Preliminary Studies on the Rearing of the West African Mangrove Oyster, *Crassostrea tulipa*, in the Laboratory

K. Yankson

Department of Zoology, University of Cape Coast, Ghana

The West African mangrove oyster, *Crassostrea tulipa* has been reared from fertilized eggs through to settled spat in laboratory cultures. The combined influence of temperature and salinity on fertilization success and larval yield was investigated in a 4 × 4 design. The early developmental stages and growth of the larvae of the species were also studied. Temperature had a more profound influence on the early life stages of *C. tulipa* than salinity. Combined temperature and salinity ranges of 25-30°C and 20-30‰ respectively, supported satisfactory fertilization and larval development. Eggs which were fertilized and incubated under these conditions developed into straight-hinge veligers within 24 h. When reared at 30°C and 30‰ salinity, the larvae attained competence from 14-18 days and settled successfully on mussel shells. The results are discussed in the light of the environmental background of the species and also compared with records on other species of *Crassostrea*.
KEY WORDS: Temperature, salinity, gametes, fertilization, veliger larva, competent larva.

L'huître de mangrove ouest-africaine, la *Crassostrea tulipa* a été élevée dans des cultures de laboratoire, depuis des oeufs fertilisés jusqu'aux naissains stabilisés. Nous avons examiné dans un cadre mesurant 4 × 4 l'influence conjuguée de la température et de la salinité sur le succès du fécondement et sur le rendement larvaire. Nous avons également étudié les premières étapes du développement et de la croissance des larves de l'espèce. La température a eu une influence plus significative que la salinité sur les premières étapes du développement de la *C. tulipa*. Une fertilisation et un développement larvaire satisfaisants ont été obtenus dans des températures et salinité variant entre 25-30°C et 20-30‰ respectivement. Les oeufs fertilisés et couvés dans ces conditions se sont métamorphosés dans 24 heures en veligers attachés à des gonds tout droits. Elevées dans des températures variant entre 30°C et 30‰ les larves ont atteint la maturité dans 14 à 18 jours et se sont installées avec succès sur des coquilles de moule. Nous discutons des résultats à la lumière de l'environnement de l'espèce; les résultats sont également comparées aux données sur d'autres espèces de la *Crassostrea*.
MOTS CLES: Température, salinité, gamètes, fertilisation, larve de veliger, larve mûre.

Introduction

The West African mangrove oyster (*Crassostrea tulipa*) is one of two bivalves (the other being *Anadara senilis*) of considerable economic potential occurring along the coast of West Africa. The species occurs naturally on the stilt roots of the red mangrove (*Rhizophora sp.*) which fringes the borders of lagoons, estuaries and sheltered bays in the region. They form a popular source of protein for many coastal villagers who harvest them from the wild. The oyster shells are used in various ways including preparation of paint, poultry feed ingredient, concrete for building, treatment of burns, etc. Hence their increased production would meet several needs in the region.

The hatchery production of bivalves, through artificial fertilization, and rearing for transplantation onto farms is a common practice in their commercial exploitation. In spite of the considerable economic potential of the West African mangrove oyster (Afinowi, 1975; Kamara *et al.*, 1972; Ajana, 1980) experiments on the culturing of the species have been limited to a few preliminary field studies (Kamara and McNeil, 1975; Ajana, 1976; 1979). There is no record, to my knowledge, on the rearing of the species in the laboratory. This may be due to the usually ready availability of spat in their natural habitat.

Nevertheless, an understanding of the requirements of the early stages of the life history in laboratory cultures could be exploited for a better forecasting of seed availability. It could also help in site selection for its farming, and in genetic studies for stock improvement.

This paper reports preliminary experiments on the culturing of the species in the laboratory. It highlights the combined effect of temperature and salinity on fertilization and larval yield; larval growth and morphological changes; and the duration of early life history stages from egg to spat.

Materials and Methods

Source of oysters

The adult oysters that provided the gametes originated from the Benya lagoon situated approximately 5°05'N, 1°20'W on the coast of Ghana. They were air-freighted to the United Kingdom and placed in a closed circuit sea water system within 48 h

of their collection from the wild. (The option of the closed circuit quarantine system was dictated by United Kingdom importation regulations.) Three separate importations of 60 mature oysters each were made for this study.

The closed circuit system consisted of a reservoir of 450 litres capacity (providing 7.5 litres per oyster) from which the sea water was circulated by means of immersion pumps through open trays (10 litre capacity) in which the oysters were held. Temperature and salinity were maintained at 18-22°C and 32-35‰ respectively. About one-third the volume of the sea water in the system was changed weekly. The animals were fed adequate quantities of a mixed algal diet of *Monochrysis lutheri* and *Isochrysis galbana* (clone T-ISO; Ewart and Epifanio, 1981).

Gamete procurement

The gametes were obtained by the stripping method (Zell *et al.*, 1979). After removing one valve of the shell, a Pasteur pipette was used to suck the sex products by piercing it through the body wall. The eggs were washed by flushing them through a 90 µm nylon screen and collecting them on a 20 µm mesh. They were then suspended in filtered (1.2 µm mesh), U.V. irradiated sea water (treated seawater) for fertilization. The extracted spermatozoa were similarly suspended in treated sea water and filtered through a 20 µm mesh to remove most of the nonspermatic tissue.

Fertilization experiments

Three separate experiments were conducted to determine the combined effects of temperature and salinity on fertilization and larval yield. In each experiment, duplicate static cultures were established at each of the 16 different temperature-salinity combinations using 250 ml tall form pyrex glass beakers. The temperature ranged from 20-35°C at 5°C intervals and the salinity from 20-35‰ at 5‰ salinity intervals. The different salinities were obtained by diluting sea water (35‰) with distilled water that had been allowed to stand for 24 h.

About 2000 eggs were suspended in 200 ml of the treated sea water and inseminated with 2 ml of the sperm suspension (ca 100 × dilution) in each beaker. The cultures were decanted after 3 hours and fresh treated sea water at appropriate temperatures and salinities was added (Yankson and Moyse, 1983). The cultures were examined 48 h after insemination to evaluate fertilization success and larval yield. To determine the percentage success of fertilization, the pair of cultures with the highest fertilization rate was designated 100% value and all other percentages were computed relative to this value (Tettelbach and Rhodes, 1981). The relative percentages of larval yield were similarly calculated. In two experiments the sizes (length) of 50 D-larvae (when available) were measured to the nearest 1.35 µm using a calibrated ocular micrometer.

Larval growth and morphological changes

Two batches of larvae obtained from fertilization and incubation performed at 30°C and 30‰ salinity were reared through to settlement. The choice of temperature and salinity was influenced by the values recorded at the time of collection from the native population. Duplicate static cultures, each consisting of 1,000 larvae (1-day-old) in 200 ml of treated sea water were set up. The larvae were fed a mixed diet of *Monochrysis lutheri* and T-ISO (equal volumes) at 100 cells μl^{-1} daily. This ration was increased to 120 cells μl^{-1} daily after the first week. The culture medium was changed every other day during the first week and then once every 4 days subsequently.

The first batch of larvae reared in this way were sampled periodically for photomicroscopy while the second batch were subsampled (30-40 larvae) on days 1, 7, 12, 14, 16 and 18 for measurement of their shell height and length to the nearest 1.35 µm. Morphological changes, namely shell height/shell length index, umbo formation and competence (eye-spot development) were observed. When over 70% of the larvae had become competent they were offered cleaned *Mytilus edulis* shells as substrate for settlement. The shell dimensions of the spat were measured 12 days later.

Results

Gametes

The mature eggs of *Crassostrea tulipa* were usually pear-shaped when freshly teased out of the gonad but they became spherical shortly after their suspension in sea water. The mature egg diameters ranged from 40 to 48 µm with a germinal vesicle (=nucleus) occupying about 60% of the egg. A distinct nucleolus was visible. Each spermatozoon measured about 70 µm total length and with a rounded head about 7 µm in diameter.

Combined effects of temperature and salinity

Fertilization: The temperature and salinity requirements for fertilization of *C. tulipa* are shown in Table 1. Using 70% as an ecologically significant value (Calabrese, 1969) satisfactory fertilization success was recorded within the dash-lined boundary circumscribed by temperatures of 25-30°C, and salinities 20-35‰. No fertilizations were recorded at 35°C for all salinities. Tettlebach and Rhodes (1981) also found 35°C to be lethal to the gametes and embryos of the Northern Bay scallop. A summary of ANOVA test performed on the results is shown in Table 2. It is seen that temperature contributed 89.85% of the total variation, while a nonsignificant variation of 3.37% was due to salinity. An estimation of the least significant difference (LSD) of the means (31.14%) for the various temperatures showed the

Table 1. Relative percentage fertilization of *Crassostrea tulipa* eggs in different combinations of temperature and salinity.

Temperature (°C)	Salinity (‰) 20	25	30	35
20	11.5	11.6	31.0	29.1
25	46.9	56.0	76.5	100.0
30	98.4	80.9	82.4	70.0
35	0	0	0	0

Note: Percentages are calculated relative to the maximal mean value for duplicate cultures from 3 experiments.

Table 2. ANOVA table showing the effect of temperature and salinity on fertilization success of *C. tulipa*.

Source of Variation	DF	MS	% of total variation	F ratio	P
Temperature	2	4290.92	89.85	13.25	**
Salinity	3	161.04	3.37	0.50	NS
Residual (error)	6	323.94	6.78	-	-
TOTAL	11	4775.90	-	-	-

** significant at P = 0.01
NS = not significant

Table 3. Relative percentage straight-hinge veliger larval yield of *C. tulipa*.in different combinations of temperature and salinity.

Temperature (°C)	Salinity (‰) 20	25	30	35
20	1.4	6.6	23.1	1.6
25	43.8	63.8	85.6	63.7
30	100.0	82.5	70.2	51.9
35	0	0	0	0

Note: Percentages are calculated relative to the maximal mean value for duplicate cultures from 3 experiments.

means for 25°C and 30°C (69.9% and 82.9% respectively) to be similar while that for 20°C (20.8%) was outstanding.

Larval yield: Table 3 indicates the temperature and salinity requirements for successful embryonic development resulting in the production of straight-hinge veligers. The dash-lined boundary circumscribed by temperatures 25-30°C and salinities from 20-30‰ shows satisfactory larval yields of ≥70% of the maximum. The relevant ANOVA test is summarized in Table 4 which shows that a significant variation of 91.16% was due to temperature. A nonsignificant variation of 3.72% was contributed by salinity. A further statistical treatment of the results showed the means for 25°C and 30°C (64.2% and 76.2% respectively) to be distinct from that for 20°C (8.2%), the LSD of the means being 29.8%.

Larval mean size: The mean sizes of the straight-hinge veligers produced in the cultures at various combinations of temperature and salinity are presented in Table 5. The summarized ANOVA test (Table 6) indicates that temperature and salinity contributed almost equally (48.92% and 49.67%, respectively) to the total variation in the sizes of the larvae. Further treatment of the means (LSD) showed that the cultures at 20°C were distinct from those at 25°C and 30°C. The means were 59.7, 65.0 and 64.7 µm, respectively; the LSD of the means being 1.75 µm. Also, cultures at 35‰ were distinct from those at 20, 25 and 30‰, the larval mean lengths being 58.0, 64.1, 65.2 and 65.1 µm, respectively. The LSD of the means was 2.02 µm. The larvae from the outstanding cultures, i.e. low temperature (20°C) and very high salinity (35‰), were generally small in size.

Developmental stages

The major developmental stages of *C. tulipa* reared at 30°C and 30‰ salinity from fertilization through to settlement are summarized in Table 7. Representatives of these stages are shown in Figure 1. The fertilized eggs (40-48 µm in diameter) developed through the various stages of cleavage, morula, blastula and gastrula within 4 h. Slowly rotating trochophores measuring 45-50 µm on the longest axis were observed from 4 to 8 h, and developed into D-shaped, shelled veliger larvae after one day. Early to late umbo larvae occurred from the 4th to the 10th day, and by the 12th or 13th day, fully grown larvae measuring about 300 µm had formed. The larvae became competent for settlement from 14 days after fertilization. The smaller size at competence was 360 µm. When cleaned mussel shells were offered as substrates on day 18, over 70% had become competent. The oyster larvae settled successfully and attained a mean shell height of 3.74mm, 12 days later (i.e. 30 days after fertilization).

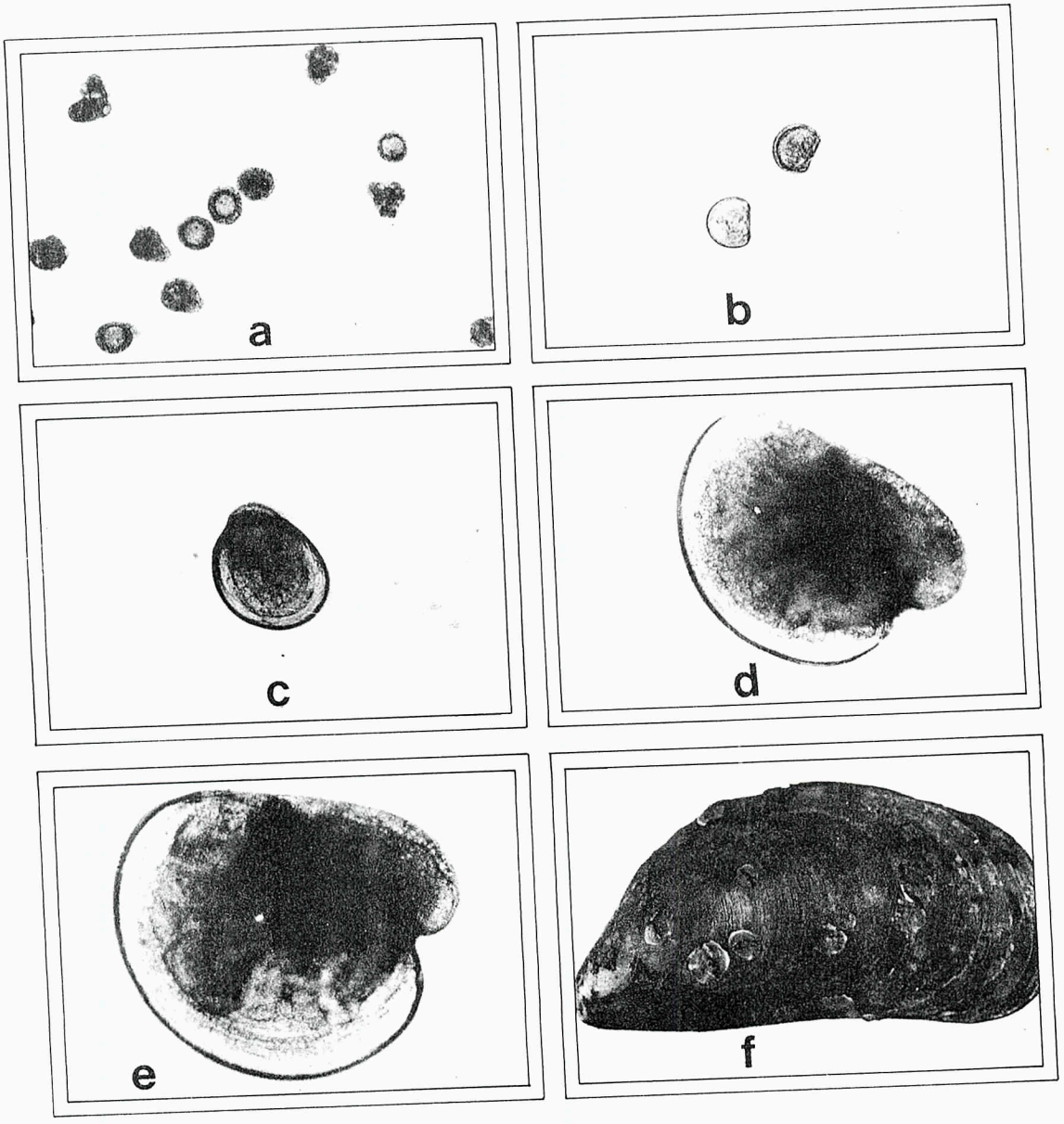

Figure 1. Developmental stages of *C. tulipa*. a: unfertilized eggs and developing embryos, 3 h after fertilization; b: straight-hinge veliger larvae, 24 h old; c: umbo larva, 7 days old; d: fully grown larva, 12 days old; e: competent larva, 18 days old; f: oyster spat, 30 days old, on mussel shell. (scale bar = 150 µm for a-e; 12.5 mm for *f*)

Table 4. ANOVA table showing the effect of temperature and salinity on larval yield of *C. tulipa*.

Source of Variation	DF	MS	% of total variation	F ratio	P
Temperature	2	5269.61	91.16	17.79	**
Salinity	3	214.90	3.72	0.73	NS
Residual (error)	6	296.14	5.12	-	-
TOTAL	11	5780.65	-	-	-

** significant at P = 0.01
NS = not significant

Table 5. Mean length (± standard deviation) of veliger larvae of *C. tulipa* (48 h old) in different combinations of temperature and salinity.

Temperature (°C)	Salinity (‰)			
	20	25	30	35
20	59.0*	61.9*	62.4 ± 1.4	55.4*
25	66.8 ± 1.4	67.0 ± 1.0	66.8 ± 1.2	59.4 ± 2.6
30	66.5 ± 2.0	66.8 ± 1.7	66.2 ± 1.8	59.1 ± 3.4

Note: The values are averages of 2 experiments. Those with * are means for < 50 larvae.

Table 6. ANOVA table showing the effect of temperature and salinity on larval size of *C. tulipa*.

Source of Variation	DF	MS	% of total variation	F ratio	P
Temperature	2	35.49	48.92	34.79	*
Salinity	3	36.03	49.67	35.32	*
Residual (error)	6	1.02	1.41	-	-
TOTAL	11	72.54	-	-	-

* significant at P = 0.001

Larval growth and changes in shell morphometry

The growth of *C. tulipa* larvae reared at 30°C and 30‰ salinity is shown in Figure 2. The 1-day-old veligers had relatively uniform shell height with a mean of 54.0 µm with a 95% confidence limit (CL) of 0.14. Growth was associated with a general increase in size variation as indicated by the various 95% CL. The larvae exhibited relatively slow growth during the first 7 days. This was followed by a faster growth till the 14th day and then a slackening till day 18 when the majority of the larvae had become competent.

Table 7. Developmental stages of the larvae of *C. tulipa* reared at 30°C and 30‰ salinity.

Stage	Size (µm)	Time after fertilization
Fertilized egg	40-48	-
Cleavae-gastrula	-	4 h
Trochophore	45-50	4-8 h
D-shaped veliger	52-54	1 d
Umbo veliger	90-290	4-10 d
Fully grown larva	300	12-13 d
Competent larva	360	14-18 d

Note: The measurements are diameters for eggs and trochophores; shell heights for the shelled larvae.

Table 8. Changes in shell index and attainment of competence in the larvae of *C. tulipa* reared at 30°C and 30‰ salinity.

Age (Days after fertilization)	Mean shell index (H/L)	Competence (% with eye-spot)
1	0.8	0
7	1.3	0
12	1.4	0
14	1.3	20.5
16	1.2	43.2
18	1.1	71.0

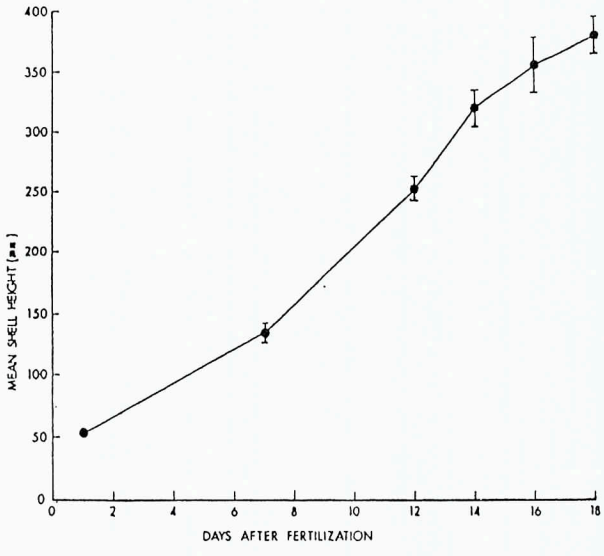

Figure 2. Growth of *C. tulipa* larvae reared at 30°C and 30‰ salinity. Vertical bars indicate 95% confidence limits.

Some changes in the shell morphometry during larval growth are shown in Table 8. The newly formed D-shaped veliger had a longer anterio-

posterior dimension (length) than height (from the straight edge to the ventral margin), resulting in a sub-unity shell index of 0.8. Subsequent growth, characterized by umbo formation and faster increases in shell height than shell length resulted in shell indices greater than unity.

Discussion

After the initial stress on the importance of analyzing the effects of multivariate environmental factors on bivalves by Kinne (1963), subsequent work on bivalve autecology has placed a premium on multifactorial rather than monofactorial analysis (Calabrese, 1969; Hrs-Brenko, 1978; Tettelbach and Rhodes, 1981; Robert *et al.*, 1988). The results in this study show that satisfactory fertilization and embryonic development of *C. tulipa* occurred only in conditions close to those characteristic of their natural habitat. Annual temperature and salinity ranges of 28-32°C and 10-40‰ respectively have been recorded for the Benya lagoon from which the oysters originated (Yankson, 1977).

The study has also shown that temperature exerts a greater influence on fertilization success and larval yield than salinity. Similar findings have been made on the early stages of other bivalves (Calabrese, 1969; Tettelbach and Rhodes, 1981; Robert *et al.*, 1988). But unlike the scallop (Tettelbach and Rhodes, 1981), on which the influence of salinity was also significant, fertilization and larval development of the West African mangrove oyster was not significantly influenced by salinity. This conforms with the wide annual range of salinity in the Benya lagoon where the species breeds continuously throughout the year (Yankson, unpublished).

The small sized larvae (<63 µm in shell length) produced in cultures at the lowest temperature (20°C), may be attributed to low metabolic activity in the developing embryos of this tropical species. In the highest salinity culture (35‰), the small larval sizes may have been due to osmotic problems which probably siphoned their energy. Although these under-sized larvae appeared normal, their fate was not known. On the other hand, successful development to settlement was achieved with straight-hinge larvae measuring ≥66 µm in shell length produced in cultures at temperatures 25-30°C, and salinities 20-30‰.

No firm conclusions can be made on the upper and lower limits of the parameters tested due to the wide intervals of 5 units used. Nevertheless, it may be inferred from the results that *C. tulipa* is stenothermal and euryhaline with respect to fertilization and embryonic development. It appears therefore, that for hatchery practices optimal yield of larvae could be achieved when fertilization and incubation are conducted within temperature and salinity ranges of 25-30°C and 20-30‰ respectively.

The developmental stages of *C. tulipa* recorded in this study are generally similar to those described for other oviparous oysters (Cahn, 1950; Tanaka, 1975; Ogasawara, 1980; Quayle, 1988). The egg sizes recorded for *C. tulipa* (40-48 µm) compare well with sizes recorded by the above authors for other oviparous oysters (46-58 µm). These egg sizes are, however, only half the sizes recorded for larviparous oysters (Cahn, 1950; Walne, 1979) in which the fertilized eggs are incubated in the supra-brachial chamber of the female till they hatch into larvae before their release into the environment. The spermatozoa length and head diameter of *C. tulipa* (70.0 and 7.0 µm , respectively) are similar to those of *C. gigas* recorded as 75 and 7.3 µm, (Cahn, 1950), and 78.0 and 7.8 µm (Tanaka, 1975).

The fertilized eggs of the mangrove oyster developed to the D-shaped veliger stage within 24 h under the present experimental conditions. Their subsequent growth was characterized by variations in shell form as evidenced by changes in the shell height/shell length ratios. The reason for such variation is not clear, but it implies a nonuniform growth in the various shell dimensions with the shell height generally increasing at a faster rate. This type of shell growth is also exhibited by adult *C. tulipa* in wild populations in Ghana (Yankson, unpublished). The general growth pattern exhibited by *C. tulipa* larvae in the laboratory cultures is typical of marine invertebrates. The apparent slackening of growth at the approach of settlement time may be attributed to decline in growth of individuals which had attained competence but had not settled due to absence of a suitable substrate. Such a decline in growth shown by competent larvae of sessile bivalves is associated with "delay in metamorphosis", a phenomenon which is believed (Bayne, 1965) to increase the chances of the larvae encountering suitable substrates for settlement.

The attainment of competence by *C. tulipa* larvae from day 14 to day 18 at shell height of about 360 µm compares with the findings of other studies. For example, settlement sizes of 330 µm in 14 days and 300 µm in 2 to 3 weeks have been reported by Cahn (1950) and Tanaka (1975) respectively for *C. gigas*. The ability of *C. tulipa* larvae to settle successfully on mussel shells indicates the potential of such material as a spat collector for future mass production of the West African mangrove oyster.

Although this study is a preliminary one, it has yielded some information on the morphology of the gametes; temperature and salinity requirements for fertilization and embryonic development in laboratory cultures; and larval growth and developmental stages of the West African mangrove oyster.

I thank the Commonwealth Commission for the award of Academic Staff Fellowship, and the University of Cape Coast for granting me study leave to undertake this post-doctoral research. I am grateful to Prof. J.A. Beardmore, Head of the School of Biological Sciences, University

College of Swansea, Wales, for providing the facilities. I also thank Dr. J. Moyse for useful discussions.

References

Afinowi, M.A. 1975. The biology of *Anadara senilis* and *Gryphaea (Crassostrea) gasar* in West African waters. In *FAO/CIFA Symposium on Aquaculture in Africa.* Rome:FAO. 386-406.

Ajana, A.M. 1976. Oyster culture in closed ponds and waters. Nigerian Institute of Oceanography and Marine Research, Lagos. *Annual Report.*

_____. 1979. Preliminary investigation into some factors affecting the settlement of the larvae of the mangrove oyster *Crassostrea gasar* (Adanson) in the Lagos lagoon. *Proc. 6th Eur. Malac. congr. Malacologia* 18:271-275.

_____. 1980. Fishery of the mangrove oyster, *Crassostrea gasar*, Adanson (1757), in the Lagos area, Nigeria. *Aquaculture* 21:129-137.

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

Cahn, A.R. 1950. Oyster culture in Japan. *Fishery Leafl. Fish Wildl. serv. U.S.* 383:1-80.

Calabrese, A. 1969. Individual and combined effects of salinity and temperature on embryos and larvae of the coot clam. *Mulinia lateralis* (Say). *Biol. Bull.* 137:417-428.

Ewart, J.W. and C.E. Epifanio. 1981. A tropical flagellate food for larval and juvenile oysters, *Crassostrea virginica* Gmelin. *Aquaculture* 22:297-300.

Hrs-Brenko, M. 1978. The relationship of temperature and salinity to larval development in mussels (*Mytilus galloprovencialis*. Lamarck). *Proc. 12th Eur. mar. Biol. symp.* 359-365. ed. D.S. McLusky and A.J. Berry. Oxford: Pergamon Press.

Kamara, A.B. and K.B. McNeil. 1975. Preliminary oyster culture experiments in Sierra Leone. Freetown, Sierra Leone Ministry of Agriculture and Natural Resources, Fisheries Division.

Kamara, A.B., K.B. McNeil and D.B. Quayle. 1979. Tropical mangrove oyster culture: Problems and prospects. In *Advances in Aquaculture,* ed. T.V.R. Pillay and W.A. Dill. F.A.O. Technical Conference on Aquaculture. Kyoto, Japan. 1976. 344-348.

Kinne, O. 1963. The effects of temperature and salinity on marine and brackish water animals. 1. Temperature. *Oceanogr. mar. Biol. A. Rev.* 1:301-340.

Ogasawara, Y. 1980. Oysters and their culture. *Japan Marine Production. Photo Materials Association.* 201-221.

Quayle, D.B. 1988. Pacific oyster culture in British Columbia. *Can. Bull. Fish. Aquat. Sci.* 218:241.

Robert, R., E. His and A. Dinet. 1988. Combined effects of temperature and salinity on fed and starved larvae of the European flat oyster *Ostrea edulis. Mar. Biol.* 97:95-100.

Tanaka, Y. 1975. Oyster culture techniques. In *Outline of Aquaculture.* Tokyo:Japan International Co-operation Agency. 73-115.

Tettelbach, S.T. and E.W. Rhodes. 1981. Combined effects of temperature and salinity on embryos and larvae of the Northern Bay Scallop, *Argopecten irradians. Mar. Biol.* 63:249-256.

Walne, P.R. 1979. *Culture of bivalve molluscs.* 2d ed. London and Tonbridge:Whitefriars Press Ltd.

Yankson, K. 1977. Some aspects of the biology of the West African bloody cockle, *Anadara senilis* (L.) occurring in some coastal lagoons in Ghana. M.Sc. Thesis. University of Cape Coast 1971. 135.

Yankson, K. and J. Moyse. 1983. Artificial fertilization and rearing of *Cerastoderma lamarcki* (Reeve) in the laboratory. *J. moll. Stud. Suppl.* 12a:209-213.

Zell, S.R., M.H. Bamford and H. Hidu. 1979. Cryopreservation of spermatozoa of the American oyster (*Crassostrea virginica*). *Cryobiology* 16:448-460.