J. moll. Stud. (1983), *Suppt.* **12A**, 209-213 Proceedings 2nd Franco-British Symposium on Molluscs

ARTIFICIAL FERTILIZATION AND REARING OF *CERASTODERMA LAMARCKI* (REEVE) IN THE LABORATORY

KOBINA YANKSON
and
JOHN MOYSE
Department of Zoology, University College of Swansea, Wales, U.K.

ABSTRACT

Additional data on artificial fertilization and rearing of the larvae of *Cerastoderma lamarcki* (Reeve) are presented. Temperature shock was used to stimulate ripe cockles to release large quantities of viable gametes. High rates of fertilization (>80%) were recorded in salinities ranging from 15 to 35‰. However, the best salinity for quick embryonic development and larval hatching success seems to be 25-30‰. No larval hatching was recorded in salinities below 15‰.

Crowding of eggs had little effect on fertilization success, but satisfactory embryonic development and larval hatching occurred at densities lower than c700 eggs/cm^2. The sizes of the newly hatched D-larvae decreased with increasing egg density in the size range 111.0-120.7 μm.

Of the five different micro-algal food organisms tested on the larvae, *Isochrysis aff. galbana* (clone T-ISO) (see Ewart & Epifanio, 1981) produced the best growth rate and minimum mortality.

This work shows *C. lamarcki* to be well adapted to bivalve hatchery procedures. The relative ease of maintaining its laboratory cultures strengthens previous claims that it would be a better choice for controlled commercial production than its sibling species *C. edule* (L.).

INTRODUCTION

Of the commercially important bivalves, cockles (*Cerastoderma* spp.) seem to have received the least attention by way of laboratory rearing experiments as a basis for hatchery practice. The cockle industry in Europe is based on natural recruitment but reports on this industry in Britain have indicated recent declines in some stocks (Pickett & Franklin, 1973; Franklin, 1976). Although some natural enemies, especially oyster catchers do a great deal of damage to cockle beds (Franklin, 1972) substantial declines in stocks can result from environmental factors such as topographical changes which hamper recruitment (Pickett & Franklin, 1973; Franklin, 1976). It is therefore becoming increasingly desirable to attempt producing seed cockles as a possible long term supplement to natural recruitment or for intensive cultivation.

Some records of artificial fertilization and rearing of cockles are found in Lebour (1938), Creek (1960), Boyden (1971) and Kingston (1973 and 1974). The above works cover the embryogenesis of *C. edule*, interspecific hybridization, the effects of temperature on fertilization and temperature and salinity on the growth of the larvae of *C. edule* (L.) and *C. lamarcki* (Reeve). Kingston (1973 and 1974) induced ripe cockles to spawn (no mention of stimulus), but the other workers used gametes "stripped" from the gonad.

The paucity of information on the laboratory rearing of cockles renders further studies into their hatchery conditions a relevant exercise for both academic and economic purposes.

The name *C. lamarcki* (Reeve) is used in this paper in preference to *C. glaucum* Bruguière in view of recent evidence for the specific status of the Mediterranean form to which the latter name now properly applies (Brock, 1980). A recent study suggests that the lagoon or brackish water cockle (*C. lamarcki*) would be a better choice for commercial farming than its sibling species, *C. edule*, the common European cockle (Ivell, 1979). In this work therefore, *C. lamarcki* has been subjected to laboratory conditions of spawning, fertilization and rearing in order to assess its adaptability to hatchery procedures.

MATERIALS AND METHODS

i. *Source of specimens*

The cockles originated from Aberthaw Power Station lagoon, the only known site for *C. lamarcki* in S. Wales. Ripe cockles were collected from the lagoon on 14th May 1981 and kept in aerated seawater (30‰) at 15°C. They were fed with cultures of *Tetraselmis suecica* (Kylin) Butch and *Phaeodactylum tricornutum* Bohlin (Lewin) and served as the parent stock.

ii. *Laboratory spawning*

The cockles were scrubbed clean and placed in 2L pyrex glass beakers containing filtered seawater

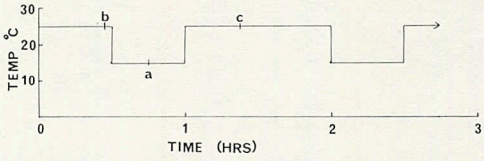

FIGURE 1 Sequence of transferring cockles between warm and cold water as a means of inducing spawning.

(2 μm). One set of beakers contained cold seawater maintained at 15°C by running tap water and ice. Another set held warm seawater at 25-26°C maintained by an electric water bath. Ten cockles were placed in each beaker and transferred between warm and cold water in the sequence illustrated in Figure 1 (a procedure suggested by M. M. Helm, pers. comm.) until spawning was elicited in some individuals.

Spawnings were achieved on two occasions using this method: On the first occasion 10 out of 40 cockles (4♂, 6♀) responded at point (a) in Figure 1. On the second occasion, one male responded at point (b). The sperm released was diluted to 200 ml and added at the rate of 10 ml/L to the seawater holding the other cockles. Thirteen more (6♂, 7♀) out of 57 responded at point (c) in Figure 1. Whenever an individual started to spawn it was transferred to a separate beaker containing 'treated' seawater (fibre glass filtered seawater which had been passed through an ultra-violet sterilization unit) where it continued to spawn.

iii. *Fertilization experiments*

Eggs from several females were pooled and fertilized with appropriate quantities of sperm suspension obtained by diluting the entire discharge of one male in 200 ml of treated seawater (30‰). A perforated plastic hand plunger was used to ensure a thorough mixing of the gametes. After 4h of incubation the culture was carefully decanted leaving the developing embryos at the bottom of the vessel. Fresh treated seawater was added and left to incubate at 18 ± 2°C till the larvae hatched within 3 days.

The effect of salinity on fertilization was investigated by pipetting about 2,000 eggs into 200 ml of treated seawater (i.e. 10 eggs/ml) in 250 ml tall form pyrex glass beakers at the salinities indicated in Table 1. Two ml of sperm suspension were added to each. The salinities were obtained by diluting treated seawater (approx. 35‰) with distilled water that had been left to stand for 24h. Two replicates were set up. After 24h and 72h of incubation 100-150 eggs from each vessel were examined to determine (a) success of fertilization; (b) level of development and (c) larval hatching success. Thirty D-larvae from each vessel were measured, using a calibrated ocular micrometer in a binocular microscope; but showed no size variation.

To determine the effect of egg density (or crowding) on fertilization the stated quantities of eggs (Table 3) were pipetted into 200 ml of treated seawater (30‰) in pyrex glass beakers measuring 23.8 cm^2 at the bottom. One ml sperm suspension was added to the lowest egg density culture and increased proportionately for the others. Two replicates were set up. After 24h and 72h of incubation 100-200 eggs from each vessel were examined respectively. The sizes of 25 D-larvae from each vessel were measured and the results are also indicated in Table 3.

iv. *Rearing of larvae*

Five different phytoflagellates were fed separately but simultaneously to the larvae of *C. lamarcki* in order to assess their relative food value. About 2,000 lrvae (4 days from fertilization and measuring 110 μm) were held in 200 ml of treated seawater (i.e. 10 larvae/ml) in 250 ml tall form pyrex glass beakers. Each culture vessel received 2 μl packed cell volume of actively growing phytoflagellates daily. This ration is equivalent to that successfully fed to oyster and clam larvae by Davis and Guillard (1958). Two replicates were set up including unfed controls. The larvae were sieved, washed and transferred into fresh treated seawater every 3 days during which 30 from each vessel were measured. The number of dead larvae from subsamples were recorded and survival rates subsequently calculated. The above operations were repeated until over 50% of the larvae had settled as spat.

RESULTS

In all the experiments the results from the various replicates were generally very similar. In Table I it is seen that fertilization was least successful in a salinity of 5‰, but high rates, above 80% were achieved over the range 15-35‰. However, complete development to the hatching of D-larvae occurred only in the range 20-35‰ with maximum hatching success at 25-30‰. The newly hatched D-larvae measured 110 μm and did not show size variation in the various salinities. It is inferred from Tables 1 and 2 that the embryos in 25-30‰ developed faster than the others since after 24h of incubation more had reached the ciliated trochophore stage and were already rotating within the gelatinous membranes. The embryos in 10-15‰ developed slowly and the majority did not proceed beyond the multicellular stage. The few which reached the ciliated trochophore stage were dead by the end of

TABLE 1 Effects of Salinity on the success of fertilization, and hatching of larvae in *C. lamarcki.*

Salinity (‰)	% Fertilization after 24h		% D-larvae after 72h	
	A	B	A	B
5	21.1	26.6	0	0
10	35.4	38.1	0	0
15	94.9	85.2	0	0
20	98.9	98.2	61.9	48.3
25	99.2	97.9	98.1	97.7
30	98.4	98.9	98.4	98.1
35	97.8	98.5	92.3	87.9

TABLE 2 Effects of salinity on the development of embryos of *C. lamarcki* 24h after fertilization (combined values of 2 replicates).

Salinity (‰)	Maximum development achieved (%)			
	Abnormal	2-8 celled embryos	Multicellular embryos	Ciliated trochophores
5	100	0	0	0
10	0	100	0	0
15	0	0	95	5
20	0	0	60	40
25	0	0	10	90
30	0	0	10	90
35	0	0	74	26

TABLE 3 Effects of egg density (crowding) on fertilization and larval development in *C. lamarcki* (combined values of 2 replicates) G.M. = gelatinous membranes.

No. of eggs per 23.8 cm^2 surface area	Corresponding egg density (No./cm^2)	% Fertilization	72h. after fertilization		
			% shelled larvae within G.M.	% Free D-larvae	Mean size of D-larvae (μm)
1,000	42	99.4	0	83.7	120.7
4,000	168	100	3.9	77.7	120.1
8,000	336	98.6	7.7	74.4	118.6
16,000	672	95.8	46.2	21.8	111.6
20,000	840	87.9	38.1	11.8	111.0

the 72h incubation period. All the embryos in 5‰ were abnormal.

The results of the egg crowding experiment show little effect of egg density on fertilization (Table 3). Probably the use of the hand plunger maximised the exposure of the eggs to the spermatozoa for fertilization. Nevertheless, complete development of the embryos decreased with increasing egg density. Some shelled larvae failed to hatch from their enveloping gelatinous membranes within 72h. Such incidence increased with increasing egg density to 672 eggs/cm^2. At 840 eggs/cm^2 the development of 50% of the embryos terminated at the multicellular stage. The size of the newly hatched D-larvae shows a decrease with increasing egg density from 120.7 μm at 42 eggs/cm^2 to 111.0 μm at 840 eggs/cm^2. The results of analysis of variance showed significant differences in sizes among the egg densities ($P<0.001$).

Figure 2 indicates the growth of *C. lamarcki* larvae fed on 5 different phytoflagellates from the 4th day after fertilization until 50% or more settled as spat. *Isochrysis aff. galbana* designated Clone T-ISO (see Ewart and Epifanio, 1981) produced the best growth followed by *Isochrysis galbana* Parke, *Monochrysis lutheri* Droop, *Tetraselmis tetrathele* (West) and *Prymnesium parvum* Carter in that order. Settlement occurred in the larval cultures fed on the first 3 micro-organisms on the 13th day after fertilization, and 3 days later in those fed on *T. tetrathele.*

The survival rates (indicated in Figure 2) were generally high except those cultures fed on *P. parvum* and the unfed controls where all the larvae died on the 12th and 14th days after fertilization respectively.

DISCUSSION

Kinne (1977) lists the artificial spawning stimuli for bivalves as biological, thermal, osmotic, electrical, mechanical, chemical and radiant. He mentions that not all bivalves are equally responsive to a certain stimulus. Also not all gametes discharged in response to all stimuli are viable (Fitt Trench, 1981). A thermal stimulus, applied either singly or in conjunction with others, appears to have been much more successfully employed than the others but the mode of its administration varies from worker to worker (see Loosanoff & Davis, 1963; Maurer & Price 1968; Bayne, 1965; Fitt & Trench 1981). The method used in this work is simple and has an advantage of eliciting spawning within a relatively short time (45-90 minutes). This is comparable to the time recorded by Bayne (1965) for *Mytilus edulis* (L.) and much quicker than that reported by Price and Maurer (1971) for *Crassostrea virginica* (Gmelin). Furthermore the gametes released were all viable, resulting in very high fertilization rates.

The results of the fertilization experiments show that although fertilization can take place at very high

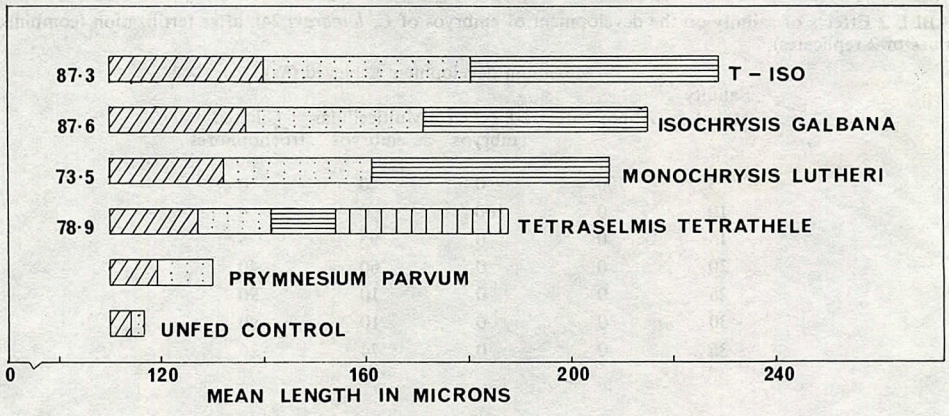

FIGURE 2 Growth of *C. lamarcki* larvae fed on 5 separate algal diets. (Figures given on the left represent percentage survival at settlement).

Oblique hatching, 4th to 7th day after fertilization; stippling, 7th to 10th; horizontal hatching, 10th to 13th; vertical hatching, 13th to 16th.

egg densities and over a wide range of salinities satisfactory embryonic development and larval hatching occurs only at lower egg densities (probably not exceeding 600 eggs/cm^2 and over narrower salinity range (25-35‰). Bacterial infection or competition for dissolved oxygen may account for the low hatching rate and smaller sizes of D-larvae at high egg densities, while the unsatisfactory embryonic development outside the optimum salinity range may be attributed to osmotic problems. The Aberthaw Power Station lagoon from which the parent stock originated has a salinity range of 19.0—24.2‰ during the breeding period of May to August (2 years observation). It is therefore possible that a very wet spring or summer could adversely affect recruitment since seawater influx is restricted to high spring tides. For hatchery purposes it seems that the use of normal or slightly diluted seawater for fertilization and incubation would be preferred to the brackish water from which the parent stock originates.

The poor results shown by feeding *C. lamarcki* larvae with *Prymnesium parvum* are in agreement with those of other workers (for instance Davis & Guillard, 1958; Walne, 1979; working with bivalve larvae) who have demonstrated that this alga is toxic to zooplankton. The apparently low food value of *Tetraselmis tetrathele* is possibly attributable to its tendency to remain at the bottom of the culture vessels and hence not readily available to the planktonic larvae in unagitated cultures. This is in contrast to *T. suecica* which swims freely and is a good food for oysters (Walne, 1979).

Isochrysis galbana and *Monochrysis lutheri* have been proved to be very good foods for the larvae of a number of commercially important bivalves (Davis & Guillard, 1958; Bayne, 1965; Walne, 1979). It is of interest to note that T-ISO produced a much better growth in *C. lamarcki* larvae than these two well known algae. The food value of T-ISO to larval oysters is not significantly different from that of *I. galbana* (Ewart & Eipfanio, 1981; M.M. Helm, pers. comm.). The results presented here show that in terms of larval survival the values of the two algae are similar but growth is possibly better on a T-ISO diet. It is known that *I. galbana* cultures are unstable at temperatures above 22°C while T-ISO grows well in temperatures up to 28°C (Ewart & Epifanio, 1981). This could be of particular importance to the culture of *C. lamarcki* whose larvae, according to Kingston (1974) show optimal growth at 30°C.

SUMMARY

C. lamarcki has been shown to be suited to bivalve hatchery procedures. Temperature shock was successfully used to obtain a large number of viable gametes from ripe individuals. High rates of fertilization and larval hatching success were achieved in the laboratory. Finally, very good larval growth and high rates of survival were obtained in cultures receiving popular food like T-ISO, *Isochrysis galbana* and *Monochrysis lutheri.*

REFERENCES

BAYNE, B. L. 1965. Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.) *Ophelia,* **2**(1) 1-47.

BOYDEN, C. R. 1971. A comparative study of the reproductive cycles of the cockles *Cerastoderma edule* and *C. glaucum. Journal of the Marine Biological Association of the U.K.,* **51**, 605-622

BROCK, V. 1980. The geographical distribution of *Cerastoderma (Cardium) edule* (L.) and *C. lamarcki* (Reeve) in the Baltic and adjacent seas related to salinity and salinity fluctuations. *Ophelia,* **19**(2) 207-214.

CREEK, G. A. 1960. The development of the lamellibranch *Cardium edule* L. *Proceedings of the Zoological Society of London,* **153**, 243-260.

DAVIS, H. C. & R. R. GUILLARD. 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. *Fishery Bulletin*, 136, **58**, 293-304.

EWART, J. W. & C. E. EPIFANIO. 1981. A tropical flagellate food for larval and juvenile oysters, *Crassostrea virginica* (Gmelin). *Aquaculture,* **22**, 297-300.

FITT, W. K. & R. K. TRENCH. 1981. Spawning, development and acquisition of zooxanthellae by *Tridacna squamosa* (Mollusca. Bivalvia). *Biological Bulletin,* **161**, (2) 213-236.

FRANKLIN, A. 1972. The cockle and its fisheries. *Laboratory Leaflet* (New Series), (26) 34 pp.

FRANKLIN, A. 1976. The Burry Inlet Cockle Fishery. In: *Problems of a Small Estuary* (E. Nelson-Smith and E.M. Bridges eds.) (3) 1-5.

IVELL, R. 1979. The biology and ecology of a brackish lagoon bivalve, *Cerastoderma glaucum* Bruguière in an English lagoon, the Widewater, Sussex. *Journal of Molluscan Studies,* **45**, 383-400.

KINGSTON, P. 1973. Interspecific hybridization in *Cardium. Nature,* **243**, p.360.

KINGSTON, P. 1974. Some observations on the effects of temperature and salinity upon the growth of *Cardium edule* and *Cardium glaucum* larvae in the laboratory. *Journal of the Marine Biological Association of the U.K.,* **54**, 309-317.

KINNE, O. 1977. Cultivation of animals — Research Cultivation. Bivalvia. In: *Marine Ecology.* (O. Kinne, ed.) III (2), 900-934

LEBOUR, M. V. 1938. Notes on the breeding of some lamellibranchs from Plymouth and their larvae. *Journal of the Marine Biological Association of the U.K.,* **23**, 119-145.

LOOSANOFF, V. L. & H. C. DAVIS. 1963. Rearing of bivalve molluscs. *Advances in Marine Biology,* **1**, 1-136.

MAURER, D. & K. S. PRICE. 1968. Holding and spawning Delaware Bay oysters *(Crassostrea virginica)* out of season. I. Laboratory facilities for retarding spawning. *Proceedings of the National Shellfisheries Association,* **58**, 71-77.

PICKETT, G. D. & A. FRANKLIN. 1973. The effect of recent topographical changes on stocks of cockles (*Cardium edule* L.) in the Burry Inlet, South Wales. *International Council for the Exploration of the Sea. C.M.K.,* 23, 4pp (Mimeo).

PRICE, K. S. & D. MAURER. 1971. Holding and spawning Delaware Bay oysters (*Crassostrea virginica*) out of season II. Temperature requirements for maturation of gonads. *Proceedings of the National Shellfisheries Association,* **61**, 29-34.

WALNE, P. R. 1979. *Culture of Bivalve Molluscs.* Whitefriars Press Ltd. 2nd Ed. London and Tonbridge.

Since this paper was written, further studies by Dr. Brock of the University of Aarhus, Denmark, (unpublished) have failed to confirm the specific separation of the Mediterranean form and the Baltic-Atlantic form. In these circumstances, the specific name *lamarcki* (Reeve) used in this paper is synonymous to *glaucum* (Bruguiere), but the latter takes precedence.