Journal of Shellfish Research, Vol. 18, No. 2, 425–429, 1999.

BIOCHEMICAL UTILIZATION DURING EMBRYOGENESIS AND METAMORPHOSIS IN THE BAY SCALLOP, *ARGOPECTEN IRRADIANS CONCENTRICUS* (SAY)

YANTIAN T. LU, NORMAN J. BLAKE AND JOSEPH J. TORRES
Department of Marine Science
University of South Florida
St. Petersburg, Florida 33701

ABSTRACT Protein, total lipid, and carbohydrate were measured for spawned eggs, D-shaped larvae, premetamorphic, and metamorphic larvae of the bay scallop *Argopecten irradians concentricus* (Say). Spawned eggs were composed of 64.0% protein, 26.5% lipid, and 9.5% carbohydrate. After 48 hours of embryogenesis, 13.6% of the protein, 46.3% of the lipid, and 20.8% of the carbohydrate mass had been lost, providing 25.5%, 69.8%, and 4.8% of the total energy expenditure of 0.176 mJ per embryo. During 48 hours of metamorphosis, lipid was utilized first, followed by a heavy consumption of protein; protein, lipid and carbohydrate lost 55.6%, 59.4%, and 67.3% of their mass respectively. Protein and lipid supplied a comparable amount of energy for metamorphosis, 47.9% and 43.5%, respectively; whereas, carbohydrate contributed only 8.6%, to the 4.35 mJ per larva metamorphic energy expenditure.

INTRODUCTION

Early survival of marine bivalves is limited by two critical developmental stages: embryogenesis and metamorphosis. Because of the difficulties in obtaining such information from the natural environment, our knowledge on these subjects comes primarily from laboratory studies and hatchery production of commercially important species. Low survival of early developmental stages in laboratory studies has often been found to be associated with embryogenesis and metamorphosis (Castagna and Duggan 1971, Heffernan et al. 1992), with fluctuations in environmental factors often increasing mortalities (Tettelbach and Rhodes 1981, Lu 1989, Lu and Blake 1996).

Embryogenesis of most marine bivalves occurs in surrounding waters rather than in the female and, thus, represents a environmental stage of negative energy balance, because embryos do not have a digestive system and cannot feed on particulates. Endogenous energy reserves of eggs are mobilized to supply the energy necessary for embryogenesis, and this process lasts to at least the formation of a digestive tract. In bivalves, this represents a development from eggs to straight hinge veligers or D-shaped larvae. Early veligers of the bay scallop *Argopecten irradians concentricus* still need to rely partially on endogenous reserves in addition to energy obtained from feeding (Lu 1996).

Metamorphosis represents the second phase of development, wherein stored energy reserves are consumed for metabolism during early development (Whyte et al. 1990). Energy reserves are accumulated by the planktonic larvae through feeding on organic particles and are subsequently used for supporting metamorphosis (Rodriguez et al. 1990, Haws et al. 1993). During metamorphosis, the larval velum disappears, and larvae lose their ability to feed until the development of gill filaments (Sastry 1965, Bayne 1965, Hickman and Gruffydd 1971).

Success in completing embryogenesis and metamorphosis is determined to a large extent by the amount of energy reserves inherited from the female and/or accumulated through larval feeding. High survival has been found in the large eggs of *Mercenaria mercenaria* and *Argopecten irradians* (Kraeuter et al. 1982), probably because of their higher energy content. Survival may depend upon the ability of embryos or larvae to complete development of feeding structures before energy reserves are depleted (Haws et al. 1993).

The southern bay scallop was reported to produce smaller oocytes (Barber and Blake 1981) than its northern counterpart. Low energy reserves of small eggs and higher metabolic demand associated with higher temperature in its natural habitat could be disadvantageous to the early development of the southern bay scallop. However, information on the energy metabolism of early development in the bay scallop is lacking. The objective of this study was to investigate changes in biochemical composition of eggs, larvae, and juveniles, and the energy expenditures associated with embryogenesis and metamorphosis of the southern bay scallop *Argopecten irradians concentricus* (Say).

MATERIALS AND METHODS

Sample Collection

Bay scallops were collected from Homosassa, Florida and were spawned in the lab of the Department of Marine Science, University of South Florida. Culturing of larvae and juveniles followed the methods described by Lu and Blake (1996). Larvae were raised at a density of 4–8 mL^{-1} and fed daily with 10,000–30,000 cells mL^{-1} of *Isochrysis galbana*, depending upon larval size. Seawater was replaced every day in the amount of 1/3 of the total volume. Daily food ration for juveniles was increased gradually from 30,000 to 100,000 cells mL^{-1} of *I. galbana*.

Fertilized eggs were collected onto a 28 μm nylon screen. A portion of the eggs were used for biochemical analysis, and the rest were released in 1.2 μm filtered seawater (25% S) and allowed to develop at 26 °C. After 48 hours, D-shaped larvae were collected on a 35-μm nylon screen. The egg and larval samples were washed with 1.2-μm filtered seawater and pipetted to a graduated cylinder. Seawater was added to bring the volume to 100 mL and total eggs or larvae were determined by counting five 0.5-mL samples. Mean egg diameter and larval shell length were determined by measuring 50 individuals each using a microscope fitted with a micrometer. Subsamples were drawn from the graduated cylinder, washed three times with 3% ammonium formate solution to remove salt, and frozen at −20 °C until analyzed for biochemical composition. Each sample for chemical analysis contains 100,000–150,000 eggs or 150,000–200,000 D-larvae.

Premetamorphic larvae (mean shell height 185 ± 7.8 μm) were kept in 2000-mL plastic beakers containing 1.2-μm filtered seawater without food. A sample of larvae was taken 24 hours later. Development was followed microscopically, and juveniles were observed in the culture at 48 hours. Veligers that settled on the

beakers were brushed into a petri dish. Juveniles were separated from the pediveligers under a microscope. Samples of larvae and juveniles were washed with 3% ammonium formate solution, quantified, and frozen at −20 °C until analyzed. Each sample for chemical analysis contains 3,000–5,000 premetamorphic larvae or 1,000–2,000 postmetamorphic larvae.

Egg and larval samples were homogenized in 1.5-mL DI water using a 4710 series ultrasonic homogenizer (Cole–Parmer Instrument Co.). Subsamples were taken from the slurry for protein, lipid, and carbohydrate analysis.

Juveniles of various shell height (1–10 mm) were collected from a 300 Liter stocking tank at various times. Sample size ranges from 1–50 individuals, depending upon size. For juveniles less than 2 mm, it is difficult to completely separate the soft body from shells; consequently, whole animals were homogenized in DI water with a tissue grinder and then an ultrasonic homogenizer. For larger juveniles up to 10 mm, only the soft body was homogenized. In all cases, samples were analyzed within 1 month of collection.

Biochemical Analysis

Under most circumstances, three samples were analyzed for eggs and larvae of each development stage. The exception is with juveniles (>2 mm shell height), which were analyzed individually.

Protein analysis followed the Folin phenol method of Lowry et al. (1951). Three samples of 20-μL homogenate each were transferred to test tubes, to which 60 μL of DI water was added to make final volumes of 80 μL each. Samples were hydrolyzed by adding 0.12 mL of 0.1 N NaOH to each test tube and heating at 100 °C for 10 minutes. After cooling, 1.2 mL Reagent B (0.5 mL 1% $CuSO_4$/$5H_2O$ + 0.5 mL 2% Na Tartrate + 50 ml 2% Na_2CO_3) was added and allowed to sit for 10 min. Then 0.12 mL reagent E (phenol reagent diluted to 1 N with water) was added and mixed immediately. After 30 min, the optical density of the blue solution was read at 750 nm on a Cary 2000 spectrophotometer with bovine serum albumin as the standard.

Lipid was extracted according to the method of Bligh and Dyer (1959). Three samples of 0.2-mL homogenate each were placed in test tubes. To each sample, 0.73 mL of 2:1 $MeOH:CHCl_3$, 0.24-mL chloroform, and 0.24-mL H_2O were added and mixed after each addition. The samples were allowed to sit for 1 h for separation. The two phase solutions were poured into 0.45-μm PTFF centrifuge filter units, and the lipid fraction passing through was collected in test tubes and dried at 30 °C under a flow of nitrogen. Total lipid content was determined using the charring method of Marsh and Weinstein (1966). To each dried sample, 1 mL of concentrated H_2SO_4 was added, followed by heating at 200 °C for 20 minutes. The charring samples were cooled in tap water and were then diluted with 3-mL H_2O. After cooling, the optical density was read at 375 nm on a spectrophotometer with stearic acid as the standard.

Total carbohydrate content was determined using the phenol-sulfuric acid method of Dubois et al. (1956). Three samples of 0.2-mL homogenate were pipetted to centrifuge tubes, washed consecutively with acetone and ether to remove lipids, and dried at 60 °C. The dried samples were hydrolyzed in 2.0 mL 5% trichloroacetic acid at 100 °C for 20 minutes. After cooling, the tubes were centrifugated at 5,000 rpm for 10 minutes and 1-mL supernatant was removed from each sample to 16 × 150-mm test tubes. To each test tube, 0.5 mL of 5% phenol and 2.5 mL of concentrated H_2SO_4 were added. After cooling for 30 minutes, the optical density of the orange-yellow solution was read at 490 nm on a Cary 2000 spectrophotometer with oyster glycogen as the standard.

Energy expenditures for embryogenesis and metamorphosis were estimated by the loss of protein, lipid, and carbohydrate during those two events. Energy conversion factors used were 20.0, 39.5, and 17.5 mJ μg^{-1} for protein, lipid, and carbohydrate respectively (Brett and Groves 1979).

RESULTS

Table 1 gives the content of protein, lipid, and carbohydrate at various developmental stages and shell lengths. The spawned eggs of the southern bay scallop had a mean diameter of 60.7 ± 0.8 μm, and were composed primarily of protein (64.0%), lipid (26.5%), and carbohydrate (9.5%). Mean energy content of an egg was 0.631 mJ. During the 48 hours of embryogenesis, all three components decreased. Protein dropped 13.6% by mass, lipid 46.3%, and carbohydrate 20.8%, respectively, leading to a loss of 22.9% of the total organic matter (defined here as the total of protein, lipid, and carbohydrate) (Table 2). Lipid was the major substrate utilized for embryogenesis, supplying 69.8% of the total energy expenditure of 0.176 mJ per embryo, while protein and carbohydrate contributed 25.5% and 4.8% respectively.

Lipid was accumulated as the larvae developed, increasing from 18.5% in D-larvae to 26.9% in premetamorphic larvae. Mean energy content of premetamorphic larvae (185-μm shell length) was 7.49 mJ per larva. During the first 24 hours of metamorphosis, protein, lipid, and carbohydrate lost 24.4%, 38.8%, and 53.6% of their original content, respectively. Lipid supplied half of the total energy expenditure, and protein and carbohydrate made up the rest. Carbohydrate was the substrate that lost the highest portion, but it only contributed 12.2% to the total energy expenditure because of its low absolute content.

During the second 24 hours of metamorphosis, more protein was lost (41.3%) than lipid (33.5%) and carbohydrate (29.4%). Protein surpassed lipid as the major substrate in fulfilling the energy demand. The role of carbohydrate as an energy reserve was further reduced.

Overall, the process of metamorphosis consumed 57.9% of the total organic substrate, contributed by 55.6%, 59.4%, and 67.3% of protein, lipid, and carbohydrate reserves respectively. Total energy expenditure during metamorphosis was 4.35 mJ per larva, with

TABLE 1.

***Argopecten irradians concentricus*. Changes in biochemical content (ng ind.$^{-1}$) during embryogenesis and metamorphosis.**

Developmental Stages	Length (μm)	Protein	Lipid	Carbohydrate	Total
Fertilized eggs	61	16.2 ± 0.5 (64.0%)	6.7 ± 0.4 (26.5%)	2.4 ± 0.1 (9.5%)	25.3
D-larvae	98	14.0 ± 0.1 (71.8%)	3.6 ± 0.2 (18.5%)	1.9 ± 0.1 (9.7%)	19.5
Premetamorphic starved 0 hrs	185	187.4 ± 6.6 (62.4%)	80.5 ± 7.3 (26.9%)	31.8 ± 0.4 (10.6%)	299.7
starved 24 hrs	187	141.7 ± 2.8 (68.9%)	49.3 ± 5.6 (24.0%)	14.7 ± 0.2 (7.1%)	205.6
Postmetamorphic	230	83.2 ± 1.9 (65.8%)	32.8 ± 1.6 (25.9%)	10.4 ± 0.2 (8.2%)	126.4

TABLE 2.

***Argopecten irradians concentricus*. Losses of biochemical substrates, their caloric equivalents and contribution to energy expenditures of embryogenesis and metamorphosis.**

	Protein	Lipid	Carbohydrate	Total
Embryogenesis				
Wt loss (ng ind.$^{-1}$)	2.2	3.1	0.5	5.8
Wt loss (%)	13.6%	46.3%	20.8%	22.9%
E equiv. (mJ ind.$^{-1}$)	0.0449	0.1229	0.0084	0.1762
E contribution (%)	25.5%	69.8%	4.8%	100%
Metamorphosis				
Wt loss (ng ind.$^{-1}$)	104.2	47.8	21.4	173.4
Wt loss (%)	55.6%	59.4%	67.3	57.9%
E equiv. (mJ ind.$^{-1}$)	2.0841	1.8909	0.374	4.3487
E contribution (%)	47.9%	43.5%	8.6%	100%

protein and lipid providing 47.9% and 43.5%, respectively, and carbohydrate contributing only 8.6%.

Protein, lipid, and carbohydrate content of juveniles are summarized in Table 3. In contrast to the larval biochemical composition, juveniles had significantly higher levels of protein ($P < .001$) and lower levels of lipid ($P < .001$). There was no significant difference in carbohydrate levels between larvae and juveniles. Protein was the major component in the biochemical composition of juveniles, constituting 72.6 ± 3.0% of the total organic matter, and mean lipid and carbohydrate components were 18.6 ± 1.1% and 8.8 ± 2.5%, respectively. As in the larval stage, carbohydrate was the least significant component in juveniles.

DISCUSSION

Embryogenesis of marine invertebrates is an energy-consuming process during which embryos rely solely on energy reserves within the eggs provided by the female. Existing information has shown that energy stored in other parts of the body is transferred to the gonads during gametogenesis (Gabbott 1976, Bayne 1976, Barber and Blake 1981) and energy stored in eggs as protein, lipid, and carbohydrate substrates are subsequently utilized for growth and development of the embryo (Gallager et al. 1986, Whyte et al. 1990). Protein forms the main constituent of eggs, followed by lipid and then carbohydrate in marine invertebrates (reviewed by Holland 1978). The present study on the eggs of the bay scallop *Argopecten irradians concentricus* shows the same trend: protein forms the main biochemical constituent (64.1%), followed by lipid (26.4%). Carbohydrate is the smallest component of the three (9.5%).

Energy reserves in the form of protein, lipid, and carbohydrate were utilized as eggs developed, which was indicated by the reduction of corresponding substrates at the end of embryogenesis. In the bay scallops, lipid was used as the principal energy source for egg development, supplying 69.8% of the total energy expenditure, more than protein (25.5%) and carbohydrate (5.5%) combined. Carbohydrate played a minor role as an energy reserve in the bay scallop because of its low content in eggs. High conversion efficiency ($W_{final}/W_{initial}$, where W is the weight of a given biochemical constituent (see Holland 1978) of protein (86.4%) and low conversion efficiency of lipid (53.7%) provide evidence that protein is conserved for the formation of planktonic larvae, and lipid is the major energy reserve fueling this process.

Information on the utilization of major biochemical constituents for embryogenesis of marine invertebrates comes mainly from studies on crustaceans. Although some species use lipid as the major energy reserve (Pandian 1967, Pandian and Schumann 1967, Shakuntala 1977), other species rely primarily on protein (Barnes 1965, Lucas and Crisp 1987). The utilization of lipid as the dominant energy reserve in developing eggs was also reported for the Pacific halibut *Hippoglossus stenolepis* (Schmidt) (Whyte et al. 1993) and the red drum *Sciaenops ocellata* (Vetter et al. 1983); whereas utilization of protein dominated in the rainbow trout *Salmo gairdneri* (Oliva-Teles and Kaushik 1987). In bivalves, 69 and 71% of total lipid was lost during embryogenesis of the clam *Mercenaria mercenaria* and the oyster *Crassostrea virginica*, respectively (Gallager et al. 1986), indicating heavy use of lipid; however, lack of information on changes in protein and carbohydrate contents prevents estimation on relative importance between lipid and protein. Whyte et al. (1990) reported that lipid and protein substrates contributed equally to the energy expenditure of embryogenesis of the rock scallop *Crassadoma gigantea*, accounting for 46.7 and 43.5%, respectively, and carbohydrate supplied only 9.8%.

During the planktonic stages of the bay scallop, larvae feed on organic particles, and their organic mass increases as larvae grow (Lu and Blake 1996). As a result, larvae build up energy reserves of protein, lipid, and carbohydrate through feeding (Table 1). Relative lipid content increased from 18.5% in D-shaped larvae to 26.9% in premetamorphic larvae.

Bay scallop larvae use lipid reserves at the beginning of metamorphosis followed by a heavier consumption of protein reserves. During the first 24 hours, metamorphosing larvae derived 50.5% of their energy from lipid and 37.3% from protein. During the next 24 hours, lipid supplied 34.4% of the total energy expenditure of metamorphosing larvae, and protein supplied the bulk of it, 61.6%. On average, protein and lipid provided similar amounts of energy, 47.9 and 43.5%, respectively, during the 48-hour metamorphosis. As in embryogenesis, carbohydrate is the least important constituent in metamorphosing larvae and contributed only 8.6% of the energy expenditure.

Disagreement remains on which substrate serves as the major source of energy for metamorphosis in bivalves. In the oyster *Ostrea edulis*, Holland and Spencer (1973) reported that over half of the neutral lipid reserves were used for metabolism during meta-

TABLE 3.

***Argopecten irradians concentricus*. Protein, total lipid, and total carbohydrate content (µg ind.$^{-1}$) in juveniles.**

Mean Height (mm)	Protein	Lipid	Carbohydrate	Total
1.07	8.8 (63.3%)	3.3 (23.7%)	1.8 (12.9%)	13.9
2.1	68.5 (71.6%)	18.0 (18.8%)	9.2 (9.6%)	95.7
2.2	81.3 (68.6%)	25.0 (21.1%)	12.2 (10.3%)	118.5
2.8	112.1 (67.9%)	30.2 (18.3%)	22.7 (13.8%)	165.0
3.0	162.4 (73.7%)	40.7 (18.5%)	17.2 (7.8%)	220.3
3.1	176.8 (74.2%)	43.8 (18.4%)	17.8 (7.5%)	238.4
3.4	248.3 (69.4%)	64.9 (18.1%)	44.7 (12.5%)	357.9
4.8	566.1 (74.7%)	140.5 (18.5%)	51.3 (6.8%)	757.9
5.1	682.8 (74.5%)	164.6 (18.0%)	69.4 (7.6%)	916.8
5.2	685.3 (75.6%)	160.8 (17.7%)	60.7 (6.7%)	906.8
5.5	837.4 (71.6%)	205.4 (17.6%)	74.2 (6.3%)	1170.0
5.8	953.7 (75.1%)	223.7 (17.6%)	92.9 (7.3%)	1270.3

morphosis, but the authors had no data for the use of protein, carbohydrate, or phospholipid. However, a later study on the same species by Rodriguez et al. (1990) found protein supplied most of the energy for metamorphosis (62%), more than twice that supplied by lipid (28%). In two other species of oysters, *Crassostrea virginica* and *C. gigas*, 50.2% and 51.1% of their total energy expenditure during metamorphosis came from lipid and 39.9% and 38.5% from protein, respectively (Haws et al. 1993). In the rock scallop *Crassadoma gigantea* (Gray), Whyte et al. (1992) reported that protein formed 59.9% of the total energy expenditure during metamorphosis and lipid 38.5%. However, their data, like those of Whyte et al. (1990), were derived from relative values and were found hard to interpret.

In the bay scallop, metamorphosis consumed 57.9% of the total organic reserves, equivalent to a total energy expenditure of 4.35 mJ per larva. This may represent the minimum level of consumable energy reserve of bay scallop larvae, below which larvae cannot complete metamorphosis without obtaining energy from their surrounding environment. Assuming that metamorphosing larvae have the same respiration rate as eyed larvae (14.664 μLO_2 $mgAFDW^{-1}$ h^{-1}; Lu 1996), energy expenditure during 48 hours of metamorphosis can be calculated to be 4.55 mJ. This value is close to the value of 4.35 mJ determined in this study, to further support our estimation on the energy requirement for metamorphosis of the bay scallop. Those values are comparable to those determined for the oyster *Crassostrea virginica* and *C. gigas* (2.13 and 4.65 mJ per larva, respectively, over a 36-hour period) (Haws et al. 1993). The oyster *Ostrea edulis* seems to lose more energy (5.62–14.65 mJ/larva over a 36–48-hour period) during metamorphosis (Rodriguez et al. 1990).

One of the main difficulties in estimating the biochemical changes and the associated energy metabolism is the lack of knowledge on when premetamorphic larvae stop feeding and for how long. Our understanding of biochemical energetics in metamorphosing larvae has been based on the assumption that metamorphosing larvae lack the capability to feed and that metamorphic energy demand comes solely from energy reserves accumulated during the planktonic stage. In a recent feeding study on the oyster *Crassostrea virginica*, Baker and Mann (1994) found that all prodissoconchal and dissoconchal metamorphs ingested the experimental microspheres, and, except for only a few hours during the settler phase, feeding was possible throughout the oyster metamorphosis. If this finding holds true for other bivalve larvae, energy obtained through feeding by metamorphosing larvae has to be taken into account in the estimation of energy budget. However, it is not clear what the quantitative contribution of feeding to the total energy expenditure of metamorphosing larvae is. Results of this and other studies (e.g., Rodriguez et al. 1990, Haws et al. 1993) demonstrate that bivalve larvae are able to complete metamorphosis based solely on the accumulated energy reserves of their biochemical substrates.

In the natural environment, larvae may cease feeding at a very late stage of metamorphosis, or larvae may stop feeding for only a few hours during metamorphosis, as observed by Baker and Mann (1994). As a result, the minimum energy level can be substantially lower, and larvae may not need as much energy reserve for metamorphosis as previously thought. Therefore, the estimation made here may represent the upper range of the metamorphic metabolic demand of the bay scallop.

ACKNOWLEDGMENT

The authors thank Joe Donnelly for his assistance in the biochemical analysis.

REFERENCES

Baker, S. M. & R. Mann. 1994. Feeding ability during settlement and metamorphosis in the oyster *Crassostrea virginica* (Gmelin, 1791) and the effects of hypoxia on postsettlement ingestion rates. *J. Exp. Mar. Biol. Ecol.* 181:239–253.

Barber, B. J. & N. J. Blake. 1981. Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). *J. Exp. Mar. Ecol.* 52:121–134.

Barnes, H. 1965. Studies in the biochemistry of cirripede eggs. 1. changes in the general biochemical composition during development of *Balanus balanoides* and *B. balanus*. *J. Mar. Biol. Ass. U.K.* 45:321–339.

Bayne, B. L. 1965. Growth and delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia* 2:419–443.

Bayne, B. L. 1976. Aspects of reproduction in bivalve molluscs. pp. 432–448. *In:* M. L. Wiley (ed.). *Estuarine Processes*. Academic Press, New York.

Bligh, E. G. & W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911–917.

Brett, J. R. & T. D. D. Groves. 1979. Physiological energetics. pp. 280–352. *In:* W. S. Hoar, D. J. Randall and J. R. Brett (eds.). *Fish Physiology*, vol. 8. Academic Press, New York.

Castagna, M. & W. Duggan. 1971. Rearing the bay scallop, *Aequipecten irradians*. *Proc. Natl. Shellfish. Assoc.* 61:80–85.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers & F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Analyt. Chem.* 28:350–356.

Gabbott, P. A. 1976. Energy metabolism. pp. 293–355. *In:* B. L. Bayne (ed.). *Marine Mussels: Their Ecology and Physiology*. Cambridge University Press, Cambridge.

Gallager, S. M., R. Mann & G. C. Sassaki. 1986. Lipid as an index of growth and viability in three species of bivalve larvae. *Aquaculture* 56:81–103.

Haws, M. C., L. DiMichele & S. C. Hand. 1993. Biochemical changes and mortality during metamorphosis of the eastern oyster, *Crassostrea virginica*, and the Pacific oyster, *Crassostrea gigas*. *Mol. Mar. Biol. Biotechnol.* 2:207–217.

Heffernan, P. B., R. L. Walker & J. W. Crenshaw, Jr. 1992. Embryonic and larval responses to selection for increased rate of growth in adult bay scallop, *Argopecten irradians concentricus* (Say, 1822). *J. Shellfish Res.* 11:21–25.

Hickman, R. W. & L. D. Gruffydd. 1971. The histology of the larva of *Ostrea edulis* during metamorphosis. pp. 282–294. *In:* D. J. Crisp (ed.). *Fourth European Marine Biology Symposium*. Cambridge University Press, New York.

Holland, D. L. 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. pp. 85–123. *In:* D. C. Malins and J. R. Sargent (eds.). *Biochemical and Biophysical Perspectives in Marine Biology*, vol. 4. Academic Press, New York.

Holland, D. L. & B. E. Spencer. 1973. Biochemical changes in fed and starved oysters, *Ostrea edulis* L. during larval development, metamorphosis, and early spat growth. *J. Mar. Biol. Ass. U.K.* 53:287–298.

Kraeuter, J. N., M. Castagna & R. Van Dessel. 1982. Egg size and larval survival of *Mercenaria mercenaria* (L.) and *Argopecten irradians* (Lamarck). *J. Exp. Mar. Biol. Ecol.* 56:3–8.

Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:65–275.

Lu, Y. T. 1989. Effect of zinc on the growth and development of larvae of

the bay scallop *Argopecten irradians*. Chin. *J. Oceanol. Limnol.* 7:318–326.

Lu, Y. T. 1996. Physiological energetics of larvae and juveniles of the bay scallop *Argopecten irradians concentricus* (Say). Dissertation, University of South Florida. 160 pp.

Lu, Y. T. & N. J. Blake. 1996. Optimum concentrations of Isochrysis galbana for growth of larval and juvenile bay scallop. *Argopecten irradians concentricus* (Say). *J. Shellfish Res.* 15:635–644.

Lucas, M. I. & D. J. Crisp. 1987. Energy metabolism of eggs during embryogenesis in *Balanus balanoides*. *J. Mar. Biol. Assoc. U.K.* 67: 27–54.

Marsh, J. B. subject D. B. Weinstein. 1966. Simple charring method for determination of lipids. *J. Lipid Res.* 7:574–576.

Oliva-Teles, A. & S. J. Kaushik. 1987. Nitrogen and energy metabolism during the early ontogeny of diploid and triploid rainbow trout (*Salmo gairdneri* R.). *Comp. Biochem. Physiol. A.* 87A:157–160.

Pandian, T. L. 1967. Changes in chemical composition and caloric content of developing eggs of the shrimp *Crangon crangon*. *Helgoländer wiss. Meeresunters* 16:216–224.

Pandian, T. L. & K. H. Schumann. 1967. Chemical composition and caloric content of egg and zoea of the hermit crab *Eupagurus bernhardus*. *Helgoländer wiss. Meeresunters* 16:225–230.

Rodriguez, J. L., F. J. Sedano, L. O. Garcia-Martin, A. Perez-Camacho & J. L. Sanchez. 1990. Energy metabolism of newly settled *Ostrea edulis* spat during metamorphosis. *Mar. Biol.* 106:109–111.

Sastry, A. N. 1965. The development and external morphology of pelagic larval and postlarval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. *Bull. Mar. Sci.* 15:417–435.

Shakuntala, K. 1977. Yolk utilization in the freshwater prawn *Macrobrachium lamarrei*. *J. Anim. Morphol. Physiol.* 24:13–20.

Tettelbach, S. T. & E. W. Rhodes. 1981. Combined effects of temperature and salinity on embryos and larvae of the northern bay scallop, *Argopecten irradians irradians*. *Mar. Biol.* 63:249–256.

Vetter, R. D., R. E. Hodson & C. Arnold. 1983. Energy metabolism in a rapidly developing marine fish egg, the red drum (*Sciaenops ocellata*). *Can. J. Fish. Aquat. Sci.* 40:627–634.

Whyte, J. N. C., N. Bourne & N. G. Ginther. 1990. Biochemical and energy changes during embryogenesis in the rock scallop *Crassadoma gigantea*. *Mar. Biol.* 106:239–344.

Whyte, J. N. C., N. Bourne, N. G. Ginther & C. A. Hodgson. 1992. Compositional changes in the larva to juvenile development of the scallop *Crassadoma gigantea* (Gray). *J. Exp. Mar. Biol. Ecol.* 163:13–29.

Whyte, J. N. C., W. C. Clarke, N. G. Ginther & J. O. T. Jensen. 1993. Biochemical changes during embryogenesis of the pacific halibut, *Hippoglossus stenolepis* (Schmidt). *Broodstock Manag. Egg Larval Quality* 24:193–201.