	132	50	10	63	31
07-NWCM12	17.40	16.10	17.00	29.00	10.00	4.30	10.10	4.50	19.00	130	49	12	61	27
07-NWCM13	18.00	16.70	17.60	31.00	10.00	4.50	10.50	4.50	15.00	139	47	11	59	28
07-NWCM14	16.70	15.40	16.40	28.00	10.00	4.00	9.30	4.00	14.00	135	56	16	64	33
07-NWCM15	17.60	16.20	17.20	30.00	11.00	4.30	10.40	4.50	15.00	132	51	14	62	26
07-NWCM16	18.00	16.80	17.20	31.00	11.00	4.90	10.80	4.50	19.00	130	59	11	60	28
07-NWCM17	18.50	16.80	17.50	33.00	9.00	4.40	10.20	4.00	17.00	136	50	12	52	24
07-NWCM18	17.20	15.90	16.50	30.00	8.00	4.20	9.40	4.00	14.00	135	69	12	53	25
07-NWCM19	18.90	17.40	18.40	35.00	10.00	4.90	10.80	4.00	18.00	136	39	11	60	29
07-NWCM20	17.90	16.40	17.30	33.00	9.00	4.50	10.70	4.00	16.00	137	52	12	57	30
07-NWCM21	19.40	17.90	19.00	36.00	12.00	4.90	11.00	5.00	18.00	150	42	12	58	26
07-NWCM22	18.60	17.10	18.00	35.00	9.00	4.40	10.40	5.00	14.00	126	56	12	61	29
07-NWCM23	18.80	17.30	18.30	32.00	8.00	4.30	11.00	4.00	15.00	135	64	11	61	28
07-NWCM24	17.80	16.40	17.30	30.00	9.00	4.00	9.90	4.00	14.00	139	56	13	61	26
07-PTHM1	8.80	8.00	8.50	18.00	6.00	2.40	5.10	2.00	8.00	88	24	15	54	25
07-PTHM2	8.30	7.50	8.10	17.00	6.00	2.30	4.80	2.00	7.00	89	33	12	52	30
07-PTMH3	7.70	7.00	7.50	16.00	6.00	2.30	4.50	2.00	7.00	87	36	14	40	25
07-PTHM4	8.70	7.80	8.40	17.00	6.00	2.80	5.00	3.00	8.00	91	45	12	32	20
07-PTHM5	7.80	7.30	7.50	15.00	6.00	2.20	4.70	2.00	7.00	99	34	13	28	25
07-PTHM6	7.40	6.60	7.10	14.00	5.00	2.00	4.30	2.00	7.00	106	33	12	56	29
07-PTHM7	7.90	7.20	7.70	18.00	5.00	2.20	4.60	2.00	7.00	97	30	12	45	25
07-PTHM8	7.30	6.60	7.00	15.00	5.00	2.00	4.10	2.00	7.00	94	31	10	58	24
07-PTHM9	7.80	7.00	7.50	15.00	5.00	2.10	4.50	3.00	7.00	121	15	14	60	29
07-PTHM10	8.15	7.50	7.90	17.00	5.50	2.40	4.90	3.00	7.00	119	15	14	58	30
07-PTHM11	8.05	7.20	7.80	17.00	6.00	2.10	4.60	3.00	8.00	133	14	14	46	31
07-PTHM12	9.00	8.30	8.90	12.00	6.00	2.40	5.40	2.50	9.00	134	10	14	58	28
07-PTHM13	7.80	7.10	7.70	17.00	4.50	2.20	4.60	3.00	8.00	124	12	14	62	28
07-PTHM14	8.50	7.20	8.20	18.00	6.00	2.30	4.80	3.00	8.50	123	15	14	60	32
07-PTHM15	9.00	8.20	8.80	18.00	6.00	2.50	5.35	3.00	7.50	130	12	14	59	32
07-PTHM16	8.00	7.40	7.80	13.00	6.00	2.20	4.70	3.00	8.00	119	12	14	62	32
07-PTHM17	7.50	6.60	7.30	15.00	6.00	2.10	4.50	3.00	6.00	96	12	16	42	21
07-PTHM18	8.50	7.70	8.10	16.00	6.00	2.10	4.90	3.00	7.00	85	16		53	24
07-PTHM19	8.00	7.30	7.50	17.00	6.00	2.30	4.50	3.00	7.00	110	11	12	67	26
07-PTHM20	8.50	7.50	8.20	15.00	5.00	2.20	4.90	3.00	5.00	102	13	17	69	30
07-PTHM21	7.60	7.00	7.20	17.00	6.00	2.10	4.50	3.00	7.00	92	17	11	54	27
07-PTHM22	7.90	6.90	7.60	19.00	5.00	2.30	4.70	3.00	5.00	86	15	12	46	20
07-PTHM23	8.50	7.60	8.00	17.00	6.00	2.20	4.80	3.00	7.00	106	14	13	61	31
07-PTHM24	7.90	7.30	7.70	15.00	6.00	2.10	4.60	3.00	7.00	97	15	12	52	27
07-PTHM25	7.50	6.60	7.20	13.00	5.00	1.70	4.50	3.00	8.00	95	6	13	--	9
07-PTHM26	7.80	7.00	7.60	17.00	6.00	1.80	4.60	4.00	9.00	97	7	13	--	12
07-PTHM27	9.80	8.90	9.40	21.00	7.00	2.60	6.10	4.50	10.00	104	5	12	70	21
07-PTHM28	9.30	8.30	8.70	20.00	10.00	2.50	5.30	3.00	8.00	105	10	18	--	24
07-PTHM29	8.90	8.30	8.50	19.00	7.00	2.30	5.30	3.00	10.00	96	11	12	80	9
07-PTHM30	8.00	7.30	7.50	16.00	7.00	2.20	4.70	3.00	9.00	100	9	11	--	6
07-PTHM31	7.90	7.10	7.50	16.00	5.00	2.00	4.40	3.00	7.00	107	12	14	64	32

Stable isotopes

Table 2. *A. dubius* stable isotope data. Sampling year, standard length are given for each sample along with the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Year	Standard Length (cm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
2006	12.6	-20.96	10.59
2006	13.2	-21.02	10.35
2006	12.2	-20.79	10.13
2006	11.6	-21.09	10.75
2006	13.2	-20.57	10.73
2006	13.2	-20.71	10.52
2006	11.2	-20.69	10.83
2006	11.2	-21.17	10.00
2006	13.2	-20.57	10.41
2006	12.9	-20.98	10.38
2006	12.5	-20.82	9.93
2006	12.2	-21.18	10.68
2006	10.0	-21.05	9.74
2006	13.0	-21.04	10.47
2006	12.4	-21.36	10.67
2006	12.7	-21.29	10.45
2006	12.2	-20.94	10.75
2006	13.3	-21.19	9.80
2006	11.7	-21.40	10.57
2006	12.2	-20.74	10.53
2006	11.7	-20.74	10.34
2006	12.4	-20.81	9.64
2006	11.5	-21.76	10.92
2006	11.7	-21.17	10.74
2006	11.5	-21.00	10.27
2006	10.8	-21.46	10.19
2006	11.1	-21.12	10.55
2006	12.8	-21.25	10.10
2006	13.0	-20.61	10.18
2006	12.4	-20.63	10.40
2006	12.8	-21.23	10.13
2006	11.5	-20.81	10.90
2006	12.0	-20.85	10.90
2006	11.9	-20.69	10.13
2006	11.4	-21.20	10.31
2006	10.9	-21.28	10.09
2006	11.7	-20.09	9.37
2006	11.7	-21.03	9.91
2006	8.9	-20.88	10.35
2006	9.9	-20.58	10.46
2006	10.5	-20.91	9.87
2006	11.1	-21.68	10.36
2006	11.1	-21.63	10.55

2006	9.1	-19.10	10.22
2006	10.0	-21.31	10.79
2006	12.0	-22.30	10.42
2006	13.6	-22.02	10.52
2006	13.0	-21.47	10.31
2006	12.8	-21.53	10.59
2006	12.3	-21.91	10.58
2006	12.7	-23.22	9.93
2006	12.5	-22.62	10.44
2006	10.6	-21.91	10.45
2007	19.7	-19.65	10.20
2007	15.3	-19.82	10.45
2007	16.7	-20.34	9.97
2007	17.3	-19.55	10.10
2007	12.9	-21.17	10.22
2007	16.3	-20.23	10.67
2007	18.4	-20.08	9.49
2007	17.6	-20.29	9.81
2007	18.2	-19.95	10.32
2007	17.4	-20.06	10.31
2007	17.3	-20.07	10.66
2007	20.8	-19.35	10.66
2007	16.9	-20.25	9.64
2007	17.1	-20.27	10.07
2007	17.3	-20.37	10.28
2007	17.0	-19.89	9.81
2007	19.8	-19.61	10.92
2007	17.2	-20.18	10.10
2007	16.2	-20.21	10.28
2007	16.3	-19.65	10.37
2007	15.8	-20.04	10.16
2007	17.5	-20.15	9.68
2007	14.8	-20.30	10.75
2007	16.1	-20.17	10.40
2007	16.3	-19.50	10.48
2007	18.0	-20.14	10.07
2007	16.8	-20.04	9.99
2007	16.3	-20.39	10.36
2007	16.4	-20.19	9.99
2007	15.6	-20.11	10.29
2007	17.1	-19.63	10.39
2007	18.0	-20.13	10.22
2007	15.7	-20.00	10.46
2007	15.4	-20.00	10.53
2007	12.7	-20.02	10.61

2007	19.2	-19.75	9.83
2007	18.4	-20.42	10.05
2007	17.6	-20.56	9.42
2007	18.2	-20.40	9.35
2007	17.2	-20.79	10.50
2007	12.9	-21.30	10.06
2007	12.0	-20.97	10.14
2007	12.4	-21.56	10.38
2007	12.2	-22.19	10.28
2007	11.3	-22.32	9.30
2007	12.1	-22.17	9.98
2007	12.5	-21.72	9.59
2007	13.4	-21.60	9.65
2007	12.0	-22.11	10.36
2007	12.8	-21.91	9.74
2007	15.5	-20.31	10.05
2007	15.6	-20.46	9.90
2007	15.2	-20.07	10.45
2007	14.7	-20.43	10.06
2007	15.5	-21.10	9.51
2007	17.6	-20.92	10.78
2007	17.0	-20.47	10.26
2007	16.1	-20.22	9.52
2007	16.6	-20.25	9.94
2007	16.5	-20.74	10.73
2007	16.5	-20.80	9.83
2007	15.6	-20.41	10.46
2007	16.0	-20.51	10.23
2007	16.2	-20.79	9.06
2007	15.4	-20.11	9.44
2007	13.8	-21.16	10.68
2007	11.9	-22.05	9.60
2007	13.7	-21.99	10.25
2007	12.3	-22.40	9.93
2007	12.1	-22.24	10.26
2007	11.9	-22.12	10.48
2007	11.9	-21.49	9.52
2007	12.6	-21.62	9.97
2007	12.3	-21.02	10.23
2007	12.2	-22.30	10.26
2007	11.6	-21.84	9.21

2007	12.8	-22.84	10.53
2007	12.2	-21.92	10.36
2007	12.9	-21.82	10.49
2007	15.2	-21.18	10.76
2007	15.0	-20.71	10.73
2007	16.0	-20.48	10.76
2007	16.0	-20.61	10.84
2007	17.5	-20.53	10.69
2007	16.8	-21.22	10.16
2007	17.6	-20.81	9.60
2008	15.9	-19.85	9.90
2008	16.9	-19.80	10.01
2008	17.5	-19.96	9.75
2008	15.3	-19.97	10.01
2008	17.4	-20.11	10.32
2008	16.7	-19.99	9.76
2008	16.0	-20.04	9.94
2008	15.1	-20.73	10.07
2008	16.5	-20.54	9.89
2008	16.2	-20.66	10.20
2008	17.0	-20.55	9.80
2008	15.5	-20.36	10.12
2008	16.8	-20.10	9.87
2008	16.5	-19.87	10.00
2008	16.8	-19.94	10.25
2008	14.9	-20.20	10.01
2008	17.4	-20.14	10.05
2008	17.7	-19.93	9.73
2008	17.7	-20.47	10.61
2008	15.9	-20.14	10.31
2008	18.0	-20.19	10.15
2008	15.7	-20.24	10.33
2008	13.5	-20.53	10.43
2008	19.8	-20.45	9.34
2008	15.4	-20.26	10.42
2008	16.1	-20.48	10.23
2008	13.3	-21.05	10.06
2008	16.1	-20.68	9.97
2008	17.0	-20.26	10.47
2008	14.9	-20.24	10.60

Table 3. Isotope data for samples of *A. americanus* taken from waters off of Provincetown, MA during the fall of 2007.

Location	Standard length (cm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Provincetown Harbor	9.2	-20.19	11.23
Provincetown Harbor	8.3	-19.93	11.07
Provincetown Harbor	7.1	-20.35	10.78
Provincetown Harbor	9.3	-20.32	10.64
Provincetown Harbor	8.5	-20.39	10.82
Provincetown Harbor	9.2	-21.50	10.34
Provincetown Harbor	7	-19.85	11.02
Provincetown Harbor	8.1	-19.95	11.01
Provincetown Harbor	8.2	-20.21	11.47
Provincetown Harbor	10.8	-20.87	10.91
Provincetown Harbor	9.9	-21.35	10.66
Provincetown Harbor	7.5	-20.74	10.79
Provincetown Harbor	7.8	-20.27	10.90
Provincetown Harbor	10.2	-20.23	10.47
Provincetown Harbor	7.8	-20.86	10.79
Provincetown Harbor	8.8	-20.36	11.17
Provincetown Harbor	8.4	-20.23	10.59
Provincetown Harbor	8.2	-20.50	11.18
Provincetown Harbor	7.8	-20.03	10.00
Provincetown Harbor	8.1	-21.13	10.74
Race Point	7.7	-20.36	11.09
Race Point	8.5	-21.04	10.99
Race Point	7.6	-20.49	10.99
Race Point	7.9	-20.12	10.17
Race Point	7.6	-20.12	10.50
Race Point	7.3	-20.79	10.92
Race Point	7.9	-20.54	10.72
Race Point	7.5	-21.31	10.59
Race Point	8	-21.35	10.19
Race Point	10.6	-19.94	11.03
Race Point	9	-20.09	10.65
Race Point	8	-21.16	10.91
Race Point	7.7	-21.57	10.41
Race Point	8	-21.19	10.72
Race Point	7.6	-21.00	10.03
Race Point	7	-21.03	10.46
Race Point	7.1	-20.71	10.55
Race Point	7.5	-20.80	10.80
Race Point	7.7	-21.19	10.73
Race Point	6.5	-21.16	10.31

6. RESULTS and CONCLUSIONS

Sampling gear development

Both the Rockport and Provincetown Harbor minnow trap array sampling trials were unsuccessful. Neither location's array caught any sand lance. Eleven trials were run with the drop frame net, five when it was fully operational. During those five trials, the mean number of sand lance caught per deployment was six (± 7.5 standard deviations). This was not sufficient for the purposes of the study.

The modified shrimp beam trawl was the most successful sampling method tested. It was found to produce replicable numbers of sand lance in each catch. During initial trials, the tow lines and foot rope were heavy chain and the net was towed on the seafloor. Video observations and catch mortality indicated that the sand lances were getting cut up by the scraping of the net on the bottom. Consequently, the net was raised two inches up the trawl tow bridle. Additionally, as sand lances were observed to react to the heavy tow chains and the heavy foot rope, those were replaced by thin cable and very light chain respectively. It was found that towing at a mean speed of 2 knots is optimal for capturing live sand lance in good condition. The modified shrimp beam trawl method captures sand lance from both swimming schools and ones that are scared up from burrows in the sand.

Field sampling

Sand lance were sampled during 20 sea days split between the two vessels, plus additional hours or days volunteered by both captains as opportunity arose; much of this time was expended in gear development, which was reported on earlier. The sand lances were captured using customized beam trawls, in four principal study areas: Northwest Corner and Southwest Corner on Stellwagen Bank (Middle Bank), the Race on the ocean side of Provincetown and Provincelands, and in Provincetown Harbor itself, mostly near the dike. Samples from *Ammodytes dubius* caught on the Northwest Corner of Stellwagen Bank were much larger in 2007 and 2008 than in 2006. Two size cohorts were caught in 2007: an under-14 cm group, hereafter referred to as "small", and an over-14 cm group, hereafter referred to as "large". We did not attempt to conduct a quantitative survey of sand lance abundance once we realized that (a) there were other more basic questions to be answered first, (b) sand lance can be appreciated on high frequency sonar and that offered a better prospect for future abundance estimates, and (c) the behavior of sand lance around the net compounds spatial variability and makes it necessary to execute an impractical number of tows to achieve statistical confidence.

Sand lances were released from the cod end after a tow, subsampled, and portions of the subsample frozen quickly for later analysis. Early in the study we also retained several thousand sand lance to be kept alive for behavioral studies. Through collaboration with The New England Aquarium, this captive population yielded substantial information on the husbandry and pathology of both species in captivity. It also resulted in the exhibition of sand lance in the Aquarium's Stellwagen Bank exhibit, and the completion of a doctoral dissertation on fungal infections of captive and wild sand lance by then veterinary student Dr. Akinyi Nyaoke of the University of Connecticut and the New England Aquarium.

Morphometrics

The first study used the assumption that plicae provide a reliable way to separate *A. americanus* from *A. dubius*, determining that the sample set included representatives of both species. Individual plicae counts averaged from both sides of the fish ranged from 109 to 141.5 indicating the presence of both *A. dubius* and *A. americanus*. Discriminant analysis models (JMP software, SAS Inc.) assigned 79.59% of individuals to one of the two species correctly using all the measured characteristics and ratios. It is possible that some of the ratios or length measurements included in this assessment may have negatively affected the accuracy of the separation and further study is necessary to determine the exact effects of individual variables. A principle

components analysis (PCA) was used to assess correlation between length ratios and the number of plicae. The PCA showed which characteristics were most strongly correlated and based on the correlations we determined which ratios to include in subsequent discriminate analyses. Using only head to standard length ratio, head to the distance from the snout to the first dorsal ray ratio, and caudal fin length as proxies for species discrimination, 20.41% of individuals were misclassified. When caudal fin length was removed from the analysis, 24.49% of individuals were misclassified showing that caudal fin length was not essential in separation. The percent of individuals misclassified was similar for both species at 25% for *A. americanus* and 24.24% for *A. dubius*. Assessed individually, the head length ratios were not nearly as effective. Head length as a percentage of standard length and head length as a percentage of distance from snout to first dorsal ray were only accurate for 67.35% and 46.94% of individuals, respectively.

The second study reexamined the possibility of using length ratios to discriminate between species, in an effort to make identifying live sand lances easier in the field and for laboratory studies. Measurements were taken of individuals from northwest corner of Stellwagen Bank (n=24), assumed to be *A. dubius* due to their offshore location and plicae counts, and of individuals captured in Provincetown Harbor (n=31), assumed to be *A. americanus* due to their inshore location and plicae counts. Discriminant analyses were run using a combination of ratios: standard length to total length, head length to standard length, snout length to head length, and head length to distance from snout to first dorsal ray. With all four ratios, the model misclassified two individuals out of 55 (3.6%). Taking out the head length to distance between snout and first dorsal ray, the new model misclassified four out of 55 (7.3%). Using only two variables, the ratios of head length to standard length and snout length to head length, a third model misclassified three out of the 55 (5.5%). Lastly, when only the head length to standard length ratio was used, eight out of the 55 (14.6%) were misclassified. While a larger sample size should be examined, it appears that the ratios of head length to standard length and standard length to total length can be used to reliably distinguish between the two species.

We are frequently asked if the two species can be separated on casual inspection. The answer is "no", except for the fact that *Ammodytes dubius* achieves a much larger size than *A. americanus*, and this can be used to roughly sort all but young-of-the-year of the two species in collections where they occur sympatrically. Careful examination of samples from our study areas strongly suggests that sand lance caught on Stellwagen Bank are almost invariably *Ammodytes dubius*, that samples from Provincetown Harbor consists principally or entirely of *A. americanus*, and that the two species overlap on the Race (outer beaches), with *A. americanus* mostly restricted to waters less than 20m deep, and *A. dubius* to waters greater than 20m deep. The two species overlap in tows taken at about 20m depth.

Feeding observations

Feeding behavior of captive sand lance indicated that they utilize techniques of body ramming, jaw ramming (prey capture effected by rapid protrusion of the upper jaw), and suction feeding on each attack on a prey item, though in varying amounts dependent on each unique feeding situation. Jaw ram feeding appears to predominate on small and elusive prey; however, more work is needed on natural, living prey. Sand lance individuals used in filming were not identified to species. Further study is necessary to determine if there are differences in feeding techniques between *A. dubius* and *A. americanus*; however, comparison of the morphology of the

premaxillae and of feeding by large versus small individuals indicate that any such differences are likely to be very subtle. Nonetheless, the spatial segregation exhibited by the two species in the field result in sharp differences in their ecological roles and respective importance to ecosystem integrity, fisheries, and wildlife.

Diet composition

Stable isotopic analyses are often used in ecological studies to determine the relative trophic position of an organism. This is accomplished by finding the ratio of heavy to light nitrogen in bodily tissues. The stable heavy nitrogen isotope, ^{15}N , is taken up preferentially by animals and incorporated into their tissues. As a result, heavy nitrogen signatures increase with increasing trophic levels in food webs. Stable isotopes can also be used to determine the base of a food web, particularly in a marine system. Photosynthetic planktonic organisms use the lighter, atmospheric carbon isotope, ^{12}C , thus food webs that have plankton as their base organisms are deficient in the heavy isotope, ^{13}C . In contrast, food webs that are based on detritivores tend to have more of the heavy isotope. In this study, these isotopes were used to compare the diets and trophic positions of *A. dubius* individuals over a three year period (2006-2008) and to compare *A. dubius* and *A. americanus* individuals sampled in 2007.

As noted above, *A. dubius* caught on Stellwagen Bank in 2006 were significantly smaller on average than both small and large ones caught in 2007 ($p < 0.001$) and those that were caught in 2008 ($p < 0.001$). Additionally, those caught in 2008 were significantly larger on average than those caught in both 2007 size classes, although the difference was not as striking as that between 2006 and the other two years ($p = 0.001$ with 2007 small, $p = 0.04$ with 2007 large). Stable isotope signatures were also significantly different (all p -values < 0.001 ; Table 4), with the following exceptions: 2007 both size classes and 2008 individuals signatures of ^{15}N ($p = 0.47$ with small and 0.11 with large), small and large 2007 groups' signatures of ^{15}N ($p = 0.14$), and 2007 large and 2008 signatures of ^{13}C ($p = 0.5$). Individuals sampled in 2006 had a mean ^{13}C of -21.14 and a mean ^{15}N of 10.36 . Small 2007 individuals had a mean ^{13}C of -20.71 and a mean ^{15}N of 10.03 . Large 2007 individuals had a mean ^{13}C of -20.19 and a mean ^{15}N of 10.14 . In 2008, sampled individuals had a mean ^{13}C of -20.26 and a mean ^{15}N of 10.09 . Though the difference in ^{15}N signatures between the 2006 individuals (the smallest of all sampled) and all other individuals was statistically significant, their trophic position is not very different from the largest of the *A. dubius* sampled (Figure 5). Trophic positions can be calculated using the following equation (Post 2002, Estrada et al 2005):

$$\text{Trophic position}_{\text{organism}} = \lambda + ((\delta^{15}\text{N}_{\text{organism}} - \delta^{15}\text{N}_{\text{base}}) / \Delta n)$$

Where λ is the assumed trophic position of the food web base organism, in this case an assemblage of microscopic phytoplankton and zooplankton and Δn is the trophic fractionation level. Here we use the accepted literature mean of 3.4% (Post 2002, Estrada et al 2005). The mean trophic position for individuals in 2006 was 2.4 (s.e. 0.01), for small and large individuals in 2007 the mean was 2.3 (s.e. 0.03 , 0.02), and for 2008 individuals the mean trophic position was also 2.3 (s.e. 0.01). Thus sand lance generally feed at the same trophic level across the board, but that does not mean they are eating the same kinds of prey items at all sizes. The smaller individuals of 2006, with lighter ^{13}C signals appear to have a diet heavy in planktonic organisms compared to those of 2007 and 2008 which may have supplemented their planktonic diets with benthic organisms, as indicated by their heavier ^{13}C signals (Figure 6).

Table 4. Summary of means on *A. dubius* samples over a three year period.

Year	Mean length (cm)	Standard error	Mean $\delta^{13}\text{C}$ ‰	Standard error	Mean $\delta^{15}\text{N}$ ‰	Standard error
2006	11.88	±0.15	-21.14	±0.09	10.36	±0.05
2007 small	12.41	±0.13	-21.71	±0.12	10.03	±0.08
2007 large	16.85	±0.18	-20.19	±0.05	10.14	±0.06
2008	16.32	±0.24	-20.26	±0.06	10.09	±0.05

Table 5. Summary of means on *A. americanus* sampled in two locations in 2007.

Sampling Location	Mean length (cm)	Standard error	Mean $\delta^{13}\text{C}$ ‰	Standard error	Mean $\delta^{15}\text{N}$ ‰	Standard error
Provincetown Harbor	8.51	±0.22	-20.46	±0.10	10.83	±0.08
Race Point	7.84	±0.19	-20.80	±0.11	10.64	±0.07

Figure 5 shows the relationship between $\delta^{15}\text{N}$ and standard length for each individual sampled. Both 2006 and 2007 samples show a slightly positive relationship between increasing size and increasing $\delta^{15}\text{N}$ signatures (2006 $R^2=0.001$, 2007 $R^2=0.009$). Samples from 2008 show a stronger, though still small, negative relationship between increasing size and increasing $\delta^{15}\text{N}$ signatures ($R^2=0.174$). Figure 6 shows the relationship between $\delta^{13}\text{C}$ and standard length for all individuals. Both 2007 and 2008 samples show positive relationships between increasing size and increasingly heavy $\delta^{13}\text{C}$ signatures, though the 2007 relationship is much stronger (2007 $R^2=0.66$, 2008 $R^2=0.11$). The 2006 samples show a negative relationship between increasing size and increasingly heavy $\delta^{13}\text{C}$ signatures ($R^2=0.05$).

Figure 5. Standard length and corresponding $\delta^{15}\text{N}$ values for each sample from 2006, 2007, and 2008.

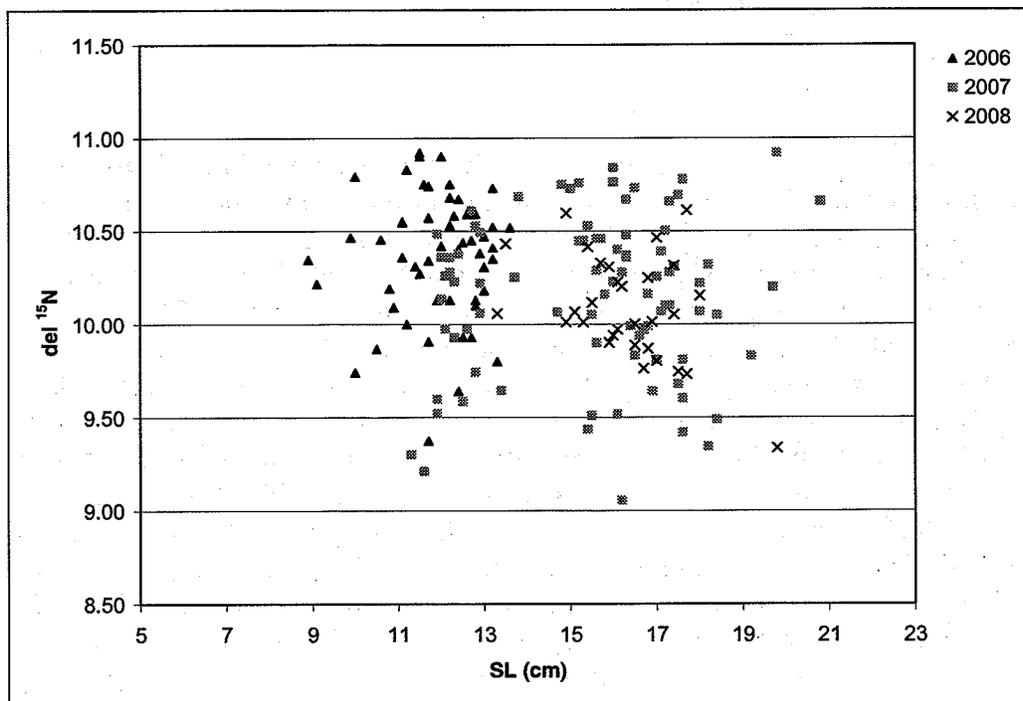
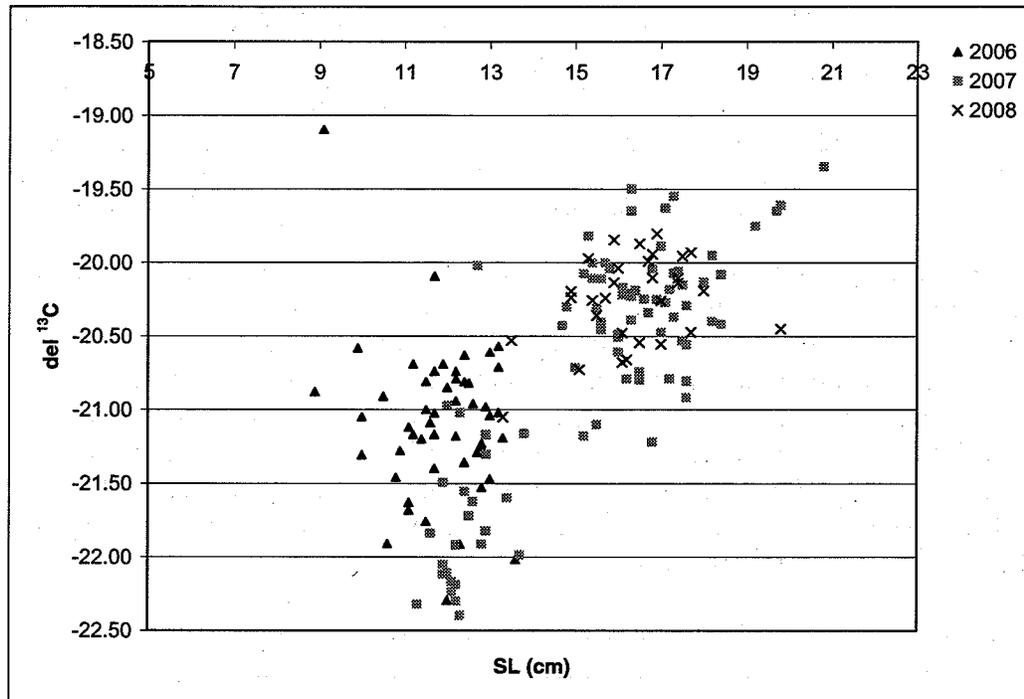


Figure 6. Standard length and corresponding $\delta^{13}\text{C}$ values for each sample from 2006, 2007, and 2008.



Comparisons were also made between *A. americanus* individuals captured in 2007 from the bayside Provincetown Harbor and the oceanside Race Point of Provincetown and between both of those locations and the *A. dubius* from the northwest corner of Stellwagen Bank, captured in 2007. Tables 4 and 5 contain summaries of group means (length, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$). Both 2007 size classes were significantly larger on average than both Provincetown groups ($p < 0.001$). Provincetown Harbor individuals were larger on average than the Race Point individuals ($p = 0.01$). Figure 7 shows the relationship between $\delta^{13}\text{C}$ and standard length for all individuals. Race Point samples show a positive relationship between increasing size and increasingly heavy $\delta^{13}\text{C}$ signatures ($R^2 = 0.22$), while the Provincetown Harbor samples show a negative relationship between increasing size and increasingly heavy $\delta^{13}\text{C}$ signatures ($R^2 = 0.14$). Figure 8 shows the relationship between $\delta^{15}\text{N}$ and standard length for each individual sampled. Race Point samples show a positive relationship between increasing size and increasing $\delta^{15}\text{N}$ signatures ($R^2 = 0.14$). Samples from Provincetown Harbor show a weak negative relationship between increasing size and increasing $\delta^{15}\text{N}$ signatures ($R^2 = 0.01$). All comparisons between stable isotope signatures, between both Provincetown sites and the northwest corner of Stellwagen Bank, were statistically significant. Race Point had the lightest ^{13}C signatures, followed by small 2007 *A. dubius* and then Provincetown Harbor, with large *A. dubius* having the heaviest ^{13}C signatures (Figure 9). Provincetown Harbor had the heaviest ^{15}N signatures, followed by Race Point, then large 2007 *A. dubius*, and lastly small 2007 *A. dubius* (Figure 10). The heavier signatures of *A. americanus*

likely result from proximity to land; heavy nitrogen runoff enters coastal systems due to sewage outfall and fertilizer use, leading to a bias in heavy nitrogen signatures in stable isotope analyses. Stomach contents were also examined for small subsets of each group. *A. dubius* individuals of both size classes were found to have copepods, krill, and hyperiid amphipods (a carnivorous zooplankton). *A. americanus* individuals were found to have mostly small copepods. More stomach samples will be examined to improve statistical power for comparative purposes.

Figure 7. Standard length and corresponding $\delta^{13}\text{C}$ values for each sample from Provincetown Harbor and Race Point.

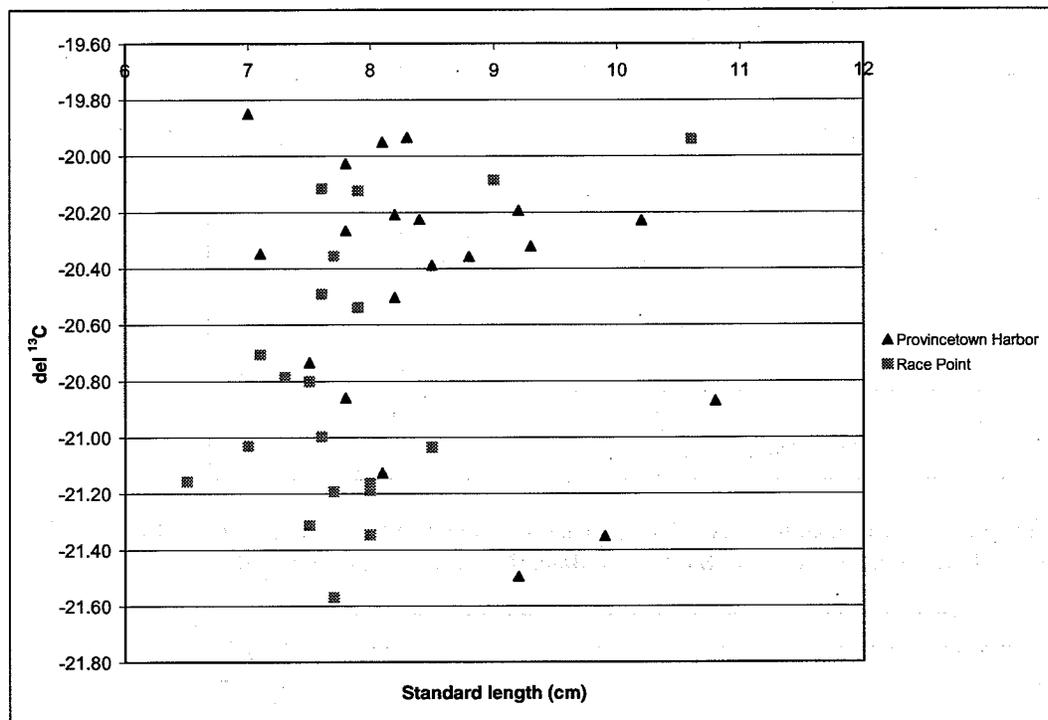


Figure 8. Standard length and corresponding $\delta^{15}\text{N}$ values for each sample from Provincetown Harbor and Race Point.

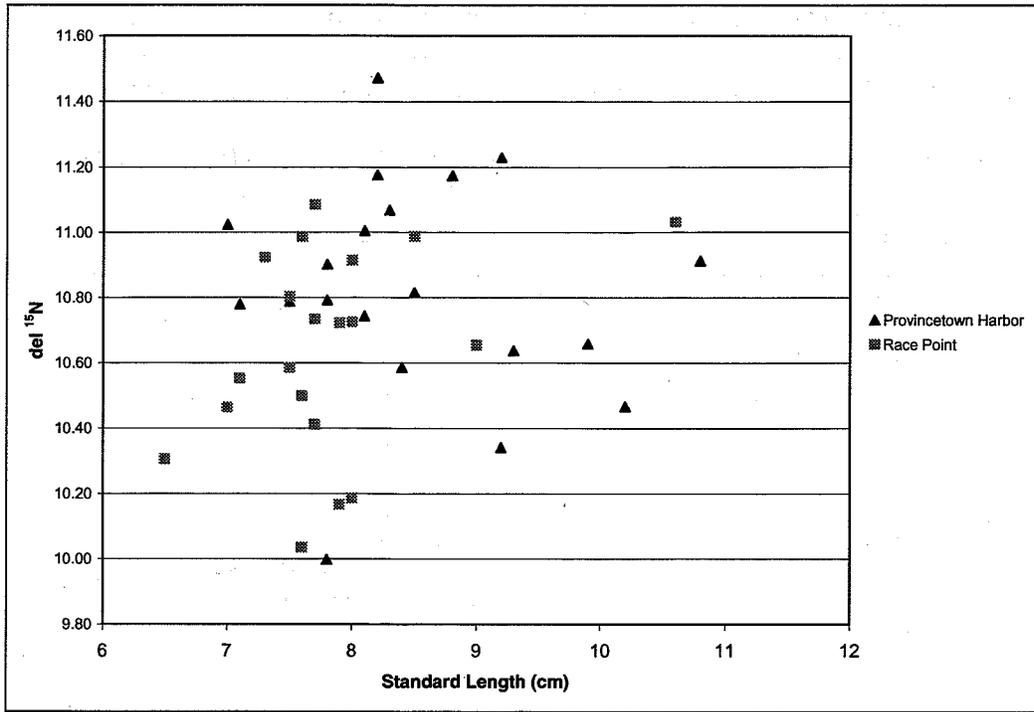


Figure 9. Standard length and corresponding $\delta^{15}\text{N}$ values for each sample taken from Provincetown Harbor (Harbor), Race Point, and the northwest corner of Stellwagen Bank (NW Corner), taken in 2007.

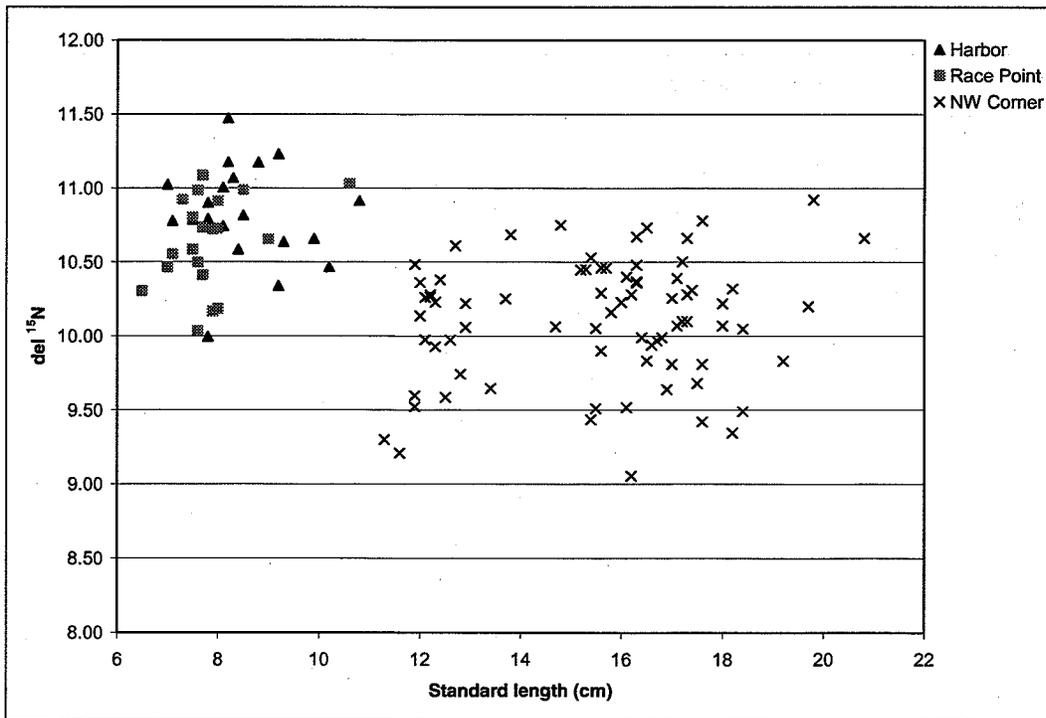
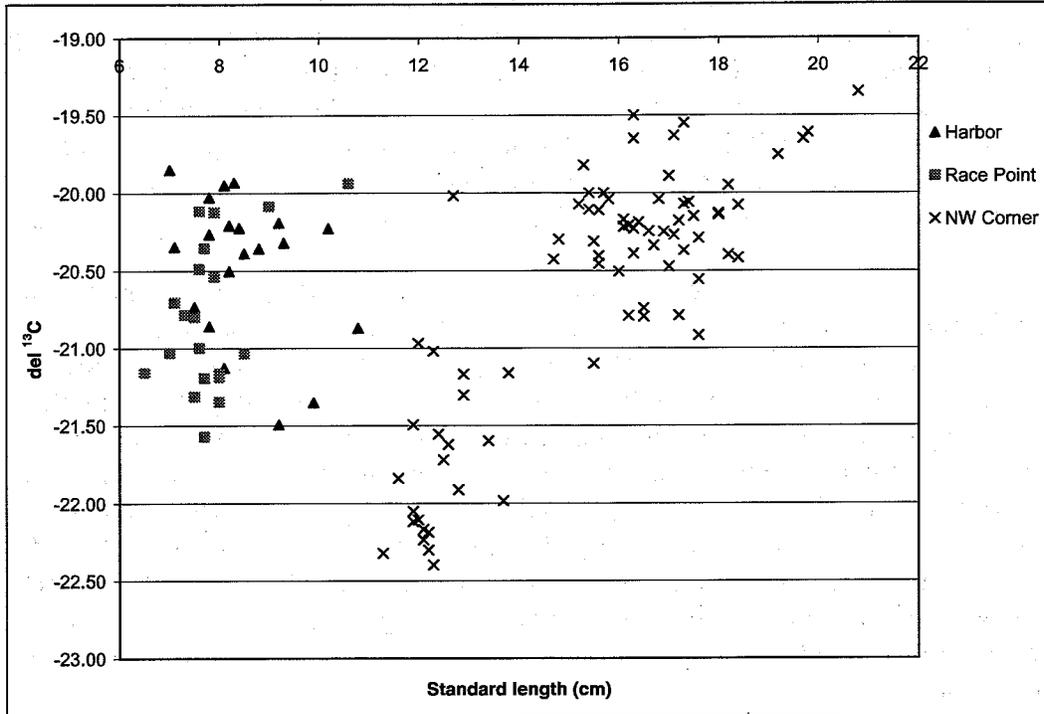


Figure 10. Standard length and corresponding $\delta^{13}\text{C}$ values for each sample taken from Provincetown Harbor (Harbor), Race Point, and the northwest corner of Stellwagen Bank (NW Corner), taken in 2007.



7. DISCUSSION

The NEC-funded work on sand lance of the Provincetown-Stellwagen Bank area resulted in:

- 1) a proven means of systematically capturing sand lance for study
- 2) a ready means to distinguish between the two species using simple hand measurements and inference regarding size and distribution
- 3) a better understanding of the prey capture mechanism of sand lance
- 4) data on diet composition and position in the food web for both species
- 5) The capture, transport, interpretation and exhibition of live sand lance to the public at The New England Aquarium in support of themes related to the Stellwagen Bank National Marine Sanctuary
- 6) New insight into fungal pathogens that attack sand lance and proliferate in captivity
- 7) Novel observations of sand lance behavior in the wild and in captivity, captured with submersible and high-speed video apparatus.

The most surprising outcomes of the research, worthy of follow-up, have to do with the feeding habits of *Ammodytes dubius* on Stellwagen Bank. The discovery and subsequent confirmation

that sand lance feed on hyperiid amphipods in upwelling zones of Stellwagen Bank, at least during the month of September, caused us to considerably rethink our concept of the pelagic food web in the Sanctuary. Hyperiiids are predatory and at maximum size are a large prey item for even adult sand lance. Concurrent with our work on sand lance, we observed humpback whales apparently feeding in hyperiid swarms during short, midwater dives. Subsequent discussion with Mason Weinrich of the New England Whale Center revealed that he had postulated that hyperiids could be an important food source for young of year humpbacks during the fall, at a time when sand lance densities may be falling, and in particular during poor sand lance years. This has led to a long-term collaboration among Boston University, our two research/fishing vessel captains, the Stellwagen Bank National Marine Sanctuary, the Whale Center, and the New England Aquarium to further explore questions related to the ecology of upwelling zones on Stellwagen Bank/Massachusetts Bay and in particular the role of sand lance.

A second important outcome of this work has been the decision by COMPASS in collaboration with the Massachusetts Ocean Partnership to declare Stellwagen Bank-Mass Bay to be Pilot Zone 1 of an experiment in the development of tools to support ecosystem-based management in our region. As a first step in this direction, we have assembled a team of participants to create a dynamic model of the upwelling process and its biological consequences, and the manner in which this process is drawn upon and influenced by various economic sectors (e.g. herring, lobster and groundfisheries, whalewatching, recreational fishing, shipping, pleasure boating). The modeling is being conducted as an exercise in MIMES (Multiscale Integrated Modeling of Ecosystem Services), with the Gund Institute for Ecological Economics, University of Vermont. Furthermore, we have submitted a proposal to the Massachusetts Ocean Partnership to model ecosystem service trade-offs to aid with zoning and conflict resolution through implementation of the Massachusetts Oceans Act.

Although the NEC support was time-limited, it has enabled us to launch a solid project and multiple collaborations capable of attracting additional extramural funds. Most important, however, is that it has enabled us to befriend and engage as close colleagues Bill Lee and Phil Michaud, two of the finest sea captains in New England, or anywhere. We look forward to continued days at sea, and join in hoping for a brighter future for collaborative marine and fisheries research in the Gulf of Maine. We also look forward to collaborating with Bill and Phil in the dissemination of our results in a variety of venues, in addition to seminar presentations that we have made (several delivered by Bill) since the grant's inception.

8. REFERENCES

- Auster PJ and LL Stewart. 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic) – sand lance. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.66). U.S. Army Corps of Engineers, TR EL-82-4. 11 pp.
- Bowman, R.; Warzocha, J.; Morris, T. 1984. Trophic relationships between Atlantic mackerel and American sand lance. International Council for the Exploration of the Sea C.M.H. 27: 1-19.
- Fogarty, MJ, EB Cohen, WL Michaels, and WW Morse. 1991. Predation and the regulation of sand lance populations: an exploratory analysis. ICES Mar. Sci. Symp. 193: 120-124.

- Furness, R.W. 1982. Competition between fisheries and seabird communities. *Advances in Marine Biology* 20: 225-307.
- Garrison, LP and JS Link. 2000. Dietary guild structure of the fish community in the Northeast United States continental shelf ecosystem. *Marine Ecology Progress Series* 202: 231-240.
- Hempel, G. 1978a. Fish eats fish: food chains and catches in the North Sea. *Umschau*. 78(9): 271-276.
- Hempel, G. 1978b. North Sea fisheries and fish stocks—a review of recent changes. *Rapports et Procès-Verbaux des Reunions Conseil International pour l'Exploration de la Mer*. 164: 57-68.
- Hendrickson, R. 1979. Prolific sand eel seen interrupting the northeast Atlantic's food chain. *Natl. Fisherman* 58(6): 14.
- Jensen, AS. 1941. On subspecies and races of the lesser sand eel (*Ammodytes lancea* s. lat.). A contribution to the discussion of the species problem in fishes. *K. Dan. Vidensk. Selsk., Biol. Medd.* 16(9): 33 pp.
- Jensen, AS. 1944. On specific constancy and segregation into races in sea-fishes. *K. Dan. Vidensk. Selsk., Biol. Medd.* 19(8): 19 p.
- Kenney, RD, MAM Hyman, RE Owen, GP Scott, and EP Winn. 1986. Estimation of prey densities required by western north Atlantic right whales. *Mar. Mammal Sci.* 2: 1-13.
- Licciardello, JJ, EM Ravesi, and MG Allsup. Keeping quality of fresh and frozen sand lance, *Ammodytes* sp. *Marine Fisheries Review* 47(1): 78-82.
- Meyer TL, RA Cooper, and RW Langton. 1979. Relative abundance, behavior, and food habits of the American sand lance, *Ammodytes americanus*, from the Gulf of Maine. *Fish. Bull. U.S.* 77: 243-253.
- Monteleone, DM and WT Peterson. 1986. Feeding ecology of American sand lance *Ammodytes americanus* larvae from Long Island Sound. *Marine Ecology Progress Series* 30: 133-143.
- Nizinski, MS, BB Collette and BB Washington. 1990. Separation of two species of sand lances, *Ammodytes americanus* and *A. dubius*, in the western North Atlantic. *Fish. Bull.* 88: 241-255.
- Payne, PM, JR Nicolas, L O'Brien, KD Powers. 1986. The distribution of the humpback whale (*Megaptera novaeangliae*) on Georges Bank and in the Gulf of Maine in relation to densities of the sand eel (*Ammodytes americanus*). *Fishery Bulletin* 84(2): 271-277.

Payne, PM, DN Wiley, SB Young, S Pittman, PJ Clapham, and JW Jossi. 1990. Recent fluctuations in the abundance of baleen whales in the southern Gulf of Maine in relation to changes in selected prey. *Fish. Bull.* 88: 687-696.

Rankine, PW, JA Morrison. 1988. Predation on herring larvae and eggs by sand-eels *Ammodytes marinus* (Rait) and *Hyperopluslancelolatus* (Lesauvage). *Journal of the Marine Biological Association* 69(2): 493-498.

Robards MD, MF Willson, RH Armstrong, and JF Piatt, eds. 1999. Sand lance: a review of biology and predator relations and annotated bibliography. Res. Pap. PNW-RP-521. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 327 p.

Sherman, K, C Jones, L Sullivan, W Smith, P Berrien, and L Ejsymont. 1981. Congruent shifts in sand eel abundance in western and eastern North Atlantic ecosystems. *Nature*. 291(5815): 486-489.

Springer, AM and SG Speckman. 1997. A forage fish is what? Summary of the symposium. In: Baxter, B.R., ed. Proceedings of the symposium on the role of forage fishes in marine ecosystems; [dates of meeting unknown]; [location unknown]. Alaska Sea Grant Program AK-SG-97-01. [Fairbanks, AK]: University of Alaska, Fairbanks: 773-805.

Ward N, 1995. Stellwagen Bank: A guide to the whales, se birds and marine life of the Stellwagen Bank National Marine Sanctuary. Provincetown, MA: Center for Coastal Studies.

Weinrich, M, M Martin, R Griffiths, J Bove and M Schilling. 1997. A shift in distribution of humpback whales, *Megaptera novaeangliae*, in response to prey in the southern Gulf of Maine. *Fishery Bulletin* 95: 826-836.

Winters, GH. 1983. Analysis of the biological and demographic parameters of the northern sand lance. *Ammodytes dubius*, from the Newfoundland Grand Bank. *Canadian Journal of Fisheries and Aquatic Sciences*. 40: 409-419.

9. PARTNERSHIPS

Massachusetts Fishermen's Partnership
New England Aquarium
Boston University Marine Program

10. IMPACTS AND IMPLICATIONS

See Above

11. PRESENTATIONS

Bill Lee presented "Gear Design for Research on the Biology of West Atlantic Sand Lances" at the ICES conference in Boston, 2006.

12. STUDENT PARTICIPATION

Graduate students

Briana Brown, graduate student, Boston University (BU)

Kathryn Kovitvongsa, graduate student, BU

Nick Gidmark, graduate student, Brown University

Undergraduate students

Andrew Fogel, undergraduate student, BU

Clare Hansen, undergraduate student, BU

Lauren Gonzalez, undergraduate student, BU

Raphael Fennimore, undergraduate student, BU

Undergraduate students in BU course BI 546, Marine Megafaunal Ecology of Stellwagen Bank and Surrounding Waters, Fall semesters 2007 and 2008.