Aquaculture, 93 (1991) 109–119
Elsevier Science Publishers B.V., Amsterdam

Realized heritability of growth rate in the hard clam *Mercenaria mercenaria*[1]

Nancy H. Hadley[a,2], Robert T. Dillon, Jr.[b] and John J. Manzi[a]
[a]*Marine Resources Research Institute, P.O. Box 12559, Charleston, SC 29412-2559, USA*
[b]*Department of Biology, College of Charleston, Charleston, SC 29424, USA*

(Accepted 29 June 1990)

ABSTRACT

Hadley, N.H., Dillon, R.T., Jr. and Manzi, J.J. 1991. Realized heritability of growth rate in the hard clam *Mercenaria mercenaria*. *Aquaculture*, 93: 109–119.

Native South Carolina wildstock clams (*Mercenaria mercenaria*) were mass-spawned to produce a large initial population of parents for a directed breeding program. At 2 years of age, the largest 10% of this population, and an equal number of mean size clams, were segregated to become selected and control-line parents. Three separate experiments were performed, usually involving 20–40 selected and control parents. Offspring were reared under standard hatchery, nursery and field grow-out conditions. Realized heritability was determined at 2 years of age. In one experiment, no response to selection was observed at 2 years, possibly due to reduced effective breeding number. Conservative estimates of realized heritability of growth rate for the other two experiments were consistent and high: 0.42 ± 0.10 and 0.43 ± 0.06. Mass selection appears to be a promising technique for improvement of hard clam broodstocks.

INTRODUCTION

The hard clam (*Mercenaria* spp.) fishery has suffered significant declines in total landings over the last 3 decades despite high demand and correspondingly high value. The enhancement of this overexploited fishery through the development of commercial hard clam mariculture has exhibited considerable promise, and commercial operations are ongoing in several states. While most of the technology necessary for successful large-scale culture of hard clams has been established, commercial efforts are still hampered by the highly variable and relatively slow growth rate of cultured stocks. Although the average time in the hatchery and nursery is rarely more than 8 months, field grow-out to market size may take up to 4 years. In South Carolina, market size is reached in 24–36 months of field grow-out. Decreasing this field ex-

[1]Contribution No. 290, South Carolina Marine Resources Center.
[2]To whom correspondence should be sent.

0044-8486/91/$03.50 © 1991 — Elsevier Science Publishers B.V.

posure time would significantly increase economic returns. Our unpublished data from South Carolina indicate that a 25% increase in growth rate could decrease grow-out time by 8 months.

Some improvement in hard clam stocks has been derived through directed breeding programs at commercial facilities (G. Petrovits, Aquaculture Research Corp., Dennis, MA, pers. commun., 1983) and pilot hatcheries (M. Castagna, Virginia Institute of Marine Science, Wachapreague, VA, pers. commun., 1983). Although no strict application of quantitative genetics to commercial-scale selective breeding has been performed, it has been a recommended line of research for many years (FAO, 1972; NAS., 1978; DOC, 1982; USDA, 1988). Recent studies have documented the value of directed breeding in bivalve culture (Haley and Newkirk, 1982; Newkirk and Haley, 1982, 1983; Newkirk, 1983; Adamkewicz et al., 1984a, 1984b; Wada, 1986). Studies on selection and growth with inbred stocks of *Ostrea edulis* at Dalhousie University (Newkirk, pers. comm., 1988) and *Crassostrea gigas* at the University of Washington (Chew, pers. comm., 1988) continue to demonstrate the value of selection in bivalve breeding. This study represents one portion of a large-scale breeding program to improve growth rates of the hard clam in South Carolina.

MATERIALS AND METHODS

Native clams were collected from the Folly River near Charleston, South Carolina, in the spring of 1983 and mass-spawned. Approximately 10 000 offspring were reared in high-density culture ($1000–1500/m^2$) to 2 years of age. In the fall of 1985, the size–frequency distribution of the population was estimated by measuring the standard length (longest anterior–posterior dimension) of 5% of the individuals (Fig. 1). This distribution was not significantly skewed but was leptokurtotic ($\bar{x}=36.89$, $s^2=40.88$; $G_1=-0.101$, $P>0.05$; $G_2=0.402$, $P<0.001$).

In mass-selection experiments, a common method of determining realized heritability is to compare the offspring to the parents at the same age. This method is not suitable for bivalves because of the substantial influence of environmental conditions on annual growth rate. When the parental population cannot be used as a baseline for determination of improvement, simultaneous control populations are generally substituted. Because a large proportion of the parental population was not reproductively mature at the time of selection, we could not spawn a random sample to serve as a control. Instead, control lines were created by spawning clams which were as close as possible to the mean size of the total population. The largest 10% of the population were segregated to serve as the parents of selected lines. A similar number of mean-sized animals were segregated as control-line parents.

In the spring of 1986, the selected and control parents were randomly sep-

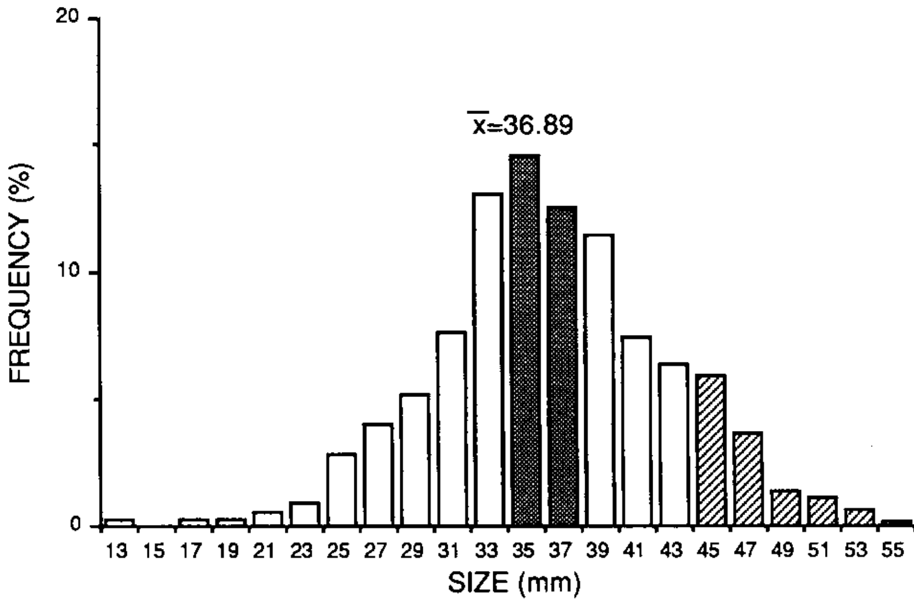

Fig. 1. Distribution of parental population from which selected (upper 10%, diagonal stripes) and control line (stippled) parents were drawn.

arated into three subgroups of 150 each and conditioned for spawning. One selected and one control-line group were each spawned on March 18, May 6 and May 13. Clams were induced to spawn in individual containers using temperature shock. Gametes were collected, rinsed and pooled prior to fertilization. Fertilized eggs were maintained in 15-l cultures at 30/ml for 24 to 48 h and then transferred to 100-l static larval culture containers. Larvae were fed Tahitian *Isochrysis galbana* at rates sufficient to maintain a density of 50 000 algal cells per ml culture water. At 1 week of age, the diet was adjusted to include up to 50% *Chaetoceros gracilis* (by cell count).

Populations were always grown in multiple-culture containers, the number of replicates depending on the total population size. During larval culture, populations were counted and measured at weekly intervals. Initial stocking densities of five larvae/ml were gradually reduced to one/ml by setting size. In order to retain all the genetic variability, no culling of small individuals was employed. When space permitted, cultures were thinned by randomly splitting the population into additional culture containers. When that was not possible, a random aliquot of appropriate volume was retained and the remainder discarded.

After setting, populations were reared in recirculating downwelling units (15 cm dia; Manzi and Hadley, 1988) suspended in a common reservoir. These were converted to upwelling units as the post-set reached 0.5 mm SL.

At weekly intervals, stocking density was estimated by measuring the packed volume of clams in each culture unit and counting subsamples. Packed volume is measured by adding clams to a graduated cylinder containing seawater and tamping gently to "pack" or settle the clams. Densities were adjusted as necessary to maintain a packed volume of less than 30 ml per culture unit.

When all individuals in all cultures reached a minimum size of 1.0 mm, the populations were moved to a flow-through nursery (Manzi et al., 1984). Flow-through units were initially stocked at no more than 0.2 ml packed volume/cm^2. At bi-weekly or monthly intervals, the cultures were sampled to monitor growth, survival, and biovolume. Culture containers were thinned as necessary to maintain densities within optimal ranges (Manzi et al., 1984).

When populations reached 6–8 mm (SL), they were planted in intertidal field-culture cages (1 m^2) at stocking densities of approximately $1000/m^2$. One such culture cage was deployed for each of the six lines. The remainder of each line was planted in backup cages (3 m^2) at densities of approximately $2000/m^2$. Densities up to $2000/m^2$ are not limiting for clams less than 30 mm SL (Manzi et al., unpubl. data). The sample cages were harvested at 18 months (after approximately 9 months in the field) for density adjustment. Two cages (Control 1 and Selected 2) had suffered from heavy predation and three (Selected 1 and 3, Control 3) were at higher densities than intended (Table 1). Densities in the sample cages were equalized to $750/m^2$ by randomly removing individuals or by randomly adding individuals from backup cages before redeployment. At 2 years of age, cages were again harvested and all individuals were measured.

Each experiment was analysed independently. Mean sizes of selected and

TABLE 1

Planting density ($\#/m^2$) at 9, 18 and 24 months. D_{adj} is the adjusted density after removal or addition from backup cages at 18 months

Experiment	Line	Cage	D_9[a]	D_{18}	D_{adj}	D_{24}
1	Selected	Sample	1060	1342	742[a]	701
		Backup	2070	770[a]	470[a]	470
	Control	Sample	1040	12	702[a]	1037
		Backup	2160	1400[a]	1090[a]	1090
2	Selected	Sample	1070	440	769[a]	810
		Backup	2000	1090[a]	970[a]	940
	Control	Sample	990	860	760	760
		Backup	2020	200	200	200
3	Selected	Sample	1000	1680	760	760
		Backup	2060	1780[a]	1250[a]	1250
	Control	Sample	1070	1250	750	720
		Backup	2000	–	940[a]	940

[a]Estimated from subsample counts.

control lines were compared at 1, 6, 8, 12, 18 and 24 months of age. There was considerable heterogeneity of variances, so all comparisons were made using two-tailed *t*-tests assuming unequal variances (Sokal and Rohlf, 1969).

Realized heritability was calculated for each of the three experiments at 2 years of age, using the corresponding control as a reference. The selection differential (S) may be estimated as the product of the standard deviation of the parental population (s_p) and i, the intensity of selection from a truncated standard normal distribution (Falconer, 1989). Then realized heritability may be calculated as R/S where R is response to selection, the difference between selected and control means ($\bar{x}_s$ and $\bar{x}_c$):

$$h^2 = \frac{R}{S} = \frac{\bar{x}_s - \bar{x}_c}{i s_p} \tag{1}$$

Note that the leptokurtosis we observed in our parental population results in an overestimate of i, and thus our estimates of h^2 will be biased downward.

Because of the potential for extreme environmental variability from one generation of bivalves to the next, some previous workers with oysters and mussels have standardized values of R and S. Newkirk and Haley (1982, 1983) divided their measure of selection differential by the standard deviation of the parental population (s_p) and their measure of response by the standard deviation of the control offspring (s_c). Since s_p cancels, this amounts to replacing s_p with s_c:

$$h^2 = \frac{\bar{x}_s - \bar{x}_c}{i s_c} \tag{2}$$

Mallet et al. (1986) multiplied S and R by their coefficients of variation, which is equivalent to dividing S by the parental mean (x_p) and R by the control offspring mean (x_c):

$$h^2 = \frac{\bar{x}_p(\bar{x}_s - \bar{x}_c)}{\bar{x}_c i s_p} \tag{3}$$

With the absence of any previous experience with *Mercenaria*, we elected to estimate h^2 by the most conservative of these three methods.

Method (1) has the further virtue of engendering the lowest sampling variance. Hill (1971, 1972) has shown that an approximation to the sampling variance of the response to one generation of selection is:

$$\sigma_R^2 \approx v_p\left(\frac{h^2}{N_{es}} + \frac{1}{M}\right) \tag{4}$$

where v_p is the phenotypic variance before selection, N_{es} is the effective number of parents selected, and M is the number of offspring measured. As we measured on the order of 10^3 offspring, $1/M$ is negligible. But in this case, the overall sampling variance of R would be the sum of the variances for both selected and control lines. We calculated the standard errors for our estimates of heritability as σ_R/S, which simplifies to:

$$\sigma_{h^2} \approx \frac{\sqrt{h^2(1/N_{es}+1/N_{ec})}}{i} \tag{5}$$

where N_{es} and N_{ec} are the effective numbers of parents from the selected and control lines, respectively.

RESULTS

Although 150 potential parents were subjected to spawning stimuli for each line in each experiment, the number of actual spawners varied from 12 to 90 (Table 2). Effective population sizes, calculated as $(4N_fN_m)/(N_f+N_m)$, were greater than 20 in all cases except the selected line of Experiment 2, where $N_{es}=9$.

Growth data for the six lines and results of t-tests are presented in Table 3. Selected lines did not consistently grow faster than controls during the first year but at 18 months selected lines were significantly larger than control lines in all experiments ($P<0.001$). However, after replenishment from the backup cage, the selected line of Experiment 2 was not significantly larger than the corresponding control line.

At 2 years of age, selected lines were significantly larger than controls in two of the three experiments. Mean size ranged from 27.49 mm for the control line of Experiment 1 to 39.25 mm for the selected line of Experiment 3. In Experiment 1, the selected line was 4.65 mm (16.9%) larger than the corresponding control ($P<0.001$); in Experiment 3, the selected line was 4.78

TABLE 2

Number of males (M) and females (F) spawning to produce each experimental line

Experiment	Selected		Control	
	M	F	M	F
1	11	12	20	11
2	3	9	17	15
3	38	21	48	42

TABLE 3

Mean size (SL, mm), standard deviation, and results of Student's t-tests comparing selected and control lines for each experiment at different ages (months)

Age	Experiment	Selected				Control			t_s	P
		N	$\bar{x}$	s		N	$\bar{x}$	s		
1	1	90	0.65	0.15	>	90	0.72	0.21	−2.66	<0.01
	2	50	0.66	0.13	<	50	0.57	0.12	3.94	<0.001
	3	30	0.58	0.07	=	30	0.59	0.11	−0.25	>0.05
6	1	51	3.75	1.32	>	49	4.88	1.38	−4.13	<0.001
	2	50	8.82	2.24	<	50	5.68	3.33	5.35	<0.001
	3	50	6.42	2.52	<	52	4.48	2.89	3.62	<0.001
8	1	50	7.48	2.34	>	50	5.94	2.21	3.38	<0.01
	2	51	7.49	2.57	=	151	7.42	3.07	0.16	>0.05
	3	101	7.61	2.93	>	100	5.10	3.15	5.85	<0.001
12	1	137	9.50	2.79	>	141	8.37	1.85	3.97	<0.001
	2	115	14.08	3.28	<	107	15.60	3.65	−3.26	<0.01
	3	105	12.65	3.36	>	100	10.29	3.19	5.17	<0.001
18	1	99	22.62	3.69	>	95	19.63[a]	4.33	5.17	<0.001
	2	102	28.26	3.02	>	100	24.24	3.95	8.80	<0.001
			24.51[b]	3.82	=		24.24		0.46	>0.05
	3	105	25.55	5.17	>	99	22.36	5.04	4.46	<0.001
24	1	701	32.14	5.01	>	1037	27.49	5.63	18.05	<0.001
	2	809	36.63	5.28	<	733	37.80	5.55	−4.23	<0.001
	3	758	39.25	5.55	>	724	34.12	5.92	16.02	<0.001

[a]Mean from backup cage.
[b]Weighted mean after replenishment from backup cage.

mm (13.9%) larger than its control ($P<0.001$), and in Experiment 2, the control line was significantly larger than the selected line ($P<0.001$).

Variances in the six experimental lines showed considerable heterogeneity at age 2, but were all substantially lower than those observed in the parental population. This did not seem to be due to variable scaling, however. The rank correlation between mean and variance over all seven populations was quite low ($r_s=0.08$). Thus, no sort of standardization would seem necessary for these data. Calculation of realized heritability by the unstandardized method (equation 1) produced the most conservative estimates of heritability of 0.42 ± 0.10 for Experiment 1 and 0.43 ± 0.06 for Experiment 3. Since $S_p > S_c$ and $\bar{x}_p > \bar{x}_c$ for both Experiments 1 and 3, heritabilities estimated by either equation (2) or (3) are larger.

DISCUSSION

There have been a number of studies which have predicted the heritability of growth rate in bivalves, but few which have reported response to selection.

Predicted heritabilities of larval growth rate have been reported for *Crassostrea virginica* based on full-sib (Lannan, 1972; Haley et al., 1975; Newkirk et al., 1977) and half-sib (Haley et al., 1975; Newkirk et al., 1977; Losee, 1979) correlation analysis. Heritability estimates reported were generally in the range of 0.2–0.5.

However, larval growth rates may not be indicative of growth to market size. Although larval and spat growth rates in *C. virginica* were highly correlated, early growth rates were not correlated with growth to market size in either *C. virginica* or *O. edulis* (Haley and Newkirk, 1977). For both *C. virginica* and *O. edulis*, Haley and Newkirk (1982) suggested that selection should be delayed until the parents were at least 3 years old. Previous work with hard clams (Manzi et al., 1991) has also demonstrated that early growth is not a good predictor of growth to market size. Table 3 shows that there was little relationship between early growth and size at 2 years. For example, the selected line of Experiment 1, which had the highest response at 18 and 24 months, showed no response at 1 month or 6 months.

Newkirk and Haley (1982, 1983) reported a large response to selection for growth to market size in *O. edulis*. First-generation selected lines averaged 23% gain over controls, with realized heritabilities ranging from 0.9 to 1.18 (Newkirk and Haley, 1982). Second-generation responses were lower with realized heritabilities of 0.09 to 0.13 (Newkirk and Haley, 1983). Wada (1986) reported response of the Japanese pearl oyster (*Pinctada fucata martensii*) to selection for several shell traits. After three generations of selection, realized heritability was estimated at 0.47 for shell width and 0.35 for shell convexity. The heritabilities of 0.42 and 0.43 reported here are in the moderate-to-high range, in line with values previously obtained for growth rates in bivalves and for production traits in livestock (Van Vleck et al., 1987).

The lack of response in Experiment 2 is almost surely in large part due to the replenishment of approximately half of the population with smaller animals from the backup cage at 18 months. Although our data showed no consistent relationship between planting density and growth (Tables 1 and 3) and the density in the backup cages was lower than the limiting threshold for clams 10–20 mm (Manzi et al., unpubl. data), the mean size in the Experiment 2 selected backup cage was significantly smaller than that of the corresponding sample cage. The mean size after replenishment was similar to that in the corresponding unselected sample cage. Although response to selection would be expected to be lowered by this replenishment, it should have been detectable by 24 months. In the other two experiments, the mean differential between selected and control lines increased approximately 2 mm over this final 6-month growth period (Table 3).

A second factor contributing to our failure to demonstrate any response to selection in Experiment 2 may have been reduced effective population size in the selected line. Even given a true heritability as high as 0.20, the substitu-

tion of $N_{es}=9$ into equation (5) results in a standard error of 0.10. Observed heritabilities as low as 0.0 would not be unusual in such circumstances. The difficulties associated with low effective breeding numbers have been reviewed by Gaffney and Scott (1984) and Dillon and Manzi (1988).

Early growth rates were somewhat slower than those previously reported for *M. mercenaria* in culture (Manzi et al., 1981, 1984, 1986; Hadley and Manzi, 1984; Malinowski, 1988). In contrast to standard nursery practice, slow growing individuals were not culled out and each entire population was retained in the hatchery or nursery until the smallest individuals were ready for transfer. After field deployment (at approximately 9 months of age), growth rates increased dramatically. The average sizes at 18 months ranged from 19.6 to 28.3 mm, which is comparable to growth obtained in commercial culture in South Carolina (Manzi et al., 1981). Culture experience in South Carolina leads us to expect an average size of 32 mm at 24 months of age (Manzi, unpubl. data; Manzi et al., 1991). In this experiment all lines except Control 1 averaged larger than 32 mm at 2 years of age.

Our data point to the importance of including simultaneous control populations in selection experiments with bivalves. Wada (1986) has reported results from several generations of selection for shell shape in the pearl oyster. In those experiments, there was often negative response or no response in comparison with parental lines, although improvement could be seen relative to contemporaneous controls. We found annual environmental variation to be so strong that only one selected line was larger than the original parents at 2 years. However, improvement is demonstrated by comparison with the contemporaneous control lines.

Dillon and Manzi (1987, 1989a,b) have shown that wild *M. mercenaria* populations are quite variable genetically. Isozyme polymorphism was high in both the parents and the offspring produced in these experiments (unpubl. data). Heritability has not generally been found to decline during the first five to ten generations of selection if genetic variation in the initial population has not been reduced (Falconer, 1989). Thus, a second generation of selection for the upper 10% of the high-selected lines of Experiments 1 and 3 should produce a response of 4–5 mm at 2 years, given favorable environmental conditions. The expected cumulative response of about 9 mm would be an increase of 25% over the mean size of our original parents at 2 years and an improvement in grow-out time of about 8 months over unselected stocks.

ACKNOWLEDGEMENTS

The authors would like to thank Nancy Peacock, Colden Battey and Marc Carter for their assistance in the production and rearing of the experimental stocks. This research was supported by NOAA-Office of Sea Grant through

the South Carolina Sea Grant Consortium under Grant No. NA85-AA-D-SG121.

REFERENCES

Adamkewicz, L.S., Taub, R. and Wall, J.R., 1984a. Genetics of the clam *Mercenaria mercenaria*. I. Mendelian inheritance of allozyme variation. Biochem. Genet., 212(3/4): 215–219.

Adamkewicz, L.S., Taub, R. and Wall, J.R., 1984b. Genetics of the clam *Mercenaria mercenaria* II. Size and genotype. Malacologia, 25(2): 525–533.

DOC (Department of Commerce), 1982. Sea Grant Aquaculture Plan 1983–1987. Publ. No. TAMU-SG-82-114. Sea Grant College Program, Texas A&M University, College Station, TX, 47 pp.

Dillon, R.T. and Manzi, J.J., 1987. Hard clam, *Mercenaria mercenaria*, broodstocks: genetic drift and loss of rare alleles without reduction in heterozygosity. Aquaculture, 60: 99–105.

Dillon, R.T. and Manzi, J.J., 1988. Enzyme heterozygosity and growth rate in nursery populations of *Mercenaria mercenaria* (L.) J. Exp. Mar. Biol. Ecol., 116: 79–86.

Dillon, R.T. and Manzi, J.J., 1989a. Genetics and shell morphology in a hybrid zone between the hard clams *Mercenaria mercenaria* and *M. campechiensis*. Mar. Biol., 100: 217–222.

Dillon, R.T. and Manzi, J.J., 1989b. Genetics and shell morphology of hard clams (Genus *Mercenaria*) from Laguna Madre, Texas. Nautilus, 103: 73–77.

FAO, 1972. Report of the first meeting of the FAO ad hoc working party on genetic selection and the conservation of genetic resources of fish, 7–10 December 1971, Rome, Italy. FAO Fish. Rep. No. 119, 9 pp.

Falconer, D.S., 1989. Introduction to Quantitative Genetics, 3rd edn. Wiley, New York, NY, 438 pp.

Gaffney, P.M. and Scott, T.M., 1984. Genetic heterozygosity and production traits in natural and hatchery populations of bivalves. Aquaculture, 42: 289–302.

Hadley, N.H. and Manzi, J.J., 1984. Growth of seed clams (*Mercenaria mercenaria*) at various densities in a commercial scale nursery system. Aquaculture, 36: 369–379.

Haley, L.E. and Newkirk, G.F., 1977. Selecting oysters for rapid growth. Proc. World Maricult. Soc., 8: 557–565.

Haley, L.E. and Newkirk, G.F., 1982. The genetics of growth rate of *Crassostrea virginica* and *Ostrea edulis*. Malacologia, 22(1–2): 399–401.

Haley, L.E., Newkirk, G.F., Waugh, D.W. and Doyle, R.W., 1975. A report on the quantitative genetics of growth and survivorship of the American oyster, *Crassostrea virginica* under laboratory conditions. 10th Eur. Symp. Mar. Biol., 17–23 Sept. 1975, Ostend, Belgium, Vol. 1. Universa Press, Wetteren, Belgium, pp. 221–228.

Hill, W.G., 1971. Design and efficiency of selection experiments for estimating genetic parameters. Biometrics, 27: 293–311.

Hill, W.G., 1972. Estimation of realized heritabilities from selection experiments. II. Selection in one direction. Biometrics, 28: 767–780.

Lannan, J.E., 1972. Estimating heritability and predicting response to selection for the Pacific oyster *Crassostrea gigas*. Proc. Natl. Shellfish. Assoc., 62: 62–66.

Losee, E., 1979. Influence of heredity on larval and spat growth in *Crassostrea virginica*. Proc. World Maricult. Soc., 9: 101–108.

Malinowski, S.M., 1988. Variable growth rates of seed clams *Mercenaria mercenaria* (Linné) in an upflow nursery system and the economics of culling slow growing animals. J. Shellfish. Res., 7(3): 359–366.

Mallet, A.L., Freeman, K.R., and Dickie, L.M., 1986. The genetics of production characters in the blue mussel *Mytilus* edulis. I. A preliminary analysis. Aquaculture, 57: 133–140.
Manzi, J.J. and Hadley, N.H., 1988.. Recent advances in nursery culture of bivalver mollusks in North America. In: A.K. Sparks (Editor), New and Innovative Advances in Biology/ Engineering with Potential for Use in Aquaculture. NOAA Tech. Rep. NMFS 70. Natl. Mar. Fish. Serv., Seattle, WA, pp. 41–46.
Manzi, J.J., Burrell, V.G., Jr. and Clawson, H.Q.M., 1981. Commercialization of hard clam (*Mercenaria mercenaria*) mariculture in South Carolina: preliminary report. J. World Maricult. Soc., 12(1): 181–195.
Manzi, J.J., Hadley, N.H., Battey, C., Haggerty, R., Hamilton, R. and Carter, M., 1984. Culture of the northern hard clam *Mercenaria mercenaria* (Linné) in a commercial-scale upflow nursery system. J. Shellfish. Res., 4(2): 119–124.
Manzi, J.J., Hadley, N.H. and Maddox, M.B., 1986. Seed clam, *Mercenaria mercenaria*, culture in an experimental-scale upflow nursery system. Aquaculture, 54: 301–311.
Manzi, J.J., Hadley, N.H. and Dillon, R.T., 1991. Hard clam, *Mercenaria mercenaria*, broodstocks: growth of selected hatchery stocks and their reciprocal crosses. Aquaculture, in press.
NAS (National Academy of Sciences), 1978. Aquaculture in the United States: constraints and opportunities. National Academy of Sciences, Washington, DC, 123 pp.
Newkirk, G.F., 1983. Applied breeding of commercially important mollusks: a summary of discussion. Aquaculture, 33: 415–422.
Newkirk, G.F. and Haley, L.E., 1982. Progress in selection for growth rate in the European oyster *Ostrea edulis*. Mar. Ecol. Progr. Ser., 10: 77–79.
Newkirk, G.F. and Haley, L.E., 1983. Selection for growth rate in the European oyster *Ostrea edulis*: response of second generation groups. Aquaculture, 33: 149–155.
Newkirk, G.F., Haley, L.E., Waugh, D.L. and Doyle, R., 1977. Genetics of larvae and spat growth rate in the oyster *Crassostrea virginica*. Mar. Biol., 41: 49–52.
Sokal, R.R. and Rohlf, F.J., 1969. Biometry: The Principles and Practice of Statistics in Biological Research. Freeman, San Francisco, CA, 776 pp.
USDA (US Department of Agriculture), 1988. Aquaculture: Situation and Outlook. U.S. Dept. of Agriculture, Economic Research Service, AQUA1, Washington, DC, 39 pp.
Van Vleck, L.D., Pollack, E.J. and Oltenacu, E.A.B., 1987. Genetics for the Animal Sciences. Freeman, New York, NY, 391 pp.
Wada, K.T., 1986. Genetic selection for shell traits in the Japanese pearl oyster *Pinctada fucata martensii*. Aquaculture, 57: 171–176.