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Overwintering hatchery-reared individuals of the soft-shell clam, *Mya arenaria* L.: a field test of site, clam size, and intraspecific density

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Abstract

Soft-shell clams (*Mya arenaria* L.) are a commercially important intertidal bivalve in Maine, USA where they are managed as a common property resource. Ten million 8–12 mm clam juveniles are reared annually at the Beals Island Regional Shellfish Hatchery and are transplanted onto publicly-owned mudflats during the fall or early winter. The success of this strategy is unpredictable because of sporadic occurrence of winter ice formation and subsequent scouring of the intertidal zone. These events, in addition to other severe winter storms, can result in complete mortality of the transplanted seed. Transplanting hatchery-reared seed in the spring, therefore, is preferable, but until now there has been no economically effective technique to hold millions of soft-shell clam seed over the winter. A field experiment designed to test the interactive effect of clam size and density on survival was conducted during the winter of 1991–1992 at a sheltered and exposed site near Beals, Maine, USA. Three sizes of hatchery-reared clam seed (Small, $\bar{X}=4.3$ mm shell length [SL]; Medium, 8.2 mm SL; Large, 11.5 mm SL) were used. Clam density was approximate and depended on clam size (Small, 18 750 ind, 37 500 ind, and 56 250 ind; Medium, 11 000 ind and 22 000 ind; Large, 4500 ind and 9000 ind). Clams of each size/density combination were added separately to nylon window screen, zippered bags (aperture = 1.8 mm). Bags containing clams were housed in wooden-framed subunits (0.45 m × 0.45 m × 0.08 m) covered with a 12 mm extruded mesh netting. Subunits were arranged vertically in groups of 4–6 and were submerged 1 m above the bottom in shallow water (3–8 m) from November 1991 to April 1992. Mean survival of medium and large size clams at both sites was 97.7%. Significant density effects were detected for both size groups at the sheltered site, although the difference between treatment levels was less than 2%. Mean survival of small clams pooled across sites was 67.8%. At the sheltered site, clams in the uppermost level of the overwintering units had

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consistently lower survival rates than those nearer the bottom. Density effects for small clams were detected only at the sheltered site where animals held at the highest density had significantly higher survival rates than animals at either of the two lower densities (75.3% vs. 60.3%). Clam loss over the winter may be reduced by suspending seed in seawater instead of transplanting it to intertidal flats.

Keywords: *Mya arenaria*; Overwintering; Density

1. Introduction

In the state of Maine, USA, soft-shell clams, *Mya arenaria* L., are a public resource and traditionally support the second most economically important marine fishery behind only lobsters, *Homarus americanus* H. Milne Edwards (NMFS, 1991). Historically, between 50% and 75% of clams landed in the state are harvested from publicly-owned intertidal flats nearest the Bay of Fundy in the eastern two counties of Hancock and Washington. From 1982 to 1991, the combined annual landings from those two counties decreased 75% from 308 200 bushels to 77 000 bushels (Maine DMR, 1992). The apparent reason for the decline is related to the paucity of newly settled spat (B. Beal, pers. obs.). Attempts to manage the resource using traditional methods have failed because these trials depend on high (i.e. successful) interannual natural recruitment (*sensu* Keough and Downes, 1982). This has lead to the development of stock enhancement programs in several communities in eastern Maine using hatchery-reared juveniles. A small-scale public shellfish hatchery and management program was established in 1987 in the town of Beals, Maine.

The Beals Island Regional Shellfish Hatchery (BIRSH) produces 10 million soft-shell clam seed (8 to 12 mm shell length — greatest anterior to posterior measurement [SL]) each year to distribute among ten coastal communities. Broodstock are spawned in the early spring and juveniles are kept in the hatchery until they have reached 3 mm SL. During May through July, groups of 15 000 3-mm clams are placed into wooden trays (1.2 m × 0.9 m × 7.6 cm deep) lined with a nylon window screening (1.8 mm aperture). Trays are taken to a protected, deep-water cove 3 miles from BIRSH where they remain floating in the top few cm of water until late October. Seawater temperatures during this time range from 8° to 16°C. At that time, clams (mean size ca. 12 mm SL) are removed from the trays and are transplanted onto intertidal mudflats, but, because seawater temperatures during November through late March fall below 4°–5°C, all somatic growth ceases (Newcombe, 1936).

For benthic bivalves like *Mya*, the risk of predation or removal from the sediments via abiotic factors decreases as the animals attain sizes larger than 30 mm SL by burrowing deeply (Blundon and Kennedy, 1982; Commito, 1982). Individuals of *Mya arenaria* < 20 mm SL live in the upper 3 cm of the substrate (Zwarts and Wanink, 1989); therefore, when transplanted in the fall, the small hatchery-reared soft-shell clams maintain their position within the substrate at extremely shallow depths (ca. 1 cm) for nearly 6 months. Investigations conducted at several intertidal mudflats in eastern Maine from 1989 to 1992 (Beal, 1991; Beal and Kraus, 1991) suggest that transplanting clams in the fall is problematical because the surface sediments of many flats become scoured by ice floes during late January through early March. Scouring either removes clams from the seeded site, or crushes and kills them. Ice may also gradually build up on some mudflats reaching ≥ 1 m by late

February (B. Beal, pers. obs.). Under these conditions, the upper few centimeters of mudflat freezes to the bottom of the ice sheet. When the ice leaves the flat, it rafts this sediment (and all its residents, including small clams) to areas usually away from the flat depending on the direction of the wind. It is not possible to predict in late fall which mudflats will be inundated by ice during the winter. Therefore, deployment of seed in spring is preferable to fall deployment.

Until recently, keeping clams alive during the winter months without transplanting them to the field was not an option. Holding clams in the floating trays during the winter months poses problems because the surface water in the upper portion of the cove usually freezes resulting in the production of icebergs (ca. 5 to 10 m diameter × 2–3 m thick) which could plow through lines of trays and easily destroy them. Keeping 10 million 8–12 mm clams inside the hatchery during the winter is cost prohibitive.

Here, we report results of an attempt to keep millions of hatchery-reared soft-shell clam seed alive over the winter months in submerged, floating units. Specifically, we tested the interactive effects of clam size and stocking density (volume) on the survival success of juveniles of *M. arenaria* held at various depths in the water column at two shallow subtidal sites near Beals, Maine from November 1991 to April 1992.

2. Methods and Materials

A pilot attempt to keep alive hatchery-reared individuals of *Mya arenaria* over the winter occurred at BIRSH during the fall of 1989. A wooden frame (0.9 m × 0.6 m × 0.45 m deep) was built using 5 cm × 10 cm spruce lumber and was lined on the outside with a heavy (extruded) 12 mm mesh netting. Four subdivisions, or compartments, were constructed within the framed unit: each measured 0.45 m × 0.3 m × 0.45 m deep. Inside the top of this enclosed box, several strips of styrofoam were added to ensure positive buoyancy. Hatchery-reared juveniles (4–12 mm SL) were placed into each compartment inside of a sealed, nylon screen bag (1.8 mm aperture and measuring 0.45 m × 0.45 m). The volume of seed added to each bag was approximately 30–50% of the volume of the mesh bag. Fifteen boxes, housing 2 million clams, were deployed during late November and early December 1989, in about 3–5 m (low tide; tidal range ca. 4 m) of water at Mud Hole Cove (MHC) — a protected site (ca. 1.2 km long × 110 m wide) located near Beals, Maine (44° 30′ N; 67° 35′W). Each wooden box and its four sealed bags of clams was anchored to its own cement weight so that the bottom of the unit was 1–2 m above the mud bottom regardless of the tidal cycle. A line ending in a surface buoy was attached to the top of each box. Boxes remained in the water column until April 1990.

More than 90% of the clams survived in the pilot study and a similar test was repeated in 1990. Nearly 4 million clams were deployed at MHC in December 1990, but in April 1991, when the sealed bags were inspected, approximately 99% of the clams had died. Many of the bags had filled with mud indicating that the boxes may have been placed too close to the bottom or that runoff from the nearby intertidal portion of the cove had overwhelmed the clams' ability to withstand periods of heavy siltation. Neither the 1989–1990 nor the 1990–1991 overwintering test was conducted using an experimental design that would allow an unambiguous assessment of which factor(s) was(were) important in successfully overwintering these clams. It seemed imperative that the mechanisms resulting in success one winter and complete failure the next be investigated because field grow-out trials indicated that successful overwintering followed by spring seeding could significantly increase first-year clam survivorship (Beal, 1991).

During the fall of 1991, two sites, one within MHC and the other near the mouth of MHC, called Weir site (WS) because of its proximity to an unused fish weir, were chosen based on differences in exposure to wind, waves, and storms. At MHC the bottom consisted of soft, poorly sorted mud (*sensu* Folk, 1974). Water depths at low tide varied from 3 to 5 m. The WS, less than 600 m from MHC, was deeper (range between 5 to 8 m at low tide) and more exposed. Substrates there were muddy, but, according to divers, firmer than those in MHC so presumably contained less silts and clays.

Beginning November 1991, approximately 3.2 million clams produced at BIRSH during the summer of 1991 were divided into three groups based on size — $\bar{X}_{Large} = 11.5$ mm ± 0.01 s.e., $n = 269$ [4.5/ml]; $\bar{X}_{Medium} = 8.2$ mm ± 0.01 s.e., $n = 344$ [17.1/ml]; $\bar{X}_{Small} = 4.3$ mm ± 0.01 s.e., $n = 449$ [75.0/ml]) (Fig. 1). New overwintering containers were designed for the 1991–1992 test. The design (Fig. 2) resembled a rigid lantern net and consisted of either four or six, square, wooden subunits stacked on top of each other approximately 5 cm apart. These subunits (0.45 m × 0.45 m × 0.08 m) comprised an overwintering container. Subunits, made using spruce strapping (1.9 cm × 7.6 cm), were covered with 12 mm extruded mesh netting (Internet Inc., North Minneapolis, MN). Each container for overwintering small clams consisted of six subunits rigidly fastened together with a piece of strapping on two sides. Overwintering containers holding medium and large clams consisted of four subunits (see below).

Clams were added to nylon screen bags (as described above) with a sewn, nylon zipper. A single bag of clams was placed inside each subunit. Small clams were added to bags at one of three approximate densities (volumes): 250 ml/bag, or 18 750 ind; 500 ml/bag, or 37 500 ind; and 750 ml/bag, or 56 250 ind. Each subunit within an overwintering container held clams from only one size group. Approximately 50 periwinkles (*Littorina littorea* L., shell height ca. 20 mm) were added to both the subunit and bag containing clams because the feeding activity of these herbivorous snails reduces algal fouling. Four round styrofoam

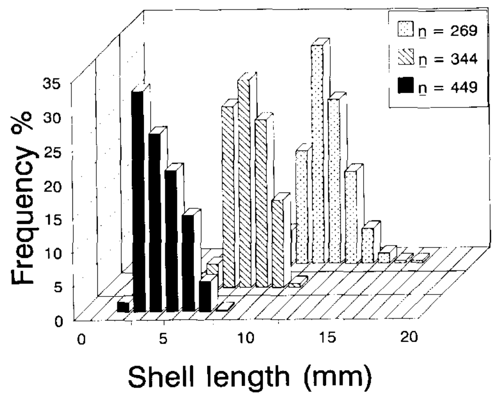

Fig. 1. Initial size-frequency distributions of hatchery-reared juveniles of *Mya arenaria* used in the overwintering field trials. $\bar{X}_{Small} = 4.3$ mm ± 0.06 s.e.; $\bar{X}_{Medium} = 8.2$ mm ± 0.06 s.e.; $\bar{X}_{Large} = 11.5$ mm ± 0.09 s.e.

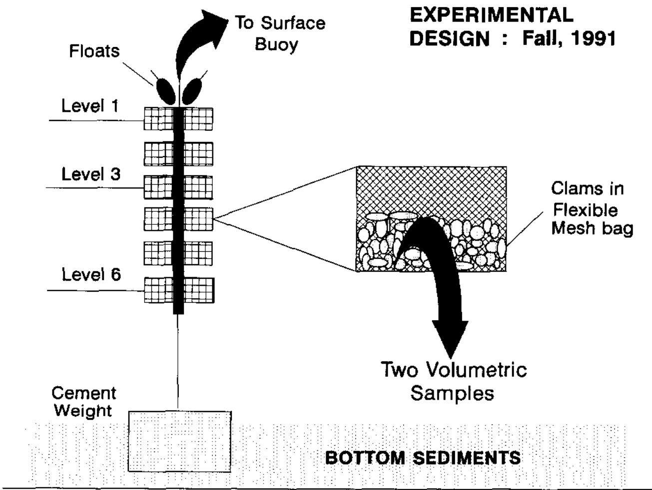

Fig. 2. Schematic of overwintering units used during the 1991–1992 field experiment. Units containing six levels were used for "Small" clams whereas units containing four levels were used for "Large" and "Medium" size clams.

floats (14 cm diameter × 8 cm wide) were attached near the top of the uppermost subunit of each container while one cement weight (0.35 m × 0.25 m × 0.25 m) was attached by rope through a hole in each of the two pieces of strapping that extended several cm below the bottommost subunit. This arrangement kept each overwintering container 1–1.5 m off the bottom regardless of tidal cycle. On some spring tides, the uppermost level of some containers may have contacted the air/surface water interface during low tide, but usually, all containers were submerged from the time of deployment on 21 November 1991, when seawater temperature was 5.5°C, to the time when they were retrieved on 28 April 1992 (seawater temperature = 4.5°C). Containers were haphazardly arrayed within a 75 m × 75 m location at each site.

Six containers per site (12 total) were deployed for holding small clams within each subunit (i.e., two containers with 250 ml/bag per subunit, two with 500 ml/bag per subunit, and two with 750 ml/bag per subunit). The number of subunits per container for large and medium size clams was reduced to four because fewer clams were available. In some cases, the lack of sufficient quantities of clams did not permit replication for a given density treatment. For large clams, two densities (volumes) were used: 1.13 l of clams/nylon bag and 2.25 l of clams/nylon bag (approximately 4500 and 9000 clams/bag, respectively). One container of each density of large clams was deployed at the beginning of the test at both sites. For medium size clams, bags were either stocked with 1 l, ca. 11 000 individuals or 2 l, ca. 22 000 individuals. At MHC, one container with medium size clams at the low density and two containers of clams at the high density were deployed in November 1991. At WS, two containers of medium size clams at the low density and one container with clams at the high density were established.

All units were retrieved on 28 April 1992. Two random 5 ml samples were taken from each nylon bag (representing a specific combination of site, level, clam size, and density) and clams sorted into living and dead categories. Dead clams were assigned two separate categories: intact individuals and disarticulated valves.

Statistical analyses

All statistical analyses are based on the percent survival from each sample. (Clams exhibited no shell growth during the overwintering interval.) Survival was determined by the following formula:

$$\text{Survival} = (\#\text{ Live animals})/(\#\text{ Live animals} + \#\text{ dead with intact shells} + [\#\text{ single, disarticulated valves}/2]) \times 100.$$

All statistical analyses of survivorship data were performed on the arcsine of the square root of the percent alive (arcsine $\sqrt{P}$). For small clams at each site the following linear model was used:

$$Y = Mu + A_i + B_j + AB_{ij} + C(A \times B)_{k(ij)} + e_{l(ijk)}$$

where: Y = transformed percent alive data, Mu = theoretical mean, and A_i = Density (250 ml/bag; 500 ml/bag; 750 ml/bag); B_j = Level (1 = Top...6 = Bottom); AB_{ij} = Density × Level (interaction); $C(A \times B)_{k(ij)}$ = Container nested within the Density × Level interaction; $e_{l(ijk)}$ = experimental error.

Several pre-planned comparisons were evaluated. For the density main effects, two, orthogonal, single-degree-of-freedom contrasts were: (1) low vs. medium, and (2) the combined effect of the lower two densities vs. the high density (i.e. [Low + Med]/2 vs. High). To better understand potential differences in clam survival due to level within a unit (i.e., distance from bottom), five, orthogonal, single-degree-of-freedom contrasts were conducted: (1) top level vs. all remaining levels; (2) second level vs. combined effect of all lower levels [i.e. 3, 4, 5, & 6]; (3) levels 3 & 4 vs. levels 5 & 6; (4) level 3 vs. level 4; and (5) level 5 vs. level 6.

The general linear model used in the analysis of the arcsine-transformed survivorship data for medium and large clams at the Mud Hole Cove site was:

$$Y = Mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + D(ABC)_{l(ijk)} + e_{m(ijkl)}$$

where: Y, Mu, A_i, B_j, AB_{ij}, and $e_{m(ijkl)}$ are as described above, and C_k = Clam size (Medium or Large); AC_{ik} = Density × Clam size (interaction); BC_{jk} = Level × Clam size (interaction); ABC_{ijk} = Density × Level × Clam Size (interaction); $D(ABC)_{l(ijk)}$ = Container nested within the three level interaction.

For the Weir site, the model used was:

$$Y = Mu + A_i + B_j + C_k + AB_{ij} + BC_{jk} + D(BC)_{l(jk)} + e_{m(ijkl)}$$

where: Y, Mu, A_i, B_j, C_k, AB_{ij}, BC_{jk}, and $e_{m(ijkl)}$ are described above, and $D(BC)_{l(jk)}$ = Container nested within the Level × Clam size interaction.

Each of the three models has a nested component. This is because the containers were not exactly the same. Using the model for small clams as an example, part of the observed variation in survival rate may be explained by slight differences between containers within a particular combination of density and level. This nested component assumes that bottom topography did not vary within the experimental site. If the bottom were heterogeneous, the effect of the container would be confounded with its depth as measured from the surface.

3. Results

Of a total of five units containing large or medium clams deployed at WS, the unit containing large clams at the high density was lost. In addition, two units containing small clams, one with clams at 250 ml/bag and another with clams at 500 ml/bag were lost. Although surface buoys clearly marked the experimental area, in all three cases, a commercial fishing vessel was responsible for interfering with the overwintering containers. No units were lost at MHC.

Small clams — Mud Hole Cove

Clams at the lower two densities did not survive as well as those at the highest density (Table 1; $\bar{X}_{low+med} = 60.29\% \pm 5.2$ s.e., $n = 24$ vs. $\bar{X}_{high} = 75.30\% \pm 2.3$ s.e., $n = 12$; $F = 10.27$, d.f. = 1,18, $P < 0.005$, Table 2). There were no significant differences in survivorship between clams held at the two lowest densities ($\bar{X}_{low} = 62.0\% \pm 4.6$ s.e., $n = 12$ vs. $\bar{X}_{medium} = 58.6\% \pm 5.7$ s.e., $n = 12$; $F = 0.42$, d.f. = 1,18, $P < 0.524$, Table 2). Although there was no significant density × level interaction ($F = 0.75$, d.f. = 10,18, $P < 0.675$), the density effect is likely due to the fact that clams in the uppermost levels of the two lower density

Table 1
Mean percent survivorship (± 1 s.e.) for "small" overwintered soft-shell clams

Level	MHC Volume (ml)			WS Volume (ml)		
	250	500	750	250	500	750
1	43.42 (11.66)	23.12 (0.48)	66.19 (0.96)	71.22	76.93	63.87 (2.16)
2	59.91 (4.55)	63.71 (20.35)	74.97 (1.14)	73.14	72.32	71.85 (2.29)
3	59.79 (20.08)	69.73 (5.42)	79.03 (6.28)	73.54	78.23	60.04 (14.39)
4	67.61 (4.63)	73.91 (0.25)	75.39 (3.06)	83.50	88.41	64.81 (13.37)
5	71.10 (11.78)	65.96 (0.23)	82.04 (2.59)	68.78	79.42	74.03 (12.20)
6	70.04 (12.62)	55.15 (3.44)	74.20 (0.85)	74.03	74.29	69.22 (5.47)

Two 5 ml samples were taken from each nylon screen bag ($A = 0.2$ m^2) within a subunit to estimate a survival rate for that level. Where s.e. is presented, $n = 2$ subunits were sampled; otherwise, $n = 1$. MHC = Mud Hole Cove; WS = Weir Site. Levels 1 and 6 are the uppermost and bottommost subunit within a container, respectively. See Methods for definitions of volume.

Table 2
Analysis of variance results on the arcsine-transformed clam survivorship data — "small" individuals overwintered in Mud Hole Cove

Source of variation	d.f.	SS	MS	F	Pr>F
Density	2	1447.76	723.88	5.35	0.0150
(Low + Med vs. High)	1	1390.68	1390.68	10.27	0.0049
Low vs. Medium	1	57.08	57.08	0.42	0.5243
Level	5	2561.09	512.22	3.78	0.0162
1 vs. 2,3,4,5,6	1	2371.85	2371.85	17.52	0.0006
2 vs. 3,4,5,6	1	61.82	61.82	0.46	0.5078
(3+4) vs. (5+6)	1	5.89	5.89	0.04	0.8370
3 vs. 4	1	15.88	15.88	0.12	0.7359
5 vs. 6	1	105.65	105.65	0.78	0.3887
Density × Level	10	1009.36	100.94	0.75	0.6754
Container (Den × Level)	18	2436.39	135.35	4.38	0.0001
Error	36	1112.15	30.89		
Total	71	8566.75			

treatments exhibited higher mortality than those individuals at the same level in the highest density treatment (Table 1).

The level of the subunit also influenced clam survivorship ($F = 3.78$, d.f. = 5,18, $P = 0.016$). Results of the pre-planned tests showed that clams in the uppermost levels had poorer survival rates (44.3% ± 6.6 s.e., $n = 12$) than the remaining levels combined (69.5% ± 1.7 s.e., $n = 60$). In fact, this one contrast explains 92.6% of the variation in the overall level effect (Table 2: SS of first contrast (2371.85)/SS level (2561.09) = 0.926). No other differences between levels were detected. There was, however, a significant container within density × level effect (Table 2). That is, containers within a given density treatment did not behave the same among levels. For example, the sum of squares (SS) for this source of variation (2436.39) can be thought of as the summation of 18 separate one-way ANOVAs, each of which tests the null hypothesis that there are no differences in percent survival between overwintering containers (2) for each level (6) within a density treatment (3). Of the 18 separate tests, five were significant ($P < 0.05$); however, no consistent pattern could be detected based on level of subunit or density treatment.

Small clams — Weir Site

ANOVA indicated no significant main effects or interactive (density × level) effects (Table 3). After pooling data across levels within a density treatment (Table 1), the mean survivorship of clams held at 250 ml/bag and 500 ml/bag was 74.0% ± 2.1 s.e. and 78.3% ± 2.3 s.e. ($n = 6$), respectively. The greatest difference in survivorship occurred for clams held at the highest density. The survival rate from one replicate overwintering container (again, pooled across levels) was 75.6% ± 2.7 s.e. whereas clams in the other, supposedly identical, container had a survival rate of 58.9% ± 3.6 s.e. The latter container had apparently lost some of its buoyancy and when divers recovered it in April 1992, it was resting on the bottom. The ANOVA (Table 3) quantifies this among container variability. Note that the source of variation labeled "Container (Density × Level)" is significant

Table 3
Analysis of variance results on the arcsine-transformed clam survivorship data — "small" individuals overwintered at the Weir Site

Source of variation	d.f.	SS	MS	F	Pr>F
Density	2	420.64	210.32	1.41	0.3147
Level	5	162.82	32.56	0.22	0.9327
Density × Level	10	356.86	35.69	0.24	0.9769
Container (Den × Level)	6	894.69	149.12	28.86	0.0001
Error	24	123.99	5.17		
Total	47	1959.00			

($P < 0.0001$) indicating that the two containers holding clams at the highest density were not behaving similarly.

Large and medium clams — Mud Hole Cove

Large and medium size clams held over the winter at MHC had excellent survival rates (Table 4). Only two of 16 estimates of survival were lower than 94% in any level of any overwintering unit (both replicates for medium size clams in the top level of the low density unit). Table 5 demonstrates that no differences in survival existed between large and medium size clams ($F = 0.13$, d.f. = 1,4, $P < 0.735$) or among levels ($F = 2.42$, d.f. = 3,4, $P < 0.206$), but that density differences did exist for both size groups. In each instance it appears that clams at the lower density did not survive as well as those at the higher density although the actual differences in estimated mean survival rates are small. For example, the mean survival rate for large clams at the low density (pooling all levels) was 96.1% ± 0.1 s.e. ($n = 4$) compared with 98.4% ± 0.1 s.e. ($n = 4$) for large clams at the higher density. Similarly, medium clams at the low density had a mean survival rate of 96.4% ± 1.7 s.e. ($n = 4$) compared with 97.6% ± 0.1 s.e. ($n = 8$) for those held at the higher density. Medium size clams at the low density in the uppermost subunit experienced poorer survivorship than those closer to the bottom (Table 4). This was also the case for similar size clams in one of the high density units (and apparently the reason for the significant container within

Table 4
Mean percent survivorship (± 1 s.e.) for "large" and "medium" overwintered soft-shell clams

Level	MHC Clam size/volume (ml)				WS Clam size/volume (ml)		
	MED/1000	MED/2000	LG/1125	LG/2250	MED/1000	MED/2000	LG/1125
1	91.34	96.85 (1.43)	95.90	98.26	98.67 (0.08)	99.13	96.14
2	98.64	97.85 (0.92)	97.38	98.65	98.82 (0.15)	98.57	97.25
3	97.36	98.24 (0.01)	94.79	98.45	98.84 (0.05)	99.11	98.58
4	98.16	97.35 (0.33)	96.25	98.35	98.25 (0.34)	98.91	98.94

Two 5 ml samples were taken from each nylon screen bag ($A = 0.2$ m^2) within a subunit to estimate survival rate for that level. Where s.e. is presented, $n = 2$ subunits were sampled; otherwise, $n = 1$. MHC = Mud Hole Cove; WS = Weir Site. Levels 1 and 4 are the uppermost and bottommost subunits within a container, respectively. See Fig. 1 for clam sizes and Methods for definitions of volume.

Table 5
Analysis of variance results on the arcsine-transformed clam survivorship data — "large" and "medium" individuals overwintered in Mud Hole Cove

Source of variation	d.f.	SS	MS	F	Pr>F
Density	1	75.83	75.83	8.18	0.0459
Level	3	67.37	22.45	2.42	0.2061
Clam size	1	1.22	1.22	0.13	0.7353
Density × Level	3	43.11	14.37	1.55	0.3324
Density × Clam size	1	18.26	18.26	1.97	0.2331
Level × Clam size	3	38.89	12.96	1.40	0.3655
Den × Lvl × Clam size	3	26.21	8.74	0.94	0.4993
Container (D × L × CS)	4	37.08	9.27	4.42	0.0101
Error	20	41.91	2.10		
Total	39	349.88			

Table 6
Analysis of variance results on the arcsine-transformed clam survivorship data — "large" and "medium" individuals overwintered at the Weir Site

Source of variation	d.f.	SS	MS	F	Pr>F
Density	1	0.94	0.94	0.39	0.5427
Level	3	9.36	3.12	2.10	0.1392
Clam size	1	15.02	15.02	16.63	0.0151
Density × Level	3	4.10	1.37	0.56	0.6490
Clam size × Level	3	29.77	9.92	10.98	0.0212
Container (Lvl × CS)	4	3.61	0.90	0.37	0.8262
Error	16	39.02	2.44		
Total	31	101.82			

density × level × clam size source of variation — Table 5), but again, the differences were extremely small.

Large and medium clams – Weir Site

Survival rates for clams in all subunits were quite high (Table 4). Estimated survivorship was higher than 95% for every replicate within every level within every overwintering container. There was a significant effect of clam size on survivorship (Table 6); however, the differences were extremely small. For example, 98.7% of the medium size clams survived compared with 97.7% for the larger clams. The ANOVA also detected a significant difference in survival of clams at the two sizes across levels ($F = 10.98$, d.f. = 3,4, $P < 0.02$; Table 6). There was a general increase in survivorship of large clams with increasing depth (Table 4), but no similar trend occurred for the medium size clams. The source of variation associated with overwintering container within level × clam size (Table 6 — i.e. the replicates of the low density treatment for the medium clams) indicated that those two overwintering containers behaved as true replicates.

4. Discussion

In Maine, USA, there now appears to be a viable alternative to transplanting hatchery-reared juveniles of the soft-shell clam during the fall. Clams larger than 8 mm in shell length can be held over the winter with high survival, at least at the densities used here. The highest densities (2 l [ca. 22 000 individuals] and 2.25 l [ca. 9000 individuals] for medium and large size individuals, respectively) practically filled the entire volume of the nylon window screen bag. Survivorship at every level of every overwintering container, regardless of site, was greater than 90% and in most cases exceeded 95%. Except for the problems associated with a commercial fishing vessel, which was responsible for the loss of several containers at WS, no apparent differences were observed between sites with respect to density effects (Table 4). The poorest survivorship (91.34%) was associated with medium size clams at the lower density in the uppermost level of one overwintering unit (Table 4). Otherwise, level did not seem to play a major role in influencing clam survival. When information from all levels of each unit at each site is combined, survivorship for those large and medium clams at MHC was 97.2% $\pm$0.4 s.e. ($n=5$) and at WS was 98.5%$\pm$0.3 s.e. ($n=4$).

Stocking density did play a role in determining the fate of the smallest overwintered clams, but only at the sheltered site — MHC (Tables 1 and 2). Clams at MHC held at the highest density (750 ml/bag) survived better than those at the lower densities. Although there was no significant density $\times$ level interaction, the reason for the apparent density effect is related to poorer survival rates among clams held in the uppermost subunits within the lower two density treatments (Table 1). This may be related to the depth of the water the containers were in. Although bottom topography within the 75 m$\times$75 m study area was not critically assessed, if containers holding clams at the two lower densities were in shallower water than their high density counterparts, it is conceivable that clams in the top levels of these containers could have been exposed to the extreme cold and wind during the spring tides of each month. The bottom topography of Mud Hole Cove is uneven, such that some subtidal areas of the cove may be as deep as 4 m at low tide while others just a few meters away may only be 2 m in depth (B. Beal, pers. obs.). Support for this suggestion comes from the deeper water (Weir) site where no similar and unambiguous effect of level was apparent (Tables 1 and 3). Another possible explanation for the lower survival of small clams at the two lower densities may be related entirely to physiology (B. Barber, pers. comm.). In winter, high densities may not be detrimental because water temperatures are so low as to preclude any metabolic constraints; in fact, there may actually be a benefit of high density in this case. Clams at high densities within the nylon screened bags probably have increased physical stability compared with their lower density counterparts. High densities decrease wave or tidally associated disturbance. Greater depths would confer a similar advantage, regardless of density.

Small clams generally did not survive as well as large and medium size clams; however, this comparison is confounded by differences in stocking densities between size classes. Differences between sites were not great. For example, 65.3%$\pm$4.5 s.e. ($n=6$) of the small clams at MHC survived whereas a 71.7%$\pm$4.3 s.e. ($n=4$) survival rate was observed at WS.

The ability to culture millions of commercially valuable bivalve mollusks in shellfish hatcheries throughout North America has become routine (Manzi, 1985; Manzi and Castagna, 1989). Commercial shellfish hatcheries in the northeastern United States usually begin to condition broodstock in the late fall or early winter so that several mass spawnings can occur during late winter. Juvenile shellfish are reared in the hatchery until they are large enough to enter a nursery and either are placed in land-based or tidally-driven upwellers or downwellers, or are moved to floating trays. All aspects of the hatchery phase are planned to take advantage of natural peaks in primary productivity.

Once bivalves have attained sizes between 15 to 25 mm SL in the nursery they typically are transplanted to a prepared bottom (*sensu* Kraeuter and Castagna, 1977). In Maine and other New England states, this usually occurs in the fall when new shell growth is minimal or has ceased. In the case of hatchery-reared American oyster seed (*Crassostrea virginica* [Gmelin]), late fall or early winter planting results in substantial losses due either to predation by eider ducks (*Somateria mollissima* [L.]) or storms (Hidu et al., 1988). Strategies have been developed to overwinter young oysters in humid air storage at temperatures between 0° and 6°C for up to 6 months with > 80% survival (Hidu et al., 1988; Hidu and Chapman, 1988). Seaman (1989a,b) found similar results with *C. gigas* (Thunberg) at temperatures near 7°C.

The goal of reducing winter (weather-related) losses of hatchery-reared juveniles of the hard clam, *Mercenaria mercenaria* (L.), has led to several experimental overwintering tests. Stevens et al. (1985), Battey (1988) and Battey and Manzi (1988) have indicated that vacated shrimp ponds in South Carolina work well as winter nurseries for hard clams. For example, 7.5 mm seed routinely increases two-fold in length between November and April with > 80% survival. Another overwinter strategy for hard clams has been used successfully by Malinowski (1988). Individuals ranging in size from 7 to 12 mm are placed in 0.7 m × 1.4 m subtidal bottom trays with plastic mesh covers and a coarse sand substrate. The following May, clams are removed from bottom trays and planted in larger (1.5 m × 3.0 m) meshed plots. Hidu et al. (1988) indicated the potential to overwinter hard clam seed in cold humid air storage, but no published reports have emerged since that time. To the best of our knowledge, no previous attempts have been made to overwinter hatchery-reared individuals of *Mya arenaria*.

Previous studies in Maine with hatchery-reared soft-shell clam seed illustrated that transplanting to intertidal mudflats and protecting juveniles from predation in early spring rather than the fall improved survival rates (Beal, 1991). Field tests have shown that it is possible to obtain 75% and higher survival to at least 40 mm (Beal, 1991; Beal and Kraus, 1991). At this size, clams are burrowed deeply enough in the sediments to discourage most green crabs (*Carcinus maenas* [L.]) and rock crabs (*Cancer irroratus* Say) and are also not as easily scoured off the flat by icebergs.

Until now, a successful method for overwintering clams so that they can be produced in one year and transplanted the next has been lacking. An economically effective overwintering strategy is crucial to the success of stock enhancement programs in Maine. The material cost to produce an overwintering container was reasonable. Each nylon bag with its own zipper cost $3.50. Materials to produce a single level within a container cost $1.00 for wood and screws and $2.50 for mesh netting. Buoys, weights, rope, and other smaller items for each container unit were approximately $10.00. The cost of materials for a container with six levels would be $46.00 and for a unit with four levels $34.00. In a safe site with adequate seawater exchange and water depths exceeding 4 m at low tide, it would be possible to stock up to 750 ml of small clams (ca. 4 mm), or 55 000 individuals, in each level over the winter. A container of five subunits or levels could house nearly 275 000 clams. To store 1 million 4 mm animals, four containers of five levels would be required. An estimate of overwinter survival rate of 70% from clams of this size is reasonable (Table 1). The cost for the units would be about $160.00. Commercial costs of the clam seed would be approximately $14 000 (1991 price list from Mook Sea Farm, Inc., Damariscotta, Maine). If labor costs are included, total costs to purchase the clams and store them for the winter would be around $14 500. Based on 1400 2-inch clams per bushel, if 70% of these survive and, once planted, 50% reach market size, clammers would need to receive about $58.00/bushel in order to recover their initial investment. Commercial harvesters in eastern Maine typically receive this price per bushel or higher from June through August.

Overwintering larger clams appears to be a lower risk. This study showed that 90–95% survivorship can be expected regardless of site. The cost to purchase 1 million 8 mm clams from a commercial shellfish hatchery would be $18 000. At a density of 20 000 per bag (level), eight units of six stacks each would be required at a material cost for the containers of approximately $370.00. With labor, estimated cost to produce these units would be $500.00 for a total investment to $18 500. If 90% of the 8 mm clams survived to be transplanted in the spring, and 50% reached market size, clammers would, similarly, have to receive nearly $58.00/bushel to break even.

These examples demonstrate the importance of survivorship not only over the winter, but also to market size. If survival rates to commercial size increase from 50% to 75%, then at $70.00 per bushel (typical of late summer prices), the 4 and 8 mm clams would bring a profit of $11 750 and $15 250, respectively. These simple calculations suggest that to optimize returns, larger clams should be held over the winter and subsequently transplanted. An alternative scenario for communities or individuals purchasing seed clams from a hatchery would be to invest in small clams (i.e., 2 mm) in the spring. For example, 1 million animals at 2 mm SL cost $6000. These could be divided into groups of 15 000 and placed within wooden trays lined with window screening (aperture = 1.8 mm). Costs to produce seventy 1.2-m × 0.9-m × 0.07-m trays are estimated at $1050 ($15.00/tray). If a suitable nursery site can be located, these clams will attain an average of 12 mm by the winter, assuming that rates are similar to those attained in eastern Maine. If 90% of these 12 mm animals survived to be transplanted the following spring, and 75% reached commercial size and were sold at $70.00/bushel, a profit of $26 200 could be realized. Although costs to protect animals from predators are not part of these calculations, it can be seen that the latter scenario would be the most cost effective.

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