Gonadal Condition of Hard Clams in a South Carolina Estuary[1]

Arnold G. Eversole, *Department of Aquaculture, Fisheries and Wildlife, Clemson University, Clemson, SC 29631*

William K. Michener,[2] *Department of Aquaculture, Fisheries and Wildlife, Clemson University, Clemson, SC 29631*

Peter J. Eldridge,[3] *South Carolina Wildlife and Marine Resources Department, P.O. Box 12559, Charleston, SC 29412*

Abstract: Gonadal condition of hard clams (*Mercenaria mercenaria*) planted at 2 tidal locations and at 3 population densities were evaluated in relation to age, size, sex, season, and culture condition. Changes in gonadal-somatic indices (GSI) reflected seasonal differences in gonadal development. Similar decreases in GSI were observed during the spring (May–Jun) and fall (Sep–Oct) spawning peaks. GSI varied significantly ($P < 0.0001$) with clam size and age. Larger clams had proportionally more gonadal tissue than smaller clams of the same age. Similarly, older clams had larger GSI than younger clams of the same size. No statistical difference ($P > 0.05$) was detected between the GSI of female and male clams of the same age and size. Clams grown at the lowest density level or at the subtidal location were larger and had proportionally more gonadal tissue than clams from higher densities or the intertidal location. Differences in size of clams among treatments explained only the variation in GSI among density treatments, but not between tidal locations.

Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 38:495-505

In previous papers it has been shown that increased population density resulted in significant reductions in growth of hard clams and when density was reduced, clams exhibited the phenomenon of compensatory growth (El-

[1] Technical contribution No. 2208, published by permission of the director, South Carolina Agricultural Experiment Station.

[2] Present address: Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, P.O. Box 1630, Georgetown, SC 29442.

[3] Charleston Laboratory, National Oceanic and Atmospheric Administration, NMFS, P.O. Box 12607, Charleston, SC 29412.

dridge et al. 1979, Eldridge and Eversole 1982). Also, clams held in a subtidal location grew significantly faster than clams in an intertidal location (Eldridge et al. 1979). In spite of these growth responses to increased density and tidal position, no statistical differences were detected in the sex ratio, in the pattern of gametogenesis, or in any quantitative measure of gonadal condition (e.g. % lumen or size of ovocytes) between density levels or tidal locations (Eversole et al. 1980). Peterson (1982) observed that a reduction in gonad mass accompanied increased levels of population density in 2 suspension-feeding bivalves, *Protothaca staminea* and *Chione undatella.* Since the amount of gonadal tissue was not initially determined, it was not possible to ascertain if hard clams, also an infaunal suspension feeder, responded in a similar way to increased density.

The objective of the present investigation was to determine the effects of size, age, sex, and culture conditions (density levels and tidal locations) on the gonadal condition (gonadal-somatic indices, GSI) of hard clams. An additional objective was to assess seasonal changes in gonadal development and GSI.

The authors thank numerous persons for helping with the field work: C. A. Aas, G. Steele, S. A. Summer, G. Ulrick, W. Waltz, and J. M. Whetstone. Dr. L. W. Grimes is acknowledged for assistance with data analysis. Financial support was provided by South Carolina Experiment Station and South Carolina Wildlife and Marine Resources Department.

Methods

Hard clams (mean shell length, SL = 13 mm) obtained from Coastal Zone Resources Corporation of North Carolina in May 1975 were planted in 20 protected trays containing 14 cm of natural sediment. Clams were the same age, approximately 5 months old at planting. Trays were planted at 3 population densities (290, 869, and 1,159 clams/m^2) and 2 tidal locations (subtidal and intertidal) in an estuarine area (mean salinity = 25 ppt) near Clark Sound, South Carolina. Initial density levels were maintained through May 1978 when trays containing clams at the 2 highest densities (869 and 1,159 clams/m^2) were adjusted to the lowest density level (290 clams/m^2). These clams were replanted and cultured until May 1980. Details of the sampling site, trays, and tray maintenance procedures were outlined by Eldridge et al. (1979) and Eldridge and Eversole (1982).

Clams were subsampled 22 times from May 1975 to May 1980: monthly in 1975, quarterly through May 1978, and once in May 1980. Clams from each density level and tidal location were subsampled and preserved in 10% buffered formalin. These clam subsamples were randomly divided, approximately half (N = 304) were used for histological studies (see Eversole et al. 1980 for details) and the other half (N = 318) were stored for gonadal-somatic index (GSI) determinations. The first female clam was detected in

September 1975 by microscopic examination of gonadal sections (Eversole et al. 1980). GSI determinations were made on clams starting on the next sampling date (Oct 1975) and continued through May 1980. Clams selected for GSI were measured for SL and total tissue wet weight before the gonad was carefully scraped from the visceral mass. Checks of gonad scrapings eliminated some of the initial fear that gonadal tissue could not be adequately separated from the rest of the visceral mass (e.g. digestive tract). Gonad and residual body tissue were dried at 90° C to constant weight. Wet and dry weights of these 2 variables were used to calculate GSI_W and GSI_D, respectively:

$$GSI_W = \frac{\text{gonad wet weight}}{\text{total tissue wet weight}} \times 100$$

$$GSI_D = \frac{\text{gonad dry weight}}{\text{total tissue dry weight}} \times 100$$

A subsample ($N = 24$) of clams from May 1978 was prepared for histological examination to determine sex. After weighing the gonad, a small sample of gonadal tissue was dehydrated in an alcohol series, cleared in xylene and embedded in paraplast. Sections were cut at 8–10 μm and stained with Harris hematoxylin and eosin. The GSI_D values for these clams were determined by converting gonad wet weight to dry weight using a conversion factor for water content in gonad tissue. Water content of gonads was determined using 84 clams from the May 1978 collection. Estimated GSI_D using the conversion factor for this subsample was determined not to be statistically different from the 84 calculated GSI_D values for May 1978.

GSI values for sizes, sexes, ages, seasons, and culture conditions (density levels and tidal locations) were calculated and compared with analysis of covariance and Student's t-tests using Statistical Analysis System (SAS) – 79) developed by Barr et al. 1979. Values for GSI appeared to be normally distributed and therefore were not transformed.

Dry weights of eviscerated bodies and gonads fluctuated less than wet weights. However, in no statistical analysis did the use of GSI_D versus GSI_W alter any conclusions. For these reasons and simplicity, GSI_D was selected as the gonadal index to report throughout the paper.

Results and Discussion

Reproductive Cycle

The seasonal pattern of monthly gonadal-somatic means averaged across years and treatments (Fig. 1*a*) was very similar to the seasonal patterns for mean % lumen (Fig. 1*b*) and for gonadal development frequency (evaluated histologically) for male and female hard clams (Fig. 1*c*). Quantitative data (not illustrated), such as % lumen filled with spermatocytes, spermatids, and

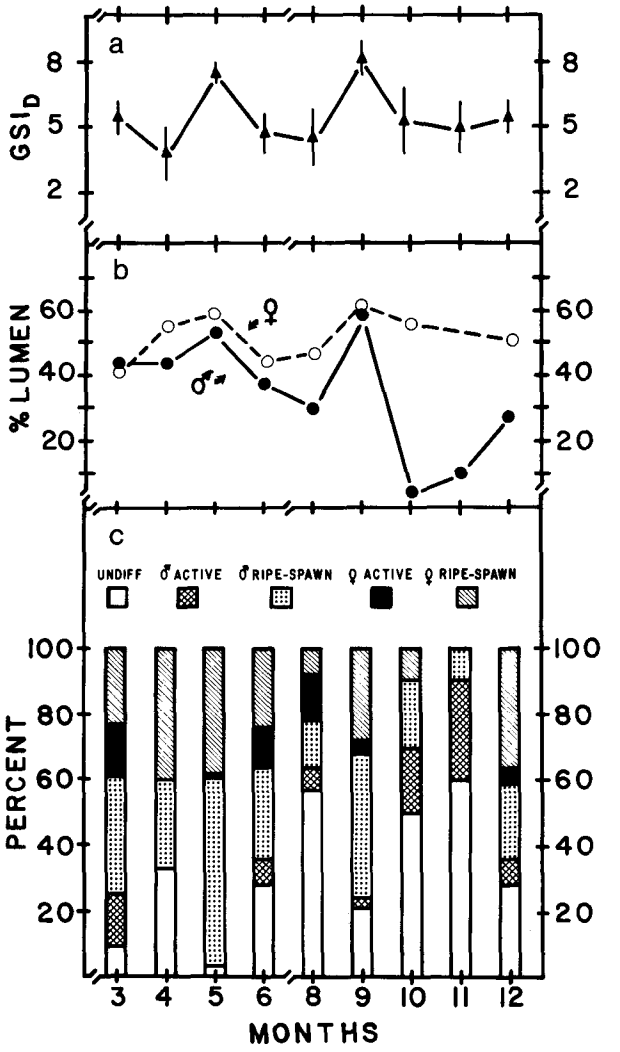

Figure 1. Composite of the developmental stages and sex of hard clams in relation to the quantitative condition of the gonad. Composite values were calculated by combining monthly values across years and treatments (density and tidal level). a.) Closed triangles with the vertical bars represent monthly means and standard errors of gonadal-somatic indices based on dry weight (GSI_D) from October 1975 to May 1980. GSI_D values for each month sampled are found in Table 1. b.) Circles represent monthly means of % lumen of male and female clams from September 1975 to May 1978 (redrawn from Eversole et al. 1980). Percent lumen was calculated by determining that area occupied by lumen (space within the follicle wall) in a standard area of gonadal tissue. c.) The length of shaded areas represents monthly frequencies (%) of clams in each developmental stage and sex from Septembr 1975 to May 1978 (after Eversole et al. 1980).

Table 1. Mean gonadal-somatic indices (GSI_D) for clams sampled from October 1975 through May 1980 (monthly means combined across treatments) and results of analysis of covariance with month (age) and shell length (SL) fit as covariates.

A. GSI_D values Month (age in months)	Mean	± SE	*N*
Oct 75 (10)	5.25	1.454	7
Nov 75 (11)	4.96	1.271	7
Dec 75 (12)	5.51	1.004	11
Mar 76 (15)	4.93	1.123	9
Apr 76 (16)	3.88	1.194	3
Aug 76 (20)	4.67	1.194	3
Sep 76 (21)	8.02	0.960	11
Dec 76 (24)	5.37	1.082	9
Mar 77 (27)	6.19	0.894	12
Jun 77 (30)	4.75	0.918	12
Sep 77 (33)	8.36	0.920	12
Dec 77 (36)	5.40	0.883	12
Mar 78 (39)	5.20	0.883	12
May 78 (41)	5.76	0.293	108
May 80 (65)	9.39	0.357	90

B. Analysis of covariance Source of variation	df	SS	*F*	*P*
Age	1	270.5509	25.06	0.0001
SL	1	244.3421	22.63	0.0001
Error	315	3,401.0698		

spermatozoa for male clams or % lumen filled with ovocytes and ovocytes number and size for females (see Eversole et al. 1980) reflect basically the same reproductive pattern as shown in Figure 1. Gonadal-somatic indices and other expressions of gonadal activity (e.g. gametogenesis) show a bimodal reproductive pattern, with spawning peaks occurring in May and June and in September and October. Spawning is marked by sharp decreases in GSI_D. The slight decline in mean GSI_D in April may be due to the small sample size (Table 1). The continued decline in GSI_D through August indicated spawning was extended. Gonadal tissue quickly regenerated after the first spawning and GSI_D reached its highest monthly mean in September. Spawning peaks appear of equivalent importance as indicated by the change of GSI_D. The average decline in GSI_D through the spring spawning peak was 2.83 and through the fall peak was 2.76. The relative importance of a second or possibly a third spawning in polymodal breeding patterns is expected to increase in the more southerly portions of the distribution of northern temperate marine invertebrates. This appears to be the case with hard clams, because in Long Island Sound (Loosanoff 1937) and Delaware Bay (Keck et al. 1975) clams have unimodal spawning patterns, in North Carolina the reproductive cycle is bimodal with the major spawning peak in June and a minor peak in September and October (Porter 1964), and in South Carolina the 2 peaks appear equiva-

lent. Reproductive data are not available for clam populations south of South Carolina, but a continuation of this trend is expected if synchronous breeding patterns are maintained.

Manzi et al. (1981) reported little differences in the trends of annual events in the reproductive cycle between intertidal and subtidal populations of oysters (*Crassostrea virginica*) in South Carolina. In this study, hard clams from both intertidal and subtidal locations, and different population densities (i.e. 290, 869, and 1,159 clams/m^2) exhibited a bimodal pattern with spawning in May and June and in September and October. However, clams from the subtidal location and the lowest density (290 clams/m^2) displayed greater changes in GSI_D during these spawning periods.

Sex Differences

No significant difference ($P > 0.05$) was found between the GSI_D values of female and male clams of the same size and age (Table 2). Though GSI_D is not a direct measure of the cost of reproductive effort, GSI_D does provide an estimate of the proportion of body tissue devoted to reproduction, and male and female clams appear to allocate similar proportions. Grahame (1973) reported similar results for the production of male and female gametes in *Littorina littorea,* a prosobranch gastropod. He suggested that successful fertilization relies on a much greater production of sperm.

Size and Age Differences

Gonadal-somatic indices varied significantly ($P < 0.0001$) with hard clam size and age (Table 3). Larger clams of the same age have proportionally more gonadal tissue than smaller clams. Similarly, older clams have proportionally larger gonads than younger clams of the same size. It is expected that increases in gonadal tissue would be accompanied by increases in gamete production. Positive correlations have been observed between female size and ovocyte or egg production in the soft-shell clam (*Mya arenaria*) (Coe and Turner 1938, Brousseau 1978) and in hard clams (Bricelj and Malouf 1980).

Table 2. Mean gonadal-somatic indices (GSI_D) for male and female clams from May 1978 sample and results of analysis of covariance with shell length (SL) fit as a covariate.

A. GSI_D values Sex	Mean	± SE		*N*
Male	5.40	0.693		7
Female	5.45	0.429		17
B. Analysis of covariance **Source of variation**	**df**	**SS**	***F***	***P***
Sex	1	0.0109	0.00	0.9521
SL	1	39.3566	13.34	0.0015
Error	23	61.9524		

However, Bricelj and Malouf (1980) found no significant difference in mean ovocyte size, % fertilization or larval survival between different sizes of hard clams. The relationship between these variables and clam age has not been investigated.

Williams (1966*a*, *b*) hypothesized that reproductive effort should increase with female age. Browne and Russell-Hunter (1978) concluded after a search of molluscan literature that Williams' (1966*a*, *b*) theoretical statements were justified. Data provided here add support to this concept in that increases in clam reproductive effort (i.e. more tissue devoted to gonads) accompanied increases in age without corresponding increases in size and vice versa. Apparently, both male and female clams exhibit the same relationship because sex ratios of the samples used in data analysis appeared equal. Also, the sex ratios of clams histologically examined were not significantly different from 1:1 in 1976, 1977, and 1978 (Eversole et al. 1980). Adult hard clams occurred equally as males and females in other populations (Loosanoff 1937, Ansell et al. 1964, Bricelj and Malouf 1980).

Tidal Location and Density Level Differences

Analysis of covariance with SL and age in months fit as covariates was conducted to detect differences in GSI_D between tidal locations and density levels (Table 3). GSI_D values from May 1980 were not included because density levels had been adjusted to the lowest density level (290 clams/m^2) in

Table 3. Mean gonadal-somatic indices (GSI_D) for clams from different population densities (290, 869 and 1,159 clams/m^2) and tidal locations (subtidal and intertidal) sampled from October 1975 through May 1978 and results of analysis of covariance with shell length (SL) and month (age) as covariates. Data from May 1980 deleted from the analysis.

A. GSI_D values Treatments	Mean	± SE	*N*
Density			
290/m^2	5.44	0.361	72
869/m^2	5.41	0.317	79
1,159/m^2	5.03	0.331	77
Tidal locations			
Subtidal	5.73	0.260	115
Intertidal	4.85	0.263	113

B. Analysis of covariance Source of variance	df	SS	*F*	*P*
Tidal	1	43.8628	5.66	0.0182
Density	2	6.9859	0.45	0.6375
Tidal-density	2	24.8950	1.61	0.2028
SL	1	256.1223	33.07	0.0001
Age	1	7.2768	0.94	0.3334
Error	220	1,703.7006		

1978. The proportion of body tissue allocated to gonadal tissue of clams the same size and age was significantly lower ($P < 0.01$) in the intertidal location than in the subtidal location.

Belding (1912) noted "that the longer the exposure, the slower the growth" of hard clams. Clams grown in the subtidal location in this study were larger ($P < 0.05$) than clams from the intertidal location (Eldridge et al. 1979). Belding (1912) suggested the time available for feeding was the important variable influencing growth. More recently, Campbell (1969) demonstrated that carotenoid content of the common mussel (*Mytilus edulis*), a filter feeder, was related to the amount of feeding which in part was controlled by the number of hours it was in the water each tidal cycle. Relationships also existed between maturation of the gonad and carotenoid content, and spawning and position in the intertidal zone (Campbell 1969). Although other environmental factors are involved, it appears gonadal growth and spawning can be influenced by an animal's position in the intertidal zone. Gonads of clams were proportionally smaller in the intertidal zone, but no significant difference was detected by Eversole et al. (1980) in any of the quantitative measures of gonad condition (e.g. % lumen, % lumen with ovocytes) between the 2 tidal locations. It therefore appears that clams in the intertidal location devoted proportionally less tissue to gonads without impairing gametogenesis. Ansell et al. (1964) reported that if the minimum amount of food and nutrient resources are available, hard clams will continue gametogenic activity. Hard clams apparently employ a reproductive strategy allowing gametogenesis and reproduction to proceed under conditions of stress (e.g. suboptimal food concentrations) but the total energy diverted to reproduction is reduced.

Figure 2 shows the change in gonadal condition (ΔGSI_D) by density and tidal location during the spring and fall spawning peaks. As mentioned earlier, spawning peaks appear to be of similar importance as indicated by the changes in GSI_D. However, the relative importance of the spawning peak varies with tidal location. The decline in GSI_D of the intertidal clams was greater during the spring than during the fall spawning peak. The opposite trend was observed with subtidal clams. The GSI_D values were similar in May for clams from both tidal locations whereas, in September, the GSI_D of clams from the subtidal location was nearly double that of the intertidal clams. It therefore appears that clams from the subtidal location were able to regenerate more completely their reproductive tissue and spawn more intensely (greater ΔGSI_D) in the fall peak than clams from the intertidal location. Clams from both tidal locations appear fully prepared for the spring spawning peak.

Growth of clams was greatest at the lowest density (290 clams/m^2) and lowest at the highest density (1,159 clams/m^2) (Eldridge et al. 1979). GSI_D values were also significantly larger ($P < 0.05$) for clams grown at the lowest density compared to clams at the two higher densities. However, when SL and age in months were fit as covariates, no significant difference ($P > 0.05$)

in GSI_D was detected among density treatments with analysis of covariance (Table 3). This implies that the stress of increased density on gonadal condition was associated with size and age of clams. When considering the effects of stress on organisms such as molluscs and fish that exhibit great degrees of phenotypic plasticity (Weatherley 1972, Russell-Hunter 1978), the differential effects of size and age must be separated if the relationship between density and the response is to be identified. Experimentally, this may be accomplished by sampling specimens of known age at 1 or 2 intervals and from a restricted size range. Peterson (1982), using basically this method, observed significantly lower growth and gonad mass in 2 suspension-feeding bivalves, *Protothaca staminea* and *Chione undatella,* at elevated densities. Variability in gonad size and condition (GSI) associated with animal size may also be minimized by statistically comparing standard or mean size animals as was done in this study.

The effect of increased density on this study's estimate of reproductive effort (ΔGSI_D) was considerable and consistent among treatments over both spawning peaks (Fig. 2). The average ΔGSI_D for clams from the intermediate (869 clams/m^2) and highest density (1,159 clams/m^2) were only 54% and 33% of the ΔGSI_D of clams from the lowest density, respectively. No significant difference was observed with any of the quantitative histological measures of gonad condition (e.g. % lumen) among density treatments (Eversole et al. 1980). These data provide further evidence that hard clams proceed with gametogenesis and reproduction but at reduced level under environmental stress (increased density). The effect of increased density, as with tidal location, was not severe enough for hard clams to alter reproductive activities (e.g. gametogenesis) and resorb its gametes.

Clams appear to be experiencing some sort of resource limitation at the intertidal location and at higher densities. Suspension-feeding bivalves are very effective filterers (Owen 1966) and can easily deplete the water of food in a short time in experimental cultures (e.g. see Bayne et al. 1976). Buss and Jackson (1981) have observed a reduction in the concentration of food particles under ambient conditions with increasing population density of suspension-feeding marine invertebrates. Obviously food could be limiting in the general vicinity of clams and be the mechanism by which density and tidal location treatments are affected. Unfortunately, the microenvironment about the trays was not monitored, so a food shortage could not be demonstrated. Nevertheless, some sort of food limitation appears to be operating because the impact of increased density on the growth and gonadal condition of hard clams was exacerbated in the intertidal location where access to food was already limited by the amount of time available for feeding. Determination of the mechanisms by which the suspension-feeding residents of soft sediments respond to environmental stress, such as increased density, deserves further study if scientists hope to understand the ecological interactions operating within natural communities and extensive culture systems.

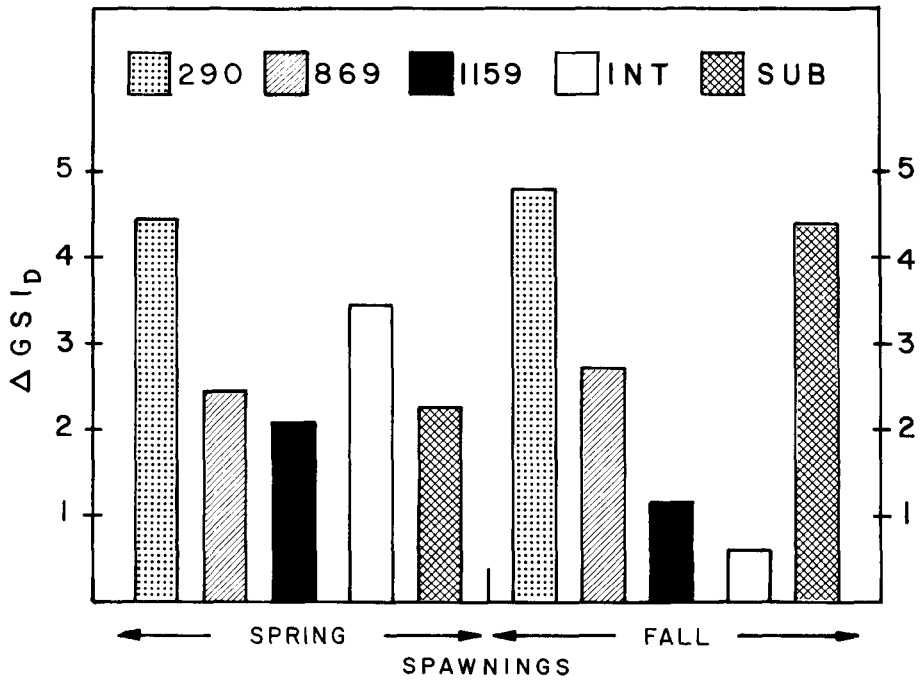

Figure 2. Average declines in gonadal-somatic index (ΔGSI_D) by density levels (290, 869 and 1,159 clams/m^2) and tidal location (intertidal and subtidal) for the spring and fall spawning peaks. Declines in GSI_D were calculated for the spring peak with GSI_D values from May ($N = 108$) through June ($N = 12$) and for the fall peak with values from September ($N = 23$) through November ($N = 11$). GSI_D values were combined across tidal locations for density values and across densities for tidal location values. GSI_D values from May 1980 were not included because density levels had been adjusted to the lowest density level in 1978.

Literature Cited

Ansell, A. D., K. F. Lander, J. Coughlan, and F. A. Loosmore. 1964. Studies on the hard-shell clam, *Venus mercenaria,* in British waters. I. Growth and reproduction in natural and experimental colonies. J. Appl. Ecol. 1:63–82.

Barr, A. J., J. H. Goodnight, J. P. Sall, W. H. Blair, and D. M. Chilko. 1979. SAS user's guide, 1979 edition. SAS Inst. Inc., Raleigh, N.C. 494pp.

Bayne, B. L., R. J. Thompson, and J. Widdows. 1976. Physiology I. Pages 121–206 *in* B. L. Bayne, ed. Marine mussels. Cambridge Univ. Press, Cambridge, Engl.

Belding, D. L. 1912. The quahaug fishery of Massachusetts. Comm. Mass. Dep. Conserv. Mar. Fish. Series. 2:1–41.

Bricelj, M. V. and R. E. Malouf. 1980. Aspects of reproduction of hard clams (*Mercenaria mercenaria*) in Great South Bay, New York. Proc. Natl. Shellfish. Assoc. 70:216–229.

Brousseau, D. J. 1978. Spawning cycle, fecundity, and recruitment in a population of soft-shell clam, *Mya arenaria.* from Cape Ann, Massachusetts. Fish. Bul. 76:155–166.

Browne, R. A. and W. D. Russell-Hunter. 1978. Reproductive effort in molluscs. Oecologia. 37:23–27.

Buss, L. W. and J. B. C. Jackson. 1981. Planktonic food availability and suspension-feeder abundance: Evidence of *in situ* depletion. J. Exp. Mar. Biol. Ecol. 49: 151–162.

Campbell, S. A. 1969. Seasonal cycles in the carotenoid content in *Mytilus edulis.* Mar. Biol. 4:227–232.

Coe, W. R. and H. J. Turner, Jr. 1938. Development of the gonads and gametes in the soft-shell clam (*Mya arenaria*). J. Morph. 62:91–111.

Eldridge, P. J., A. G. Eversole, and J. M. Whetstone. 1979. Comparative survival and growth rates of hard clams, *Mercenaria mercenaria,* planted in trays subtidally and intertidally at varying densities in a South Carolina estuary. Proc. Natl. Shellfish. Assoc. 69:30–39.

——— and A. G. Eversole. 1982. Compensatory growth and mortality of the hard clam, *Mercenaria mercenaria* (Linnaeus, 1758). Veliger. 24:276–278.

Eversole, A. G., W. K. Michener, and P. J. Eldridge. 1980. Reproductive cycle of *Mercenaria mercenaria* in a South Carolina estuary. Proc. Natl. Shellfish. Assoc. 70:22–30.

Grahame, J. 1973. Breeding energetics of *Littorina littorea* (L.) (Gastropoda: Prosobranchiata). J. Anim. Ecol. 42:391–403.

Keck, R. T., D. Maurer and H. Lind. 1975. A comparative study of the hard clam gonad development cycle. Biol. Bul. 148:243–258.

Loosanoff, V. L. 1937. Seasonal gonadal changes in adult clams, *Venus mercenaria* (L.). Biol. Bul. 72:406–416.

Manzi, J. J., V. G. Burrell, Jr., and M. Y. Bobo. 1981. Comparative gametogenesis in subtidal and intertidal oysters (*Crassostrea virginica*) from Bulls Bay, South Carolina. J. Shellfish. Res. 1:119 (Abstract).

Owen, G. 1966. Feeding. Page 1–51 *in* K. M. Wilbur and C. M. Yonge, eds. Physiology of molluscs. Vol. 2. Academic Press, New York.

Peterson, C. H. 1982. The importance of predation and intra- and interspecific competition in the population biology of two infaunal suspension-feeding bivalves, *Protothaca staminea* and *Chione undatella.* Ecol. Mongr. 52:437–475.

Porter, H. J. 1964. Seasonal gonadal changes in adult clams, *Mercenaria mercenaria* (L.) in North Carolina. Proc. Natl. Shellfish. Assoc. 55:35–52.

Russell-Hunter, W. D. 1978. Ecology of freshwater pulmonates. Pages 335–383 *in* V. Fretter and J. Peake, eds. Pulmonates, Vol. 2A. Academic Press, New York.

Weatherley, A. H. 1972. Growth and ecology of fish production. Academic Press, London, Engl. 293pp.

Williams, G. C. 1966*a*. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am. Nat. 100:687–690.

———. 1966*b*. Adaptation and natural selection. Princeton Univ. Press. Princeton, N.J. 307pp.