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Growth and Survival of Larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) Relative to Broodstock Conditioning and Lipid Content of Eggs

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ABSTRACT

Gallager, S.M. and Mann, R., 1986. Growth and survival of larvae of *Mercenaria mercenaria* (L.) and *Crassostrea virginica* (Gmelin) relative to broodstock conditioning and lipid content of eggs. *Aquaculture*, 56: 105—121.

Artificial conditioning of broodstocks of *Mercenaria mercenaria* L. and *Crassostrea virginica* (Gmelin) was initiated at various times throughout the year under both laboratory and commercial hatchery conditions. Attempts were made to spawn broodstocks at weekly intervals, and data were collected on the initial total egg lipid content of eggs by analytical procedures and by visual inspection after staining with Oil Red O. The physical parameters of egg diameter and density (estimated by density gradient centrifugation) were correlated with egg lipid content. Significant correlations were evident between the initial egg lipid content and survival to both straight hinge (24 h) and pediveliger stages ($P < 0.01$); however, egg lipid content did not correlate with subsequent larval growth rate. A characteristic of high survival to the straight hinge stage (i.e., > 75%) was catabolism of a minimum of about 4.5 ng total lipid during the embryonic stages (i.e., fertilization through to prodissoconch I shell formation) in both bivalve species. Good survival to the pediveliger stage (i.e., > 1%) was always predicated by eggs containing a minimum of 12% lipid of the ash-free dry weight (AFDW); however, relatively high lipid levels did not guarantee good larval survival. Species-specific egg density profiles were clearly evident; eggs of *C. virginica* banded unimodally at 1.075 $g \cdot cm^{-3}$ while eggs of *M. mercenaria* banded trimodally at 1.038, 1.059 and 1.073 $g \cdot cm^{-3}$. Egg lipid was logarithmically related to egg diameter. The data suggest that there is a minimum, size-related, threshold lipid level in eggs necessary for optimal survival through the non-feeding embryonic stages; but environmental or genetic factors other than egg lipid content are responsible for a considerable fraction of the mortality during this period. Variations in the broodstock conditioning protocol induced large fluctuations in egg lipid levels, suggesting that strict attention should be paid to conditioning if optimal culture potential is desired. Minor variations in egg lipid levels (i.e., 4-8% AFDW) were visually discernible by staining with Oil Red O in both laboratory and hatchery environments. Evaluating eggs at the time of spawning for their potential for survival using the lipid-staining technique can provide valuable real-time information for both research and commercial hatchery applications.

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INTRODUCTION

Routine artificial conditioning of bivalve broodstock may be effected by manipulating the physical (temperature) and nutritional (phytoplankton supply) environment of bivalve stocks removed from ambient waters at various times of the year (Loosanoff and Davis, 1963; Walne, 1970). Only recently has attention been given to details of the conditioning procedure to reduce adult stress (Bayne, 1972) and maximize the viability and survival of resultant larvae (Lannan et al., 1980). The latter authors and Lannan (1980) presented data on *Crassostrea gigas* (Thunberg) which suggest that both genetic and environmental parameters are important sources of variability often associated with culture performance.

The natural gametogenic cycle in bivalve molluscs is closely linked to cycles of glycogen storage and subsequent *de novo* synthesis of lipid during vitellogenesis in the spring at the expense of stored glycogen (Gabbott, 1975). Interruption of this cycle, by artificial conditioning at elevated temperature, may force the development of eggs before sufficient glycogen has accumulated for the synthesis of lipid. Thus, the consequence would be the production of either fewer eggs or eggs of suboptimal quality.

Although the results obtained by Lannan et al. (1980) illustrate the importance of seasonal timing in artificial conditioning systems, the authors had no explanation for the mechanism governing the environmental component of egg quality or the subsequent variability in larval survival. Working with the brooding species *Ostrea edulis* (L.), Helm et al. (1973) found that maximal viability and survival of cultured larvae was directly related to the initial lipid content (specifically neutral lipid) at the time of release. Moreover, the initial lipid content of released larvae and subsequent survival declined during extended conditioning periods. In similar studies, Bayne (1972) and Bayne et al. (1975) reported reduced growth in larvae of *Mytilus edulis* (L.) that developed from gametes of nutritionally stressed adults.

With these studies as a foundation, the present work was designed to test the hypothesis that the level of high energy lipid reserves provided in eggs during vitellogenesis could account for a significant fraction of the variability associated with larval growth and survival in two commercially important species, *Crassostrea virginica* and *Mercenaria mercenaria*. It follows that if the broodstock conditioning protocol influences the supply of energy reserves to developing eggs, then by manipulating the protocol, a powerful tool becomes available that assures the production of eggs in optimal condition.

Collaborative studies with commercial hatcheries were an important component of the present study. Two hatcheries provided a large data base for comparison with results obtained in the laboratory and on-site evaluation of the lipid-specific staining technique (Gallager et al., 1986) as an index of culture performance. The staining technique was also evaluated for its effective-

ness in predicting culture success from the level of visible lipid reserves in newly spawned eggs.

MATERIALS AND METHODS

Laboratory study

Unconditioned broodstocks were obtained from Bluepoints Co., W. Sayville, NY (*Mercenaria mercenaria*) in the months of May, June and October 1982, and from Frank M. Flowers and Sons, Inc., Bayville, NY (*Crassostrea virginica*) in February, May and September and were immediately placed in a flow-through seawater conditioning system. Each group received unfiltered seawater heated or cooled to $15 \pm 2°C$ (*M. mercenaria*) or $22 \pm 2°C$ (*Crassostrea virginica*) and a continuous addition of the flagellate *Isochrysis* aff. *galbana* (clone T-ISO) from two 1000-l culture vessels as a food supplement. Final algal concentration in the broodstock tanks was maintained between 1×10^3 and 5×10^4 cells$\cdot$ml^{-1} except in a few instances when malfunctions of the algal culture system forced 2- to 3-day periods of no algal supplement.

Attempts to spawn each group were made at intervals of 1 to 3 weeks by thermal stimulation at an elevated temperature no more than 10°C above the conditioning temperature. If spawning followed, females were isolated into individual containers and allowed to spawn to completion. Numerals painted on female *Crassostrea virginica* allowed calculation of individual fecundity after each spawn.

Both species of larvae were reared by the methods of Mann and Gallager (1984) and Gallager et al. (1986) in 200-l Nalgene tanks with the exception that the screen size for retention of larvae during water changes was held at 37 μm to allow calculation of total survival (see below). Water was filtered to 0.2 μm and changed three times per week. Cultured algae (*Isochrysis* aff. *galbana* (Clone T-ISO)) were added to a final concentration of 5×10^4 cells$\cdot$ml^{-1}.

Subsamples of larvae were removed at each water change for the following procedures:

(1) morphological measurements – shell length (anterior-posterior) and height (dorso-ventral),
(2) estimate of mortality – percentage of dead or moribund larvae,
(3) staining specifically for lipid content as an indicator of culture condition,
(4) total lipid content by analytical methods.

Survival of larvae was calculated two ways: percentage of eggs developing to the first shelled straight-hinge stage, and percentage of eggs surviving through development to the pediveliger stage. These were compared with the instantaneous growth rate (K) after Crisp (1974),

$$K = 100\,[(\ln L_2 - \ln L_1)/t]$$

where L_1 and L_2 are shell lengths at two points in development separated by time (t) in days.

The procedure for visually assessing the lipid content of shelled larvae followed that of Gallager and Mann (1981) and Gallager et al. (1986). The technique was modified for staining bivalve eggs by briefly fixing eggs in 10% v/v buffered formalin followed by centrifugation in 6-ml flat-bottom vials at $100 \times g$ for 5 min between the staining and clearing steps. Although not necessary, centrifugation allowed more rapid processing of egg samples by reducing the time required for sedimentation between steps; without centrifugation, this required up to 30 min in the viscous glycol solutions. Since hypertonic conditions prevailed in the staining solutions, the egg chorion became somewhat irregular; however, this did not interfere with the staining procedure or visual observation of egg lipid content.

Analytical procedures for determining lipid content were conducted as described in Mann and Gallager (1984) and Gallager et al. (1986) with minor modification. Egg samples were rinsed with isoosmotic ammonium formate (3% w/v) to remove salt and divided into two groups. One group was frozen at $-20°C$ while the other was subsampled for a total egg count and vacuum filtered onto a tared, Millipore filter (5 μm retention), oven-dried at 60°C and weighed on a Cahn electrobalance. Total lipid and ash weight were determined from the first group after lyophylization and combustion at 500°C for 8 h, and the dry weight per egg was estimated from the second. Egg lipid was recorded as a percentage of ash-free dry weight (AFDW) and as total lipid per egg.

The physical parameters of egg diameter and density were estimated in eggs spawned from laboratory-conditioned broodstock of *Crassostrea virginica* during May and of *Mercenaria mercenaria* during July when egg lipid levels were highest in both species (see Figs. 1 and 2). The silica-sol Percoll was combined with sorbitol, Tris, $MgCl_2$ (Pharmacia Fine Chemicals) and seawater filtered to 0.2 μm to prepare density gradients isoosmotic with seawater at 32‰ and 22°C by the procedure of Price et al. (1978). Gradients were produced in 25-ml round-bottom centrifuge tubes by sequential mixing of the Percoll mixture with seawater using a commercial gradient mixer. Egg samples were layered on the surface of the gradients prior to centrifugation at about $2000 \times g$ for 5 min in a swing-out rotor. Precautions were taken to ensure gradient stability by slow acceleration and deceleration (Price et al., 1978). Gradients were unloaded from the bottom up via a peristaltic pump that delivered 0.5-ml aliquots into separate wells of tissue culture plates for egg diameter measurements and enumeration on a Zeiss inverted microscope.

The density of each gradient fraction was estimated by refractive index (Rf) on a bench top B&L refractometer. Initial calibration of density by direct weight measurements on a Cahn Model 29 electrobalance against Rf produced the following linear relationship: density = Rf (6.99) -8.32 ($r^2 = 0.99$, $n = 21$) at 22°C between densities of 1.000 and 1.120 $g \cdot cm^{-3}$. Eggs were washed free of

Percoll with 3% *w/v* ammonium formate and lipid levels were assessed by the procedure previously described.

Statistical analysis of laboratory-generated data consisted of testing the null hypothesis that no correlation existed between initial egg lipid content and subsequent growth and survival with the Pearson product–moment correlation coefficient for two independent variables (Sokal and Rohlf, 1969). Critical levels of significance were taken to be either at 5% ($P<0.05^*$) or 1% ($P<0.01^{**}$).

Hatchery study

The two aforementioned commercial bivalve hatcheries participated in this study by subsampling eggs from each of their broodstock spawns conducted throughout a single season. The eggs were immediately frozen at $\sim -20°C$ and stored for up to 2 months before transfer to the laboratory for analysis. Data were collected on initiation date and duration of the conditioning period for each broodstock, and number of spawning females, and estimates of subsequent survial to pediveliger were assigned to one of four categories (i.e., zero, poor, good and excellent). Subsamples of larvae were also removed throughout development for application of the lipid-specific staining technique as an indicator of culture performance. Egg samples were frozen in the hatcheries without individual egg weight determination so lipid levels were expressed as a percentage of the AFDW only.

RESULTS

Fig. 1A shows the lipid level of newly spawned eggs of *Crassostrea virginica* over a complete season under both laboratory and hatchery conditions. When conditioning was initiated in February, laboratory oysters spawned eggs within 2 weeks with an average lipid level of 16% of the AFDW. Egg lipid content reached a maximum value of 21% when the same broodstock spawned 3 weeks later; lipid content subsequently decreased to 12% within the following month. Highest lipid content in laboratory-spawned eggs, 21.5%, occurred from the first spawn of a broodstock after 2 weeks of conditioning which had commenced in May; egg lipid again decreased in subsequent spawns. Despite numerous attempts, oysters conditioned in the fall did not spawn for over 6 weeks, and then only very poorly, with egg lipid contents averaging only 6.5%.

Contrary to the laboratory spawning protocol that was designed to examine the effect of prolonged sequential spawning on single broodstocks, hatchery protocol usually dictated that subsamples of each broodstock be spawned only once. The first subsample of the broodstock initiated in February at the Frank M. Flowers Oyster Company released eggs with a low lipid content of 6%; 3 weeks later a second subsample from the same broodstock spawned eggs containing 14.5% lipid. A maximum lipid content of 25.5% was produced by brood-

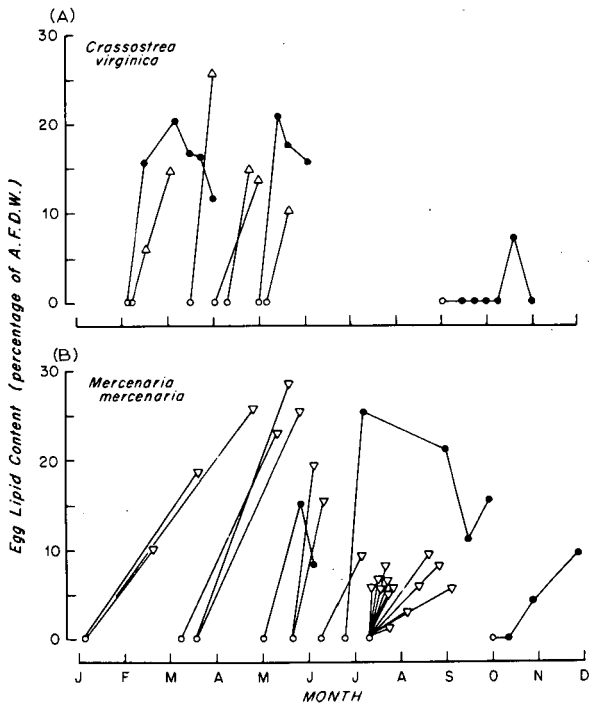


Fig. 1A. Total lipid contents in newly spawned eggs of *Crassostrea virginica* over a complete spawning season. Laboratory data, ●; data from the Frank M. Flowers and Sons hatchery, △; date on which conditioning was initiated for a particular broodstock, ○.
Fig. 1B. Total lipid contents in newly spawned eggs of *Mercenaria mercenaria* over a complete season. Symbols are as in Fig. 1A except ▽ denotes samples from Bluepoints Co.

stock that had been initiated in March and conditioned for 1 month. The egg lipid content in spawns subsequent to this decreased over the next 2 months.

A similar pattern was evident during conditioning of *Mercenaria mercenaria* (Fig. 1B). Egg lipid was high in each of the first spawns of laboratory-conditioned broodstock initiated in May and June and decreased in spawns subsequent to this. A shorter conditioning period was necessary to achieve maximal egg lipid contents in broodstock initiated at the end of June compared to those initiated in early May. Although not determined directly, this was probably a reflection of the gametogenic stage attained by the broodstock at the time conditioning was initiated.

Hatchery-conditioned broodstock of *Mercenaria mercenaria* (Fig. 1B) produced spawns of eggs with maximal lipid levels during the month of May. This was followed by a general decline in egg lipid even though three different broodstocks were used between May and August. Numerous cultures were started during July and August from a single broodstock; all spawns possessed low lipid contents and, as discussed below, resulted in poor survival. Both this and the

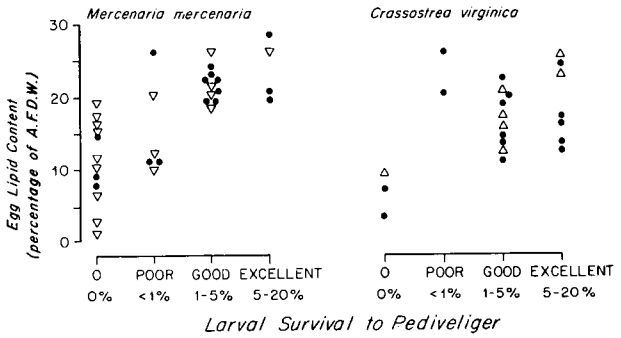


Fig. 2. The relationship between total lipid contents in newly spawned eggs of *Mercenaria mercenaria* and *Crassostrea virginica* and larval survival to the pediveliger stage. Laboratory data, ●; data from Bluepoints Co., ▽; data from the Frank M. Flowers and Sons hatchery, △.

broodstock initiated in June were not conditioned in the hatchery; rather, they were removed from ambient water and held at 15°C with a food supplement for a short period before spawning. It is likely, therefore, that the gametogenic stage of these clams was representative of naturally conditioned organisms. This would strongly imply that natural larval growth and recruitment of *Mercenaria mercenaria* was also poor during these months. Indeed, such unusual conditions were reported by hatchery operators monitoring natural larval populations (K. Kurkowski and C. Strong, Bluepoints Co., pers. commun., 1982).

The above data on egg lipid contents over time have been plotted in Fig. 2 with respect to the subsequent performance of each culture. Where possible, the hatchery survival index has been equated with percentage ranges from laboratory data. Egg lipid, as a percentage of the AFDW, was variable in both bivalve species under both laboratory and hatchery conditions. Although eggs of *Mercenaria mercenaria* that had lipid contents ranging from 2 to 26% yielded poor to zero survivorship in some cases, good to excellent survival, i.e., >1% egg to pediveliger, was not obtained unless egg lipid exceeded ~18%. Mortality of larvae developing from eggs with high lipid contents may have been effected through any number of environmental, physical or nutritional perturbations, but the corollary is that in no instance did eggs with low lipid contents yield cultures with survival greater than 1%.

As a partial explanation, Table 1 provides laboratory results of relationships between the initial egg lipid content, lipid catabolized during embryogenesis and subsequent survival. Survival to day 1 was significantly correlated with both the initial egg lipid content and the quantity of lipid catabolized during embryogenesis (i.e., that period between fertilization and prodissoconch I formation) in both bivalve species ($P<0.01$). Cultures yielding greater than 75% survival to day 1 were characterized by a minimum catabolism of 5.1 ng lipid (*Crassostrea virginica*) or 4.0 ng lipid (*Mercenaria mercenaria*) during embryonic development. Cultures generated from eggs containing less than this

TABLE 1

Fecundity of individual female *Crassostrea virginica* conditioned and spawned in the laboratory. Conditioning was initiated on 2 December 1982

Female no.	Days conditioned	Number of eggs spawned	Egg lipid level (% of AFDW	Egg diameter (μm, $X \pm$ S.D.)	Survival: Egg to pediveliger (%)
1	14	400 000	17.0	51 ± 8	0
	21	0			
	28	0			
	35	0			
	42	0			
2	14	4 180 000	16.2	48 ± 6	0
	21	0			
	28	0			
	35	0			
	42	0			
3	14	690 000	17.5	54 ± 9	0
	21	0			
	28	0			
	35	2 905 000	19.4	57 ± 13	5
	42	0			
4	14	10 710 000	14.5	42 ± 10	0
	21	3 640 000	17.9	54 ± 8	8
	28	0			
	35	0			
	42	0			
5	14	12 790 000	12.1	44 ± 8	0
	21	2 235 000	20.0	56 ± 14	6
	28	0			
	35	0			
	42	0			
6	14	0			
	21	2 300 000	23.0	58 ± 13	2
	28	1 900 000	18.3	54 ± 7	16
	35	0			
	42	0			
7	14	0			
	21	0			
	28	6 000 000	16.9	53 ± 9	12
	35	210 000	17.5	55 ± 6	18
	42	0			
8	14	0			
	21	0			
	28	0			
	35	3 780 000	16.1	46 ± 8	4
	42	4 000 000	11.7	41 ± 11	0

TABLE 2

Lipid content of bivalve eggs from various laboratory spawns and its effect on larval growth rate and survival

Egg lipid content ($ng \cdot egg^{-1}$)	Lipid content at day 1 ($ng \cdot larva^{-1}$)	Lipid catabolized during embryogenesis ($ng \cdot larva^{-1}$)	Survival: egg to day 1 (%)	Growth rate: egg to pediveliger (K)	Lipid content of pediveligers ($ng \cdot larva^{-1}$)	Survival: egg to pediveliger (%)
Crassostrea virginica						
9.2	4.1	5.1	90	5.4	92.6	9
4.3	1.0	3.3	35	1.2	54.9	0.01
7.0	0.4	6.6	80	2.6	108.0	8
6.8	1.1	5.1	75	4.7	81.3	10
4.7	0.9	3.8	20	1.6	29.3	0.6
6.7	0.3	6.4	78	0.9	74.5	6
Mercenaria mercenaria						
8.0	4.0	4.0	85	2.6	142.8	7
8.3	2.6	5.7	87	7.8	180.6	5
2.3	0.8	1.5	20	4.4	57.1	0.9

"threshold" level had poor survival, i.e., $\leq 35\%$ to day 1; apparently, since there was no correlation between the endogenous lipid remaining at day 1 and subsequent survival ($P > 0.05$), the quantity of lipid available for catabolism during embryogenesis was more important to success than the lipid contents remaining after this period.

Instantaneous larval growth rates (K) (Table 2) were not significantly correlated with either the initial egg lipid content or lipid catabolized during embryogenesis. This was not unexpected since fluctuations in environmental conditions, exogenous nutrient supply or ingestion and assimilation of food material after endogenous lipid has been completely catabolized should have a relatively greater influence on growth than the initial egg lipid contents. Survival from egg to pediveliger was not correlated with growth rate but was correlated with the initial egg lipid content ($P < 0.01$) suggesting a decoupling between growth rate and survival

The data in Table 1 give estimates of individual female fecundity, resultant egg lipid contents and larval survival from spawns of a single broodstock of *Crassostrea virginica* over a 42-day period. When spawning of a single female occurred more than once, the number of released eggs was generally greater in the first spawn. Although not a consistent feature of the egg lipid contents, in 60% of the spawns, egg lipid appeared higher in the second spawn, but not significantly so ($P > 0.05$). A negative correlation existed between egg lipid and number of eggs spawned at any given time ($P < 0.01$). This relationship was similar to that seen in Fig. 2; although eggs resulting from small spawns could concomitantly be low in lipid, eggs from large spawns were always lower in lipid than those from smaller spawns.

Species-specific density banding patterns were produced by eggs of each bivalve species (Fig. 3). Fifty percent of the eggs from nine separate spawns of

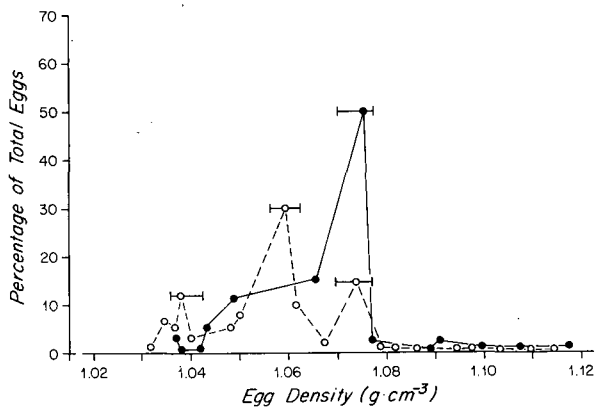


Fig. 3. Mean species-specific density distributions of eggs from three spawns of *Crassostrea virginica*, ●-●, and two spawns of *Mercenaria mercenaria*, ○--○. Horizontal bars indicate range of density peaks.

Crassostrea virginica banded unimodally at 1.075 $g \cdot cm^{-3}$ with a range from 1.070 to 1.076. A broad plateau occurred between 1.049 and 1.065 $g \cdot cm^{-3}$. Six spawns of *Mercenaria mercenaria* contained eggs with a consistent trimodal distribution with maxima at 1.038, 1.059 and 1.073 $g \cdot cm^{-3}$; these were 12, 30 and 15% of the total, respectively.

Logarithmic relationships between egg density, diameter and percentage lipid level for both species tested are given in Table 3 and the egg diameter versus lipid level is graphically illustrated in Fig. 4. Immediately evident is the almost two-fold greater Y intercept for eggs of *Mercenaria mercenaria*, reflecting the larger egg diameter of this species in general (Loosanoff and Davis, 1963). The shapes of the two curves in Fig. 4 are remarkably similar but only overlap at the largest egg diameter of *Crassostrea virginica* and the smallest diameter of *M. mercenaria*. At this size, 60 μm, there was a 10-fold difference in percentage

TABLE 3

The relationships between egg density (ρ), diameter (D), and percentage lipid level (L) in eight spawns of *Mercenaria mercenaria* and twelve spawns of *Crassostrea virginica*

Variable	Species	
	M. mercenaria	*C. virginica*
ρ	$=1.1-0.01\ (\ln L)$ $r^2=0.94$	$=1.1-0.03\ (\ln L)$ $r^2=0.98$
ρ	$=1.9-0.21\ (\ln D)$ $r^2=0.98$	$=1.6-0.14\ (\ln D)$ $r^2=0.98$
D	$=52.9+4.7\ (\ln L)$ $r^2=0.96$	$=29.2+7.8\ (\ln L)$ $r^2=0.82$

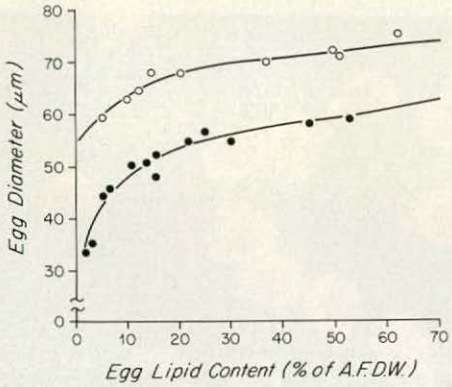


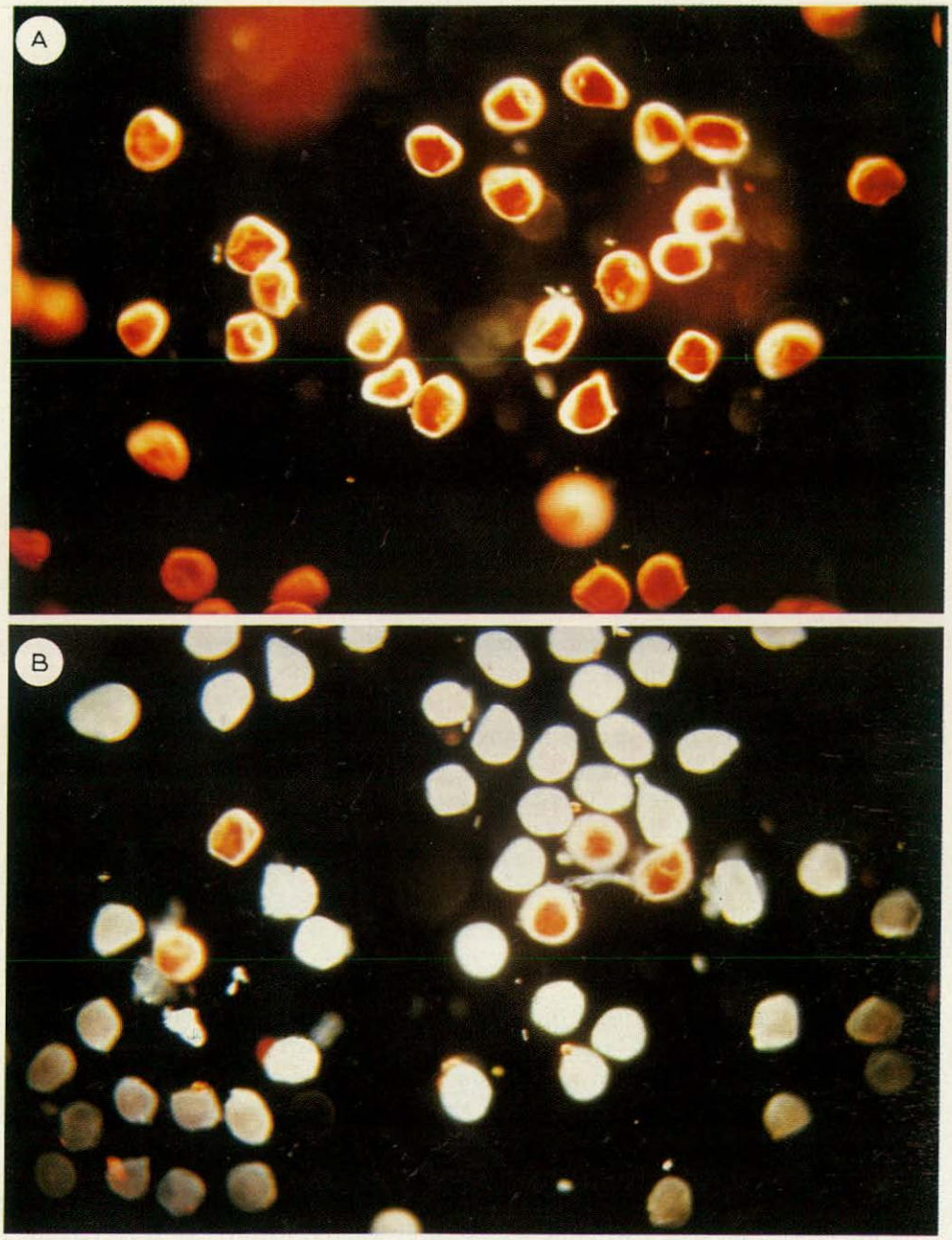
Fig. 4. The relationship between egg lipid as a percentage of the ash-free dry weight and egg diameter determined after separation by density gradient centrifugations; ●, *Crassostrea virginica*; ○, *Mercenaria mercenaria*.

lipid between species; *C. virginica* had a higher lipid level relative to egg diameter compared with *M. mercenaria*. The diameter at which eggs of both species consisted of about 50% lipid was 60 μm for *C. virginica* and 74 μm in *M. mercenaria*. The cubic relationship of the radius of a sphere in volumetric analyses explains how a difference in diameter of only 14 μm may result in a doubling of the absolute quantity of lipid in the larger egg.

The photographs in Plate I depict the visual difference in lipid levels of eggs after staining with Oil Red O. Group A had a mean lipid level of 24% AFDW while the variability between individuals was low. Group B contained 8% AFDW lipid and had a high degree of individual variability. Marked differences between eggs within a sample were a consistent feature of groups that yielded cultures with poor survival. Although the photographs illustrate an extreme situation, differences of just 4 to 8% could be distinguished; this is equivalent to ~0.32 to 0.7 ng total lipid per egg.

DISCUSSION

Inherent physiological and biochemical variability among sibling bivalve larvae can always be expected; understanding the source of that variability is crucial to reducing it in culture situations. By employing diallel analysis of the gametogenic condition of broodstock and subsequent larval survival in 180 random matings of *Crassostrea gigas*, Lannan (1980) concluded that a significant proportion of the variability in culture success was attributable to factors other than those under genetic control. Lannan and co-workers further proposed the existence of a window temporally situated within the gametogenic cycle when eggs of optimal "quality" were produced (Lannan et al., 1980). Prior to these studies, Helm et al. (1973) reported a similar decline in larval growth rate and vigor resulting from broodstock of *Ostrea edulis* that had been


A
B

spawning over an extended season. The results of the present study substantiate those obtained by the above authors; growth and survival of larvae of *Mercenaria mercenaria* and *C. virginica* were directly associated with both the time of the year when the conditioning procedure was initiated and the duration of the conditioning procedure. Laboratory and hatchery results showed maximal larval survival to occur only after a relatively long conditioning period when conditioning was initiated in winter months, while a shorter period was necessary when the gametogenic cycle was further advanced in the spring. Survival, in turn, was highly correlated with the initial quantity of endogenous total lipid supplied in eggs during vitellogenesis.

Poorly conditioned broodstock of *Mercenaria mercenaria* under natural conditions (Fig. 1B, July), or spawning of a single broodstock over an extended period of time, consistently produced eggs with low lipid levels (i.e., $\leq$ 10% AFDW) and subsequent poor larval survival. Crisp (1974) theorized that optimal reproductive strategy in bivalves should lead to allocation of sufficient energy per egg to allow normal development without an abundant energy surplus. Realistically, this is seldom the case since variability within the vitellogenic process is compounded by exogenous influences such as food supply and temperature. For example, Bayne et al. (1982) found that when adult mussels (*Mytilus edulis* L.) were conditioned under both high and low food rations, more ^{14}C-carbon from a pulse feeding with labelled algae was incorporated into developing eggs at the low food ration; conversely, glycogen became heavily labelled at the high ration. This suggests that although vitellogenesis normally proceeds at the expense of stored glycogen reserves (Gabbott, 1975; Mann, 1979; Bayne et al., 1982) a shift may occur in poorly conditioned broodstock that forces egg production directly from biochemical components of the nutrient supply. In the same study by Bayne et al., reduced larval growth rate was demonstrated to be a product of eggs spawned under nutrient stress.

Embryogenesis in molluscan larvae (i.e., that period between fertilization of the egg and development to the prodissoconch I stage; sensu Thorson, 1946) is characterized by extensive morphological change and a concomitant loss in energy reserves. Pandian (1969) calculated that the oxidation of endogenous lipid reserves during embryogenesis in the slippershell, *Crepidula fornicata* Say, was 65.3% of the total energy expended while 18.8 and 6.3% were contributed by protein and carbohydrate, respectively. Unfortunately, there are no comparable published data sets for any bivalve species where losses in all three biochemical substrates have been measured simultaneously.

Gallager et al. (1986) reported the loss of 48 and 55% of the total endogenous egg lipid in *Mercenaria mercenaria* and *Crassostrea virginica*, respectively,

PLATE I.

Lipid contents in newly spawned eggs of *Crassostrea virginica* indicated by the visual stain Oil Red O. Mean total lipid levels were 24% AFDW in group A, and 8% AFDW in group B.

within 24 h of fertilization at 25 °C. The major lipid class catabolized during embryogenesis was triacylglycerol in both of these species. The authors stressed the importance of endogenous triacylglycerols during early development after histochemically describing the disappearance of lipid droplets in embryos prior to the initiation of feeding. Once feeding began, parentally derived lipid in peripheral tissues was lost and the digestive gland became filled with lipid from the nutrient source.

Consistent with the above studies, the present data identify an apparent threshold for endogenous egg lipid below which normal metabolism and development were impeded. The rationale for a threshold lipid level in eggs follows from the consideration that the developing embryo progresses through a series of non-feeding stages. Lucas and Rangel (1983) used microepifluoroscopic techniques to demonstrate that embryonic stages of *Crassostrea gigas* do not feed on algal cells prior to complete shell formation 24 h after fertilization at 24 °C, and 30 h at 21 °C.

Coupled with data for the loss of biochemical components, the inability to feed on particulates suggests a starvation condition during embryogenesis; however, utilization of dissolved organic material (DOM) should not be overlooked. Studies by Manahan (1983) and the present authors (unpubl. data, 1984) provide evidence for uptake of dissolved free amino acids by active transport in fertilized eggs and developing larvae of *Crassostrea gigas* and *Bankia gouldi* (Bartsch), respectively. DOM, including free fatty acids, may aid embryos lacking the minimum energy requirement, but its quantitative nutritional importance must await further efforts.

In the study of Bayne et al. (1975), the lipid of unfertilized mussel eggs correlated directly with early larval development, but was not correlated with growth of older larvae once feeding had been initiated. Similarly, a significant correlation between lipid content of day 1 larvae and subsequent survival was not found in the present study. It follows that the influence of endogenous egg lipid should be more profound during the non-feeding embryonic stage when metabolism is dependent on stored energy reserves than during larval development of feeding stages. As suggested by Bayne et al. (1975), the results obtained by Helm et al. (1973) would therefore appear to have been a product of egg quality and metabolism during embryogenesis rather than a direct result of lipid levels in larvae capable of feeding at the time of release.

The physical dimensions of eggs have been an important consideration when attempts have been made to dichotomize bivalve reproductive strategies into major developmental types; e.g., lecithotrophic – large eggs (>90 μm in diameter) containing a high quota of lipid to fuel development, and planktotrophic – small eggs (40–90 μm), containing low lipid levels and larvae feed in the plankton (Thorson, 1946; Ockelmann, 1965). Within this wide range of egg sizes, oviparous species usually produce eggs with diameters ranging from 40

to 80 μm; intraspecific variation, however, is reportedly much less (Loosanoff and Davis, 1963).

Reinforcing the work of Bayne (1972) and Bayne et al. (1975), Kraeuter et al. (1982) found statistically significant correlations between egg size (graded by sequential Nitex sieves) and larval survival (to 48 h) in two bivalve species, *Mercenaria mercenaria* and *Argopecten irradians* Say. The study demonstrated that unequal distribution of cellular material during vitellogenesis (the authors presumed it was lipid) can lead to increased variability in larval survival, a conclusion alluded to but unsubstantiated by Lannan et al. (1980).

In another laboratory-based study on *Mercenaria mercenaria*, Bricelj (1979) demonstrated that the equivalent spherical volume of spawned eggs fits a bimodal distribution that became more skewed toward smaller eggs late in the reproductive season. A third peak of small material occurred occasionally in Bricelj's analyses but she attributed this to fragmented debris of otherwise viable eggs (pers. commun., 1984).

Unfortunately, in the present study we only subsampled eggs for density gradient analysis from spawns conducted during the peak of the reproductive season as indicated by egg lipid content. Therefore, no trend in egg density with spawning period could be established. However, from the relationships between egg lipid, diameter and density generated in the present study we assume that the decline in egg lipid as broodstock conditioning progressed was accompanied by a concomitent increase in egg density and decrease in egg diameter.

The species-specific density banding patterns of eggs seen in the present study suggest that although intraspecific variation may be low (note the range values for the density peaks in Fig. 3), interspecific variation can be high. It is tempting to speculate that this is a function of evolutionary adaptation of reproductive strategies designed to fill specific ecological niches. Certainly egg density will play a major role in dispersal of embryonic stages prior to development of larval sensory mechanisms that ultimately govern behavior. Indeed, the trimodal egg distribution of *Mercenaria mercenaria* may confer more hydrodynamic flexibility on the mechanism by which transport and dispersal take place in unstable environments compared with the relatively high unimodal density of *C. virginica*. However, the latter species would be at an advantage in stable environments that favored smaller and more dense eggs. An example might be estuaries that tend to retain particulate material in deep, saline water during counter-current density-driven flow (Boicourt, 1981). Obviously, we would need to include many more species and habitats to test this hypothesis.

Perhaps a more descriptive parameter than egg diameter for interspecific comparisons is egg volume. Volume is proportional to the cube of the radius of a sphere, so even very small changes in egg diameter will lead to substantial volumetric changes of 50% or more. A large egg with the same *percentage* of

lipid as a smaller egg can contain more than double the absolute quantity of lipid. The extent of the variability seen in the physical properties of eggs in the present study can easily account for groups of eggs not achieving or exceeding the minimum threshold lipid level necessary for normal embryogenesis.

The utility of the lipid-specific staining technique for estimating the probability of larval survival from the lipid content of newly spawned eggs is only diminished by the extra centrifugation steps necessary to ensure rapid sedimentation between fixation, staining, and clearing. Both laboratory and hatchery personnel were able to distinguish between groups of eggs with lipid levels greater than ~18% and less than ~10% AFDW by the stain technique. Moreover, the degree of variability between individual eggs within a sample provided as much insight into subsequent culture performance as did an estimate of average lipid content. Absolute and relative lipid levels in individual eggs may be estimated by microdensitometry (Gallager and Mann, 1986), enabling statistical evaluation of the distribution of lipid within a population of eggs.

Armed with the ability to detect major fluctuations in lipid content in newly spawned eggs, and an appreciation of the salient features of Fig. 3 (i.e., high egg lipid level does not guarantee good culture performance, but the latter cannot occur without high egg lipid), the researcher and commercial hatchery operator should be able to save valuable time and expense by properly conditioning broodstock and screening spawns for their optimal potential for success.

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