A unique shell marker in juvenile, hatchery-reared individuals of the softshell clam, *Mya arenaria* L.

Brian F. Beal
University of Maine at Machias
9 O'Brien Avenue, Machias, Maine 04654
E-mail address: bbeal@acad.umm.maine.edu

Robert Bayer
University of Maine
Hitchner Hall, Orono, Maine 04469

M. Gayle Kraus
University of Maine at Machias
9 O'Brien Avenue; Machias, Maine 04654

Samuel R. Chapman
University of Maine
Darling Marine Center, Walpole, Maine 04573

The ability to identify individual bivalve mollusks in field populations is fundamental to understanding potential population regulatory mechanisms (such as the influence of population density, tidal height, and initial shell size on growth and survival rates, and fecundity schedules). Softshell clams, *Mya arenaria* L., have been commercially harvested from the soft-bottom intertidal zone in Maine since the mid-1800s and form the basis of an extensive fishery along its entire coast. Dramatic declines in landings during the past decade in Maine (Wallace, 1997), however, have resulted in attempts to use hatchery-reared juveniles to supplement wild stocks (Beal, 1994). In the past, distinguishing between cultured and wild bivalves in the field in order to follow their fate has been performed by using alizarin staining techniques (Newell and Hidu, 1982), by tagging individuals (Brousseau, 1979), or by applying colored marks (e.g. paint dots) to the valves (Peterson and Beal, 1989). Here, we describe a natural and unique shell marker for juvenile, hatchery-reared softshell clams that forms on the outer valves of individuals once they are placed in the field. The distinctive mark appearing on the surface of each valve obviates the need to apply physical tags to individuals and also eliminates the stress that small clams otherwise undergo when being tagged.

Methods

Clams were reared during the summer of 1983 at a 4-H clam hatchery in Jonesboro, Maine, and were held in running seawater in sediment-free trays at the Darling Marine Center, Walpole, Maine, until 25 May 1984. On that date, 5000 individuals (mean shell length [$\bar{x}_{SL}$ ±1 SD]=10.4 ±1.52 mm; range= from 6.7 to 14.6 mm; n=200) were transported back to the Jonesboro hatchery where the clams were divided into five groups of 100, 200, 300, and 400 individuals (i.e. a total of 20 groups). All but ten individuals from each group (20 groups× 10 individuals=200 clams) were marked with a single, group-specific, color-coded dot (Mark-Tex Corp. paint) on both valves near the umbo. The remaining 200 clams were painted with two dots to ensure individual recognition and measured (greatest shell length) to the nearest 0.1 mm by using vernier calipers. Marking was performed at this time because it was not known whether a distinctive mark would form naturally on the surface of each valve once clams began adding new shell in the field. Clams were maintained overnight without seawater at the University of Maine at Machias in a walk-in cooler (ca. 5°C).

On 25 May 1984, a matrix consisting of two rows of ten 0.25-m^2 plots was established near the midtide level of an intertidal flat (the Narrows) located along the western shore of the Chandler River (44°39'04"N; 67°33'10"W) near the town of Jonesboro, Maine. Sediments consisted of poorly sorted muds with a graphic mean ±1 SD of 3.6 ±0.34ϕ (n=2). The matrix was located approximately 55 m from the extreme high water mark and 35 m from the mean low water mark. Both rows were parallel to the shore and were spaced 1 m apart. Black rigid-mesh enclosures (DUPONT Vexar, 6.4 mm aperture), approximately 0.25 m^2 and 15-cm wide, were installed around each plot to restrict lateral movements of the small clams (Baptist, 1955). Because the enclosures had no mesh roofs, they allowed epibenthic and infaunal predators (*sensu* Commito and Ambrose, 1985) access to the clams (Peterson and Beal, 1989). The mesh of each enclosure wall was

Manuscript accepted 5 May 1998.
Fish. Bull. 97:380–386 (1999).

attached by staples to a 40 × 2.5 × 2.5 cm wooden stake at each corner. This square, fenced assembly was then pushed 8 to 10 cm into the sediments leaving a mesh wall projecting from 5 to 7 cm vertically.

Groups of clams were assigned randomly to the open enclosures on 26 May 1984. This completely random design resulted in five replicates of each of four evenly spaced planting densities ranging from 400 to 1600/m^2. The fate of the 200 clams marked with two dots is reported here (see Beal [1994] for results of increasing intraspecific density on the fate and growth of the remaining clams). To ensure that seagulls (*Larus* spp.) did not prey on the clams before they had the opportunity to burrow, one of us remained at the site until the tide had completely covered the clams and the enclosures. During this interval (ca. 2 hours) clams burrowed into the sediments approximately 20 minutes after planting. No clam was visible at the sediment surface at the time of tidal inundation.

Live and dead clams were recovered from each of the twenty open enclosures after 99 days (1 September 1984) by collecting the top 15 cm of sediment from each enclosure and sieving the sediment through a 0.5-mm mesh. For each live clam (marked with two dots or not), an obvious mark parallel to the entire shell circumference was visible on the shell at or near the size the animal was on 26 May 1984 (see "Results" section). This mark permitted us to estimate a shell growth index for every live clam:

$$\textit{Relative growth index} = [(\textit{final length} - \textit{initial length}) / \textit{initial length}] \times 100.$$

Index values greater than 100% indicate at least a doubling in growth.

Shells from this 1984 field experiment were not saved, but results from subsequent field trials in eastern Maine with hatchery-reared softshell clam juveniles (Beal, 1994) revealed that this shell mark remains visible for periods up to 1.5 years after planting. We used scanning electron microscopy (SEM) to describe the gross shell structure across the distinctive pre- and postplanting regions of the outer shell of juvenile, hatchery-reared clams. We used the valves of cultured clams (initial size similar to those reported above) that had been reared during 1989 at the Beals Island Regional Shellfish Hatchery (BIRSH), Beals, Maine, planted at the Narrows in May 1990 and recovered in October 1990. We also used SEM to examine the outer shell structure of wild juvenile clams (ca. 15 mm) taken from a nearby mudflat in Roque Bluffs, Maine, in September 1992.

Results

When viewed macroscopically, every live hatchery-reared clam taken on 1 September 1984 (regardless whether or not it had been marked with two dots) had a distinct line on its shell surface that appeared to correspond to its size at planting on 26 May 1984. Scanning electron microscopy (SEM) of the gross shell structure of wild juvenile clams (Fig. 1, A and B) revealed sharp ridges (=lines viewed macroscopically) approximately 15-microns apart punctuated by a relatively smooth region. In sharp contrast, the valves of cultured clams (Fig. 1, C and D) consistently revealed three distinct zones: an inner region from the umbo to a distinct area (line) of demarcation that was approximately 50-microns in width (Fig. 1D) and an outer zone from that line to the ventral margin. The inner zone was characterized by a pitted, amorphous surface with interrupted ridges, whereas the outer zone of the cultured clams (Fig. 1C) appeared similar to the entire surface of wild clams. Observations from experimental or stock enhancement outplantings of cultured clams from BIRSH in numerous coastal communities in Maine since 1987 indicate that the shell mark may be present for up to 1.5 years after planting (Beal, 1994).

To test whether the obvious line, or shell mark, could be used quantitatively to distinguish initial size at planting from subsequent shell growth, we examined closely the 57 surviving clams of the 200 that had been marked with two dots on 25 May 1984. We used calipers to estimate the initial size of the clam delimited by the obvious surficial shell mark (line). Next, we compared, for each clam, this estimate of initial (i.e. predicted) shell length with the one recorded on 25 May. If the hypothesis that the line is formed at or near the time of transplanting is correct, then the mean difference between actual (i.e. recorded) and estimated size should not be significantly different from zero. Results of a one-sample, two-tailed *t*-test demonstrated that the average difference was not significantly different from zero (–0.047 mm; t=–1.505; P=0.138; n=57). An alternative statistical approach is to plot the predicted initial shell length (y-axis) against the actual initial shell length (x-axis; Fig. 2) and to determine whether the straight line (y= –0.229 + 1.017x; r^2=0.97) is significantly different from the line $y = x$. The slope of the least squares equation for that line was not significantly different from unity (F=1.38; df=2, 55; P=0.26), and the intercept was not significantly different from zero (F=0.81; df=1, 55; P=0.37).

The mean relative growth index for the period 26 May to 1 September for the 57 marked survivors was 86.9 ± 2.26% SE (Fig. 3A). Mean final length of the same in-

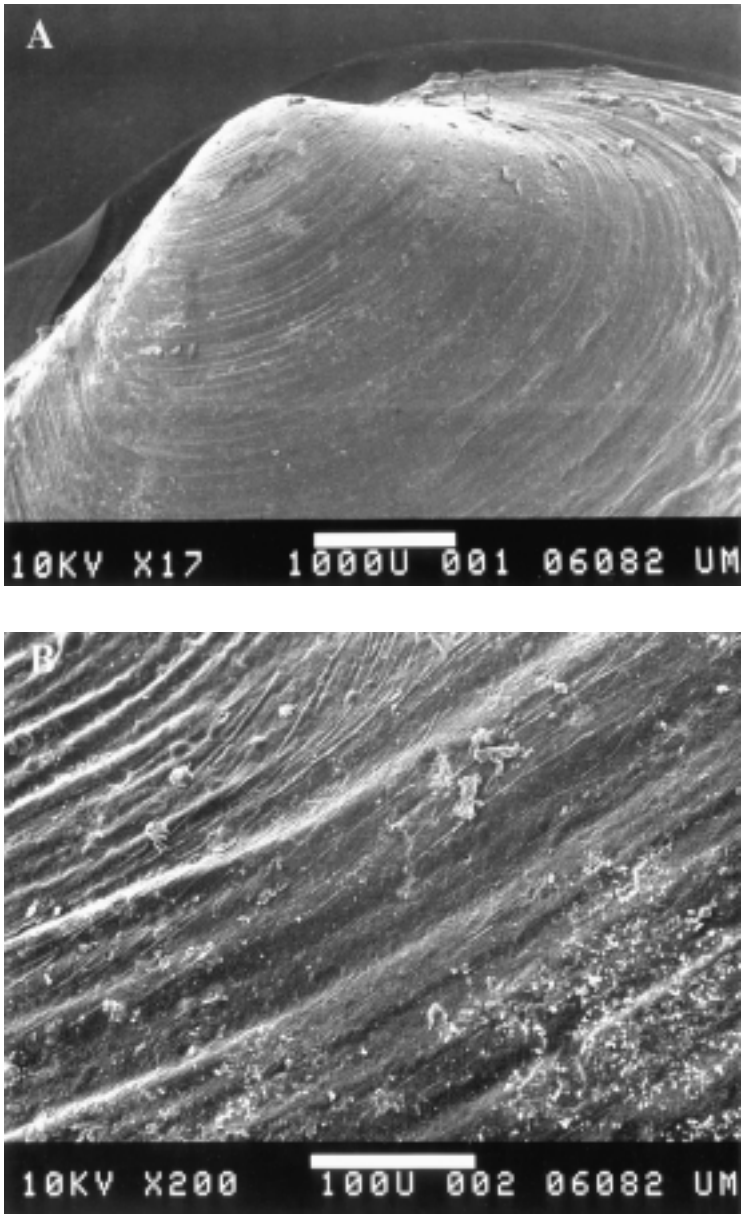

Figure 1

Scanning electron microscopy (SEM) of the shell surface of a wild (**A** and **B**) and cultured (**C** and **D**) juvenile softshell clam, *Mya arenaria* (length ca. 15 mm). The wild clam was sampled from a mudflat in Roque Bluffs, Maine, in September 1992. The cultured clam was reared at the Beals Island Regional Shellfish Hatchery and planted in May 1990, in a mudflat at the mouth of the Chandler River, near the town of Jonesboro, Maine. Photo B, taken near the umbo region of the wild clam, shows uninterrupted concentric ridges and a lack of pits and amorphous grooves. Photo C is an example of "the hatchery mark." It is a continuous, ca. 50–micron groove that extends along the ventral slope (anterior to posterior) of both valves and appears at that point on the shell surface associated with the time the animal leaves the hatchery and is placed in the soft-bottom benthos. Photo D, was taken along the anterior–posterior groove that appears at the interface before the period when new shell is deposited once the individual is transplanted to the field. The white bar indicates a length of 1000 μ for A and C and 100 μ for B and D.

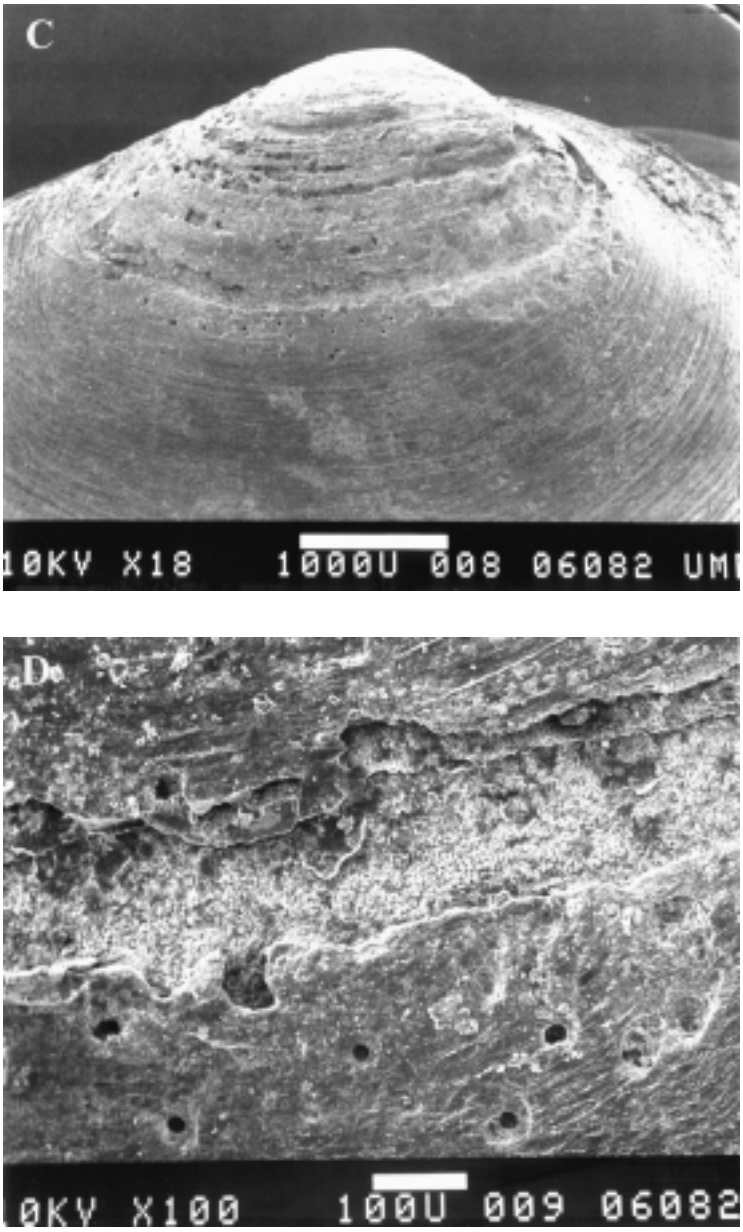

Figure 1 (continued)

dividuals was 19.6 ±0.24 mm SE (Fig. 3B), which equates to an average growth rate of 0.093 mm/day.

Discussion

Our data provide evidence of a distinctive mark or line being formed on the outer surface of the valves of juvenile hatchery-reared softshell clams. We have observed from other field tests and stock enhancement trials in Maine that the mark may be present for up to 1.5 years after planting, after which, the line disappears as the umbo region of the shell becomes increasingly worn, presumably on account of sediment abrasion from burrowing or seasonal repositioning (Zwarts and Wanink, 1989).

The formation of the line on the shell surface between the umbo and the ventral margin allows one to distinguish easily between wild and cultured juvenile clams and can be a valuable tool for resource

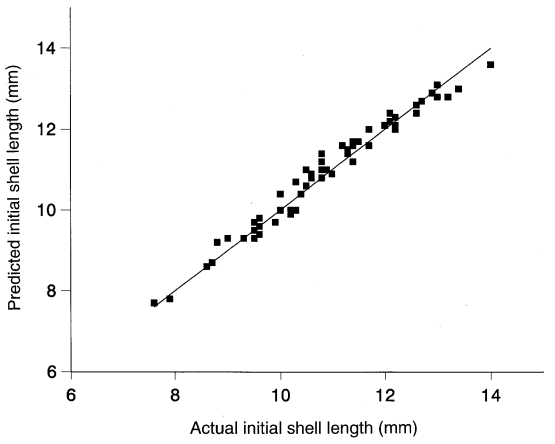

Figure 2

Predicted initial shell size versus actual initial shell size for the 57 hatchery-reared individuals of *Mya arenaria* marked with two dots that survived in open 0.25-m^2 field enclosures during the period from 26 May to 1 September 1984.

managers wishing to assess the fate of hatchery seed for stock enhancement purposes (Beal, 1994). In addition, it obviates the need to mark (physically or chemically) individuals or a group. If initially unmarked, juvenile, hatchery-reared softshell clams are planted in the field and examined within an 18-month period, the distinctive shell mark allows one 1) to determine initial planting size of individual hatchery-reared clams (Fig. 2); 2) to determine relative or absolute growth rates (Fig. 3, A and B); and 3) to estimate the time (e.g. season) when death occurred for those individuals that suffered mortality after planting. Similar disturbance lines were observed on juvenile, hatchery-reared northern quahogs, *Mercenaria mercenaria* (L.), planted from the laboratory to various subtidal and intertidal sites in North Carolina (Beal, 1983).

The origin of the shell mark may be related to conditions within the hatchery environment. After the field experiment described above, cultured softshell clam juveniles from BIRSH were planted in intertidal flats along the coast of Maine (1987–94) during the months of April through October for both manipulative tests and community-based stock enhancement efforts (Beal, 1994). In addition, cultured clam stock produced in a commercial shellfish hatchery (Mook Sea Farm, Inc., Walpole, Maine) were used in stock enhancement programs in Gloucester and Ipswich, Massachusetts (Whitten[1]). Regardless of

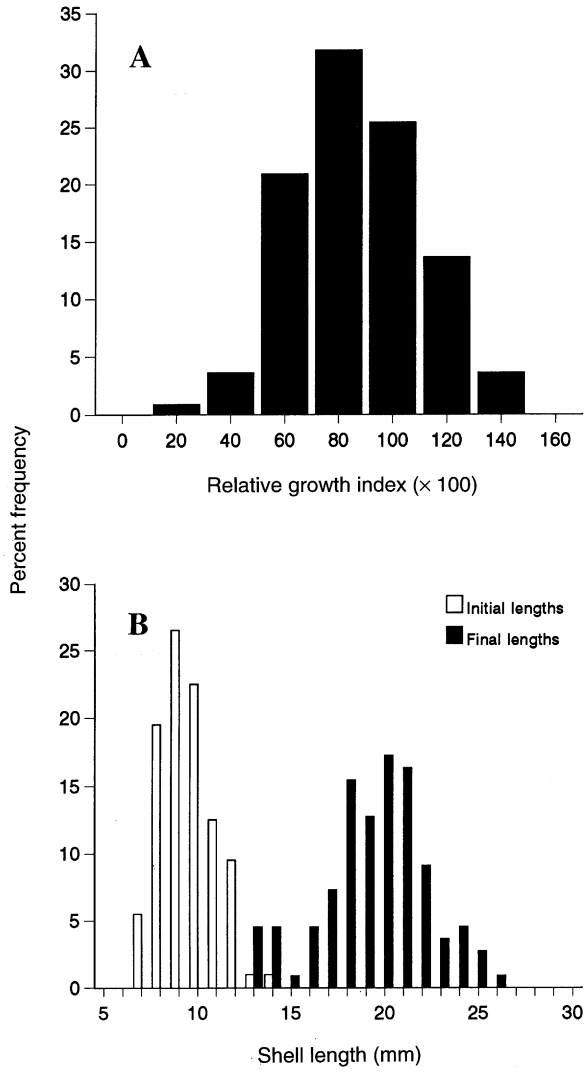

Figure 3

(**A**) Frequency distribution of relative growth rate index for the 57 hatchery-reared softshell clams marked with two dots (26 May to 1 September 1984). An index of 100% indicates a doubling of shell length. (**B**) Frequency distribution of initial (26 May 1984; n=200) and final shell lengths (1 September 1984; n=57) of hatchery-reared softshell clams.

hatchery origin, the same line appeared on the shells of all survivors planted in the soft-bottom intertidal zone at all locations, as well as on shells of those that grew before dying.

The mark has been observed in clams planted at shell sizes as small as 3 mm and as large as 25 mm (Beal, 1994). In addition, this mark appears regardless whether clams are transplanted to the field directly from the hatchery or are overwintered in submerged, floating cages (*sensu* Beal et al., 1995) before transplanting to the field.

[1] Whitten, J. 1996. Merrimak Valley Planning Commission, Haverhill, MA. Personal commun.

Although not specifically tested, at least two competing hypotheses may explain the mechanism that creates this shell marker. The first hypothesis is disturbance, for clams in the hatchery are grown in sediment-free trays with fine-mesh screening (150–1500 μ), handled (sieved) weekly, fed tropical species of microalgae such as *Isochrysis galbana* (Tahitian variety), and are grown at temperatures between 20 and 25°C—well above those normally experienced in the wild in eastern Maine. Once they have left the hatchery environment, clams are planted on flats and immediately burrow into sediments where 1) hatchery-produced disturbance ends, 2) the clams begin feeding on natural phytoplankton assemblages, and 3) they experience seawater temperatures that typically do not exceed 17°C. The new shell that grows is always distinctly whiter than the older shell. This difference in coloration may indicate a release from competition for calcium or aragonite which may be limited under hatchery conditions (Barber[2]). The second hypothesis is bacterial damage that may also relate to conditions in the hatchery where elevated levels of marine bacteria such as *Vibrio* spp. frequently occur. Qualitative tests for the presence of *Vibrio* spp. (using Difco TCBS agar [Elston et al., 1981]) at BIRSH are made regularly and show a general presence of these bacteria in the seawater within tanks holding juvenile clams, in the cultured algae, and on the valves of juvenile clams. These gram-negative, oxidase-positive, fermentive rods have been observed similarly in commercial bivalve hatcheries in the coastal northeastern United States (Elston, 1984) where they have been described as coating the shell surface of cultured juvenile northern quahogs, oysters, *Crassostrea virginica* (Gmelin), *Ostrea edulis* L., and bay scallops, *Argopecten irradians* (Lamarck). Bacteria in the family Vibrionaceae can erode and perforate areas on the surface of bivalve shells through the production of a variety of acidic metabolites that are inimical to normal deposition of calcium carbonate (Elston et al., 1982). The SEM photograph of the valve of the hatchery-reared softshell clam (Fig.1, C and D) clearly shows pitting and amorphous grooves that may indicate a bacterial origin.

During the past decade, clam landings in eastern Maine have declined by nearly 75% (Wallace, 1997). Communities that manage their clam stocks in this and other regions along the coast are beginning to use cultured softshell clams to enhance clam production. Testing the biological and economic efficacy of hatch-and-release programs is critical for the development of sensible management programs. Results presented here demonstrate the ease of distinguishing cultured from wild *Mya* and will allow scientists as well as clam harvesters a rapid assessment of field planting programs.

[2] Barber, B. 1994. University of Maine, Orono, ME. Personal commun.

Acknowledgments

Support for this work was provided by a Maine/New Hampshire Sea Grant research grant to B. F. Beal and M. G. Kraus (R/FMD-191; NA89AA-D-SG020). Additional support was provided by the Department of Animal, Veterinary, and Aquatic Sciences at the University of Maine and by the University of Maine at Machias. We thank all those that helped in the 4-H hatchery and in the field, including M. Alley, A. Chapman, J. Chapman, J. Cox Jr., J. Cox Sr., D. Engels, C. Gay, R. Look, S. Renshaw, and T. White. We also thank W. Congleton, S. Fegley, R. Hawes, A. Lewis, R. Vadas Sr., K. Vencile, and three anonymous reviewers for their useful comments on previous drafts of this note.

Literature cited

Baptist, J. P.
1955. Burrowing ability of juvenile clams. U.S. Fish Wild. Serv. Spec. Sci. Rep. 140:1–13.

Beal, B. F.
1983. Effects of environment, intraspecific density, predation by snapping shrimp and other consumers on the population biology of *Mercenaria mercenaria* near Beaufort, North Carolina. M.S. thesis, Univ. North Carolina, Chapel Hill, NC, 180 p.
1994. Biotic and abiotic factors influencing growth and survival of wild and cultured soft-shell clams, *Mya arenaria* L., in eastern Maine. Ph.D. diss., Univ. Maine, Orono, ME, 499 p.

Beal, B. F., C. D. Lithgow, D. P. Shaw, S. Renshaw, and D. Ouellette.
1995. Overwintering hatchery-reared individuals of the soft-shell clam, *Mya arenaria* L.: a field test of site, clam size, and intraspecific density. Aquaculture 130:145–158.

Brousseau, D. J.
1979. Analysis of growth rate in *Mya arenaria* using the von Bertalanffy equation. Mar. Biol. 51:221–227.

Commito, J. A., and W. G. Ambrose Jr.
1985. Predatory infauna and trophic complexity in soft-bottom communities. *In* P. E. Gibb (ed.), Proceedings of the nineteenth European marine biology symposium, p. 323–333. Cambridge Univ. Press, Cambridge, England.

Elston, R.
1984. Prevention and management of infectious diseases in intensive mollusc husbandry. J. World Maric. Soc. 15:284–300.

Elston, R., E. L. Elliot, and R. R. Colwell.
1982. Conchiolin infection and surface coating *Vibrio*: shell fragility, growth depression and mortalities in cultured oysters and clams, *Crassostrea virginica*, *Ostrea edulis*, and *Mercenaria mercenaria*. J. Fish Diseases 5:265–284.

Elston, R., L. Leibovitz, D. Relyea, and J. Zatila.
1981. Diagnosis of vibriosis in a commercial oyster hatchery epizootic: diagnosis tools and management features. Aquaculture 24:53–62.

Newell, C. R., and H. Hidu.
1982. The effects of sediment type on growth rate and shell allometry in the soft-shell clam *Mya arenaria* L. J. Exp. Mar. Biol. Ecol., 65:285–295.

Peterson, C. H., and B. F. Beal.
1989. Bivalve growth and higher order interactions: importance of density, site, and time. Ecology 70:1390–1404.

Wallace, D. E.
1997. The molluscan fisheries of Maine. *In* C. L. MacKenzie Jr., V. G. Burrell Jr., A. Rosenfield, and W. L. Hobart (eds.), The history, present condition, and future of the molluscan fisheries of North and Central America and Europe, vol. 1, Atlantic and Gulf coast, p. 63–86. U.S. Dep. Commer., NOAA Tech. Rep. 127.

Zwarts, L., and J. Wanink.
1989. Siphon size and burying depth in deposit- and suspension-feeding benthic bivalves. Mar. Biol. 100:227–240.