# EFFECT OF SALINITY, TEMPERATURE, AND FOOD VALUE OF FOUR MICROALGAE TO OYSTER, *Crassostrea iredalei* LARVAL GROWTH

Achmad Sudradjat

**ABSTRACT**

Published accounts of *Crassostrea iredalei* are only of its distribution in the Philippines. In Indonesia, this species is known to occur on the coast of South Sulawesi as well as in Banten. The purposes of the present studies were to investigate effect of salinity, temperature and food value of four microalgae to *C. iredalei* larval growth. Fine filtration of water was carried out using Sartorius capsule filter cartridge (1.2 ìm and 0.2 ìm) and sterilization was achieved by passing the water through an ultraviolet light unit. Low-salinity water was prepared by diluting filtered seawater with distilled water. High-salinity water was made by adding synthetic sea salts. All cultures were kept in constant temperature baths. Experiments of 8-days (for temperature and salinity trials) and 10-days (for diet trial) duration were duplicated in 500 mL glass beakers with larval density of $10^4$ per liter. Seawater was changed every 48 h. The algae, *Isochrysis galbana, I. galbana* clone T-ISO, and *Pavlova lutheri* were added to the glass beakers at a rate of 100 cells/iL; cell density of *Chaetoceros calsitrans* was 250 cells/il at the start of the experiment and after every water change. Using thermostat chambers, 5 temperatures were tested, ranging from 14° to 34° in 5 steps. Four salinities were used, they ranged from 10 to 35‰ in 5‰ steps. For environmental condition trial, *I. galbana* as food was used. In diet trials, 4 species of algae were tested e.g. *I. galbana, I. galbana* T-ISO, *P. lutheri, C. calcitrans* and a mixture of algae, T-ISO/*C. calcitrans.* The optimum salinity range for growth of larvae was recorded at 20‰—30‰ at which the mean shell length was 85.1—87.7 ìm. The highest survival rate was recorded at salinity of 25‰—30‰, it was 91.6%—92.7%. There were significant differences in larval growth between temperature treatments. The optimum temperature for larval growth was at 24°C—29°C, with survival rate of 91.6%—93.0%. *P. lutheri* and *I. galbana* proved to be of equal value as diet for larval growth, with survival rates of 89.4%—90.6%. The best algal food was *I. galbana* clone T-ISO, which resulted in mean shell length 107.7 ìm and survival rate 86.7%.

**KEYWORDS: temperature, salinity, microalgae, larval growth, oyster**

## INTRODUCTION

One of the predominant groups of exploited bivalves throughout the world is the oyster, an important protein source in many countries. Total world production of bivalves reached 12 mmt in 2001, which compared to 3.5 mmt in 1976. This impressive growth came exclusively from higher aquaculture production, and the major bivalve exporters are China dan Thailand (Josupeit & Jacobsen, 2003).

The slipper oyster, *Crasosstrea iredalei* (Faustino) is a tropical oyster. Much of the literatures on larval studies consist of reports on temperate oysters. A few studies on larval rearing of tropical oysters include Coeroli *et al.* (1984) and Ver (1986).

The autecological research on larval bivalves has focused mainly on the effects of single environmental factors, such as salinity or temperature. The combined effect of salinity and temperature on an organism, and the need

Central Research Institute for Aquaculture, Jakarta

to consider these factors jointly, has been emphasized by Kinne (1970). Studies on the environmental conditions and food for the culture of oyster larvae have been reviewed by Loosanoff & Davis (1963), Walne (1974; 1981). Various authors have reported studies on the individual and combined effects of temperature and salinity on larvae for a wide variety of commercially important bivalve species, including *Ostrea edulis* (Davis & Ansell, 1962; Davis & Calabrese, 1969; Robert *et al.*, 1988), *Crasosstrea gigas* (Helm & Millican, 1977; Neil & Holliday, 1988), and on *Crassostrea virginica* (Davis & Calabrese, 1964).

Most of the studies on food and feeding of larvae of bivalves include information on the growth and mortality of unfed cultures, often used as controls (Davis & Guillard, 1958; Walne, 1963; Wilson, 1978). Several authors have reported that by feeding bivalve larvae on mixed algal diet, growth is often better than when each of the constituent species of the diet was fed individually (David & Guillard, 1958; Walne & Spencer, 1968; Helm, 1977; Helm & Laing, 1987).

The literature on the effects of salinity, temperature and diet on larvae of *C. iredalei* is sparse. Oyster larval studies in the tropics have been carried out mostly at ambient salinity and temperature. Coeroli *et al.* (1984) reared larvae of *Saccostrea echinata* at temperatures and salinities of 25°C—29°C and 20‰—30‰. They used as diet, mixtures of *Isochrysis* aff. *galbana* clone T-ISO, *Pavlova lutheri* and *Chaetoceros gracilis*. Ver (1986) studied early development of *C. iredalei* at temperatures and salinities of 26°C—30°C and 25‰—32‰. *I. galbana* and *P. lutheri* were used as food.

The purpose of the present studies were to investigate effects of salinity, temperature, and food value of four microalgae to *C. iredalei* larval growth.

## MATERIALS AND METHODS

### *Water Treatment*

Fine filtration of water was carried out using Sartorius capsule filter cartridge (1.2 µm and 0.2 µm) and sterilization was achieved by passing the water through an ultraviolet light unit. Low-salinity water was prepares by diluting filtered seawater with distilled water. High-salinity water was made by adding synthetic sea salts. All cultures were kept in constant temperature baths. The pH level of seawater was 7.2—8.1, i.e. within the range regarded as optimal for oyster embryo's development (Calabrese & Davis, 1966).

### *Source of Specimens*

Adult oysters for these experiments were collected from Lada Bay (Banten). These oysters placed in a special room, this had a closed circuit seawater system and space heaters were used to maintain a room ambient temperature of about 24°C—27°C. A mixture of cultures microalgae of two species was used as oyster diets. These were *Isochrisis galbana* (Clone T-ISO) and *Pavlova lutheri*.

Gametes were obtained by stripping mature oysters. Male and female gametes were placed separately in glass beakers. Seawater salinity was 25‰, at 24°C ± 1°C. To eliminate excesspieces of gonadal tissue, eggs were sieved through 80 µm nylon mesh screens and collected on 35 µm mesh screens; sperm were sieved through 25 µm mesh screens.

### *Microalgae Culture*

Planktotrophic larvae are conveniently reared on culture algae. Marine bivalve larvae fed in the laboratory are usually offered living unicellular algae as food. Some experiments have been made with artificial food (see Jones *et al.*, 1974; Chu *et al.*, 1982; Laing, 1987), but they are a poor substitute for live algae. In the present study four species of microalgae were used (see Table 1). These four are ones which appear to be generally satisfactory for oysters and whose laboratory cultivation is well defined. A description of each of these species follows.

### Isochrysis galbana *Parke*

This golden brown alga has biflagellate cells that can change shape but are usually ellipsoidal (Parke, 1949). Their size is about 5—6 µm x 2—4 µm x 2.5—3 µm. This alga tolerates temperature of 0°C—13°C (Corkettt, 1972), but it grows well at 14°C—23°C and optimum at 18°C—20°C. Ukeles (1961; 1976) stated that algal cells are most actively motile at 15°C, and settle out at 27°C. *I. galbana* was defined as an excellent single food for oyster larvae, and widely used as a standard food in several laboratories and hatcheries (e.g. Davis & Guillard, 1958; Loosanoff & Davis, 1963; Walne, 1963; Helm & Millican, 1977; Nell & Holliday, 1988).

Table 1. Microalgae which were used as food

| Class and species | Approximately cell volume (μm)* | Source |
|---|---|---|
| Haptophyceae | | |
| *Isochrysis galbana* Parke | 57 | U.C. Swansea |
| Isochrysis aff. galbana, T-ISO | 51** | Plymouth |
| Chrysophyceae | | |
| *Pavlova (monochrysis) lutheri* Droop | 32 | U.C. Swansea |
| Bacillariophyceae | | |
| *Chaetoceros calcitrans* (Paulsen) Takano | 40 | Conwy |

Sources: * Walne (1981)
** Volkman *et al.* (1989)

### *Isochrysis aff. galbana Green (clone T-ISO)*

This tropical flagellate commonly referred to as *Tahiti Isochrysis* or T-ISO, has largely replaced *I. galbana* Parke as a food species in the culture of bivalve larvae (Helm & Laing, 1987) This strain does not grow well at 12.5°C but thrives at 17.5°C—27.5°C (Ewart & Pruder, 1981). Ewart & Epifanio (1981) suggested that for bivalve cultures at temperature above 22°C, T-ISO may be substituted for *I. galbana* Parke.

### *Pavlova lutheri (Droop) Green (formerly Monochrysis lutheri)*

A yellow naked flagellate which has shell about 7 x 2.5 μm (Davis & Guillard, 1958). This species has a high food value approximately aqual to *I. galbana* for the larvae of bivalves (Davis & Guillard, 1958; Walne, 1970). Growth is nil at 8°C—9°C, poor at 12°C and 27°C, and good at 14°C—25°C.

### *Chaetoceros calcitrans (Paulsen) Takano*

This species is a small diatom algae, approximately 4 μm in diameter. *Chaetoceros* is a little more difficult to culture. Walne (1970), Nascimento (1980), Helm & Laing (1987) found that this species is a good food for the larvae of bivalve molluscs.

The methods of culturing algae, formulae for media and details of various techniques are given by Stein (1973). In this present study, culture apparatus and techniques were mainly based on those described by Walne (1966; 1981). Conwy medium was used for all algal culturing (see Table 2). Filtered seawater is sterilized by autoclaving and after cooling, the salts are added aseptically. The salinity as adjusted between 25‰ and 30‰. In the case of *Chaetoceros*, the salinity should be adjusted to about 20‰ and the enrichment added double the quantities shown in Table 2. Sodium metasilicate was added to diatom cultures. Apart from the stock culture, all algae were batch cultured in 2 and 5 liter conical flasks with air supply and artificial lighting (three rows of 40 W fluorescent tubes). The temperature of the culture was kept at about 24°—27°C. Cell counts of the cultures were made using a haemocytometer. All the algae used were in the exponential phase of growth.

### *Experimental Methods*

One-day-old D-larvae were obtained by pipetting gametes from opened mature oysters. Experiments of 8-days (for temperature & salinity trials) and 10-days (for diet trial) duration were duplicated in 500 mL glass beakers with larvae density of 104 per liter. Seawater was changed every 48 h with the larvae being strained off on a 45 μm nylon screen. The algae, *I. galbana*, *I. galbana* Clone T-ISO, and *P. lutheri* were added to the glass beakers at a rate of 100 cells/μL; cell dencity of *C. calcitrans* was 250 cells/μL at the start of the experiment and after every water change.

Table 2. The compositions of Conwy medium (Walne, 1981)

| | | | |
|---|---|---|---|
| **Stock enrichment solution** | | | |
| (i) | $FeCl_3\ 6H_2O$ | | 2.60 g |
| | $MnCl_2\ 4H_2O$ | | 0.72 g |
| | $H_3BO_3$ | | 67.20 g |
| | E.D.T.A. (Na salt) | | 90.00 g |
| | $NH_2PO_4\ 2H_2O$ | | 40.00 g |
| | $NaNO_3$ | | 200.00 g |
| | Trace metal solution | | 2.00 mL |
| | Distilled water | to | 2 L |
| | 1 mL is added to each litre of seawater | | |
| (ii) | The trace metal solution has the following composition | | |
| | $ZnCl_2$ | | 2.1 g |
| | $CoCl_2\ 6H_2O$ | | 2.0 g |
| | $(NH_4)\ 6Mo_7O_{24}\ 4H_2O$ | | 0.9 g |
| | $CuSO_4\ 5H_2O$ | | 2.0 g |
| | Distilled water | to | 100 mL |
| | It is necessary to acidity this solution with HCl to obtain a clear liquid | | |
| (iii) | Vitamin stock solution | | |
| | B12 | | 10 mg |
| | B1 (Thiamine) | | 200 mg |
| | Distilled water | to | 200 mL |
| | 10 mL is added to each 100 litres of seawater | | |

Rations of single species or a mixed diet of T-ISO and *C. calcitrans* were based on the ash-free dry weights of the two algae such that larvae were fed on half the quantity of each in the mixture (see Helm & Laing, 1987).

Using thermostat chambers (in water batch), 5 temperatures were tested, ranging from 14°C to 34°C in 5°C steps. Four salinities were used, they ranged from 10‰ to 35‰ in 5‰ steps. For environmental condition trials, *I. galbana* as food was used. In diet trials 4 species of algae were tested e.g. *I. galbana*, *I.* aff. *galbana* T-ISO, *P. lutheri*, *C. calcitrans* and a mixture of algae, T-ISO/*C. calcitrans*.

At the end of experiments, the larval samples were preserved in a solution of 5% formalin in seawater. The percentage of live and dead larvae at the time of preservation was assessed by microscopic examination. The length of 50—100 preserved larvae per glass beaker was measured. To test variations of treatment, one way analysis of variance (Sokal & Rohlf, 1981) were used. The Student-Newman-Keuls method was used to test mean variations of shell length between experiment.

## RESULTS AND DISCUSSION

### *Effect of Salinity*

The optimum salinity range for growth of *C. iredalei* larvae was recorded at 20‰—30‰ (see Table 3 and 4) at which the mean shell length was 85.1—87.7 µm. At salinity of 20, 25‰ and 36‰ the larval growth differences were not significant ($P>0.05$). The highest survival rate was recorded at salinity of 25‰—30‰, it was 91.6%—92.7%. At the salinity of 10‰ and 15‰, the larval survival was only 7.0% and 33.5%.

Salinity has little effect on the growth of *Ostrea edulis* larvae, over the wide salinity range of 25‰—35‰ (Davis & Ansel, 1962; Robert *et al.*, 1988). The optimum salinity for development of *Crassostrea gigas* larvae was suggested to be from 23‰—28‰ (Korringa, 1976), and from experiments it was reported to be approximately 25‰ (Helm & Millican,

Table 3. Mean shell length and survival rate of D-larvae at different salinities over 8 days

| Salinity (‰) | Mean shell length (µm) | Survival rate (%) |
|---|---|---|
| 10 | 74.6 | 7.0 |
| 15 | 80.4 | 33.5 |
| 20 | 85.1 | 86.7 |
| 25 | 85.2 | 92.7 |
| 30 | 87.7 | 91.6 |
| 35 | 83.4 | 62.0 |

Table 4. Student-Newman-Keuls test between the mean length of larvae at different salinities over 8 days. Value united by lines do not differ significantly (P>0.05)

| 10 | 15 | 35 | 20 | 25 | 30 |
|---|---|---|---|---|---|
| 74.6 | 80.4 | 83.4 | 85.1 | 85.2 | 87.7 |
| ±3.0 | ±2.9 | ±4.0 | ±4.2 | ±4.0 | ±4.6 |

1977) and 19‰—27‰ (Nell & Holliday, 1988). *Saccostrea commercialis* larvae appear to grow most rapidly at salinities of 23‰—29‰, even though this oyster's larvae settle in more oceanic environments. Coeroli *et al.* (1984) studied a tropical oyster, *S. echinata* in Tahiti, and reported that the best results of rapid growth and low mortality were generally obtained within the salinity range of 20‰—30‰. The optimum salinity for growth of *C. iredalei* larvae from West Java were 20‰—30‰. These optimum salinity range resulted in a survival rate of more than 80%. The optimum salinities for *C. iredalei* larvae were wide, unsurprising since *C. iredalei* is distributed in estuaries with salinity range from 0‰—34‰. *C. iredalei* has a marked adaptation to reduced salinities. It seems likely that in practice the best salinity for growth with low mortality for larvae of *C. iredalei* is between 25‰—30‰.

### *Effects of Temperature*

Effect of temperature on *C. iredalei* larvae are shown in Table 5 and 6. There were significant differences in larval growth between temperature treatments (P<0.001). The optimum temperature for larval growth was at 24°C—29°C (87.7—88.7 µm), with survival rate of 91.6%—93.0%. The highest growth recorded was at a temperature of 34°C (91.7 um), but it was associated with low survival rate (74.0%).

Published studies on the effects of temperature on oyster larvae growth are mostly reported for temperate oysters. Davis & Calabrese (1969) observed *O. edulis* larvae with grow well at 20°C—22.5°C, and are capable of tolerating temperature as high as 27.5°C—30°C but with reduced survival. In the same species, from the Mediterranean coast of France, Robert *et al.* (1988) reported that the best growth of larvae is at the higher temperature tested of 30°C. They suggested that differences in tolerating temperature may be related to the geographical range of the species. *C. gigas* larvae show a maximum growth peak at 28°C (Helm & Millican, 1977). They also stated that with algal foods which will survive even higher temperatures it is possible that *C. gigas* larvae may grow at an even greater rate at 32°C. In a study on *S. echinata* larvae, Coeroli *et al.* (1984) reported that the best results of growth and survival were obtained at 29°C. At a slightly lower temperature (25°C), the end of the rearing was characterized by high mortality and poor growth, leading to no settlement.

Table 5. Mean shell length and survival rate of D-larvae at different temperature over 8 days

| Temperature (°C) | Mean shell length (μm) | Survival rate (%) |
|---|---|---|
| 14 | 72.2 | 65.0 |
| 19 | 76.7 | 87.0 |
| 24 | 87.7 | 91.6 |
| 29 | 88.7 | 93.0 |
| 34 | 91.7 | 74.0 |

Table 6. Student-Newman-Keuls test between the mean length of larvae at different temperatures over 8 days. Value united by lines do not differ significantly (P>0.05)

| Temperature (°C) | 14 | 19 | 24 | 29 | 34 |
|---|---|---|---|---|---|
| Mean ± C.I. | 72.2 ±1.6 | 76.7 ±1.9 | 87.7 ±4.6 | 88.7 ±4.7 | 91.7 ±3.5 |

In the present study, larvae of *C. iredalei* survived at a temperature range 14°C—34°C. The optimum temperature for growth and low mortality were at 24°C—29°C. This temperature range corresponds to the natural conditions in the Lada Bay areas. At low temperature (14°C) growth of larvae was slow and survival rate was also low. The failure of bivalve larvae to grow at low temperatures appeared to be caused by their inability to digest availability food, as has been suggested by Davis & Calabrese (1964).

### *Effects of Diets on Growth and Survival Rate*

Four algae species were tested as food for *C. iredalei* larvae over 10 days. In Table 7 and 8 are shown the results of diet experiments on larvae. *P. lutheri* and *I. galbana* proved to be of equal value as diet for larval growth (P>0.05), with survival rate of 89.4%—90.6%. The best algal food was *I. galbana* clone T-ISO, which resulted in mean shell length 107.7 um and survival rate 86.7%. The mixture of T-ISO + *C. calcitrans* was the poorest diet.

The utilizing of microalgae in aquaculture is related to the cell size, to the ease and reliability of large-scale culture and to their relative food value. Naked flagellates, *Isochrysis galbana* and *Pavlova lutheri* are the best food for larvae of *Ostrea edulis* and *Crasssostrea virginica* (Davis & Guillard, 1958; Loosanoff & Davis, 1963; Walne, 1965). In feeding trials by Davis (1953), *I. galbana* did not rank among the better foods for larvae of *C. virginica*. At the Conwy Fisheries Laboratory, *I. galbana* was used only as a constituent of mixed algal diet in the culture of *C. gigas* (Helm & Millican, 1977). Ewart & Epifanio (1981) reported that *Isochrysis* aff. *galbana* clone T-ISO is a suitable substitute for *I. galbana* for both larval and juvenile *C. virginica*. Recently, Helm & Laing (1987) observed that T-ISO was a poor food for *C. gigas* and *C. rhizophorae* larvae. They added that *Chaetoceros calcitrans* is shown to be very good food for both these *Crassostrea* spp. larvae as well as for clams, *Mecenaria mercenaria*. The same findings heve been reported by Millican & Helm (1973) and Nascimento (1980) that *C. calcitrans* is the best food for *C. gigas* larvae. Of other food tested for bivalve larvae, it has been reported that food mixtures provide a better balanced diet than either of the constituent species when fed alone (e.g. Helm, 1977; Helm & Laing, 1987).

Results of the present study show that the best food for *C. iredalei* larvae is T-ISO. Mixed algal food of T-ISO and *C. calsitrans* did not show as the best food for these two oyster species larvae. This result supports that of Ewart & Epifanio (1981) who report that T-ISO is

Table 7. Mean shell length and survival rate of D-larvae at different diets over 10 days

| Diets | Mean shell length (µm) | Survival rate (%) |
|---|---|---|
| T-ISO | 107.7 | 86.7 |
| *P. lutheri* | 100.5 | 90.6 |
| *I. galbana* | 98.8 | 89.4 |
| *C. calcitrans* | 86.8 | 62.4 |
| T-ISO + *C. calcitrans* | 80.9 | 78.7 |
| Control | 74.7 | 15.2 |

Table 8. Studen-Newman-Keuls test between the mean length of larvae at different diets over 10 days. Value united by lines do not differ significantly (P>0.05)

| Diets | Ctl | Tl + Cc | Cc | Ig | Pl | T-ISO |
|---|---|---|---|---|---|---|
| Mean ± C.I. | 74.7 ± 2.7 | 80.9 ± 3.8 | 86.8 ± 4.8 | 98.8 ± 4.9 | 100.5 ± 5.9 | 107.7 ± 6.2 |

Note: Ctl = Control; Tl + Cc = T-ISO + *Chaetoceros calcitrans*; Cc = *Chaetoceros calcitrans*; Ig = *Isochrysis galbana*; Pl = *Pavlova lutheri*

a suitable substitute for *I. galbana* for larvae and juvenile food; and provides a practical alternative to use of *I. galbana* in mass culture, because temperature used may vary from 15°C to 30°C. *P. lutheri* was a moderate larval food and *C. calcitrans* was a poor diet for *C. iredalei* larvae at room temperature 24°C—27°C. In the high temperatures of the tropics, it is probable that *C. calcitrans* as larval food could be substituted by *Chaetoceros gracilis*. *C. gracilis* has been reported by Coeroli *et al.* (1984) as a good larval diet in mass larval culture for *S. echinata* in Tahiti. They have shown the best larval growth of *S. echinata* was obtained with combination of T-ISO and *C. gracilis*, two tropical species adapted to high temperature and mass culture. For the successful larval culture of tropical oysters, the first priority is good algal diet species which survive in high temperature and give relative high food value.

## CONCLUSIONS

Optimal temperature and salinity for larval development are best around ambient temperature and salinity of about 24°C—29°C and 25‰—30‰.

In the tests for the most suitable diet with four algal species, *Isochrysis* aff. *galbana* (T-ISO) gave the best larval growth, was significantly better than *Pavlova lutheri*, *Isochrysis galbana*, *Chaetoceros calcitrans*, or mixed algal diet of T-ISO + *C. calcitrans*.

## REFERENCES

Calabrece, A. and H.C. Davis. 1966. The pH tolerance of embryos and larvae of *Mercenaria mercenaria* and *C. virginica*. *Biol. Bull.*, 131: 427—436.

Chu, F.E., K.L. Webb, D. Hepworth, and M. Roberts. 1982. The acceptability and digestibility of microcapsules by larvae of *Crassostrea virginica*. *J. Shellfish Res.*, 2: 29—34.

Coeroli, M., D. De Gaillande, and J.P. Landert. 1984. Recent innovations in cultivation of molluscs in French Polynesia. *Aquaculture*, 39: 45—67.

Corkett, C.J. 1972. Development rate of copepod eggs of the genus *Calanus*. *J. Exp. Mar. Biol. Ecol.*, 10: 171—175.

Davis, H.C. 1953. On food and feeding of larvae of the American oyster, *C. virginica*. *Biol. Bull.*, 104: 296—307.

Davis, H.C. and A.D. Ansell. 1962. Survival and growth of larvae of the European oyster, *O. edulis*, at lowered salinities. *Biol. Bull.*, 122: 33—39.

Davis, H.C. and R. Guillard. 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. *Fish. Bull.*, 136: 293—304.

Davis, H.C. and A. Calabrese. 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. *Fish. Bull.*, 63: 643—655.

Davis, H.C. and A. Calabrese. 1969. Survival and growth of larvae of the European oyster (*Ostrea edulis* L.) at different temperature. *Biol. Bull.*, 136: 193—199.

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

Ewart, J.W. and G.D. Pruder. 1981. Comparative growth of *Isochrysis galbana* Parke and *Isochrysis* aff. *galbana*, clone T-ISO at four temperatures and three light intensities. *J. World Maric. Soc.*, 12(1): 333—339.

Helm, M.M. 1977. Mixed algal feeding of *Ostrea edulis* larvae with *Isochrysis galbana* and *Tetraselmis suecica*. *J. mar. boil. Ass.* U.K., 57: 1,019—1,029.

Helm, M.M. and P.F. Millican. 1977. Experiment in the hatchery rearing of Pacific oyster larvae (*C. gigas* Thunberg). *Aquaculture*, 11: 1—12.

Helm, M.M. and I. Laing. 1987. Preliminary observations on the nutritional value of "Tahiti Isochrysis" to bivalve larvae. *Aquaculture*, 62: 281—288.

Jones, D.A., J.G. Mumford, and P.G. Gabbott. 1974. Microcapsules as artificial food particles for aquatic filter feeders. *Nature* (London), 247: 233—235.

Josupeit, H. and I. Jacobsen. 2003. Aquaculture and trend. *Global Aquaculture Advocate*, 6(6): 23—25.

Kinne, O. (Ed.). 1970. Marine ecology. Vol. 1. Environmental factors, Part 1. Willey-Interscience, N. York, 681 pp.

Korringa, P. 1976. Farming the cupped oysters of the genus *Crassostrea*. Elsevier, Amsterdam, 264 pp.

Laing, I. 1987. The use of artificial diets in rearing bivalve spat. *Aquaculture*, 65: 243—249.

Loosanoff, V.L. and H.C. Davis. 1963. Rearing of bivalve molluscs. *Adv. Mar. Biol.*, 1: 1—136.

Millican, P.F. and M.M. Helm. 1973. Preliminary observations on the culture requirements of the larvae of the Pacific oyster, *Crassostrea gigas* Thurnberg. ICES CM 1973/K: 33, 10 pp (mimeo).

Nascimento, I.A. 1980. Growth of the larvae of *Crassostrea gigas* Thurnberg, fed with different algal species at high cell concentrations. *J. Cons. Int. Explor. Mer.*, 39: 134—139.

Nell, J.A. and J.E. Holliday. 1988. Effects of salinity on the growth and survival of Sydney rock oyster (*Saccostrea commercialis*) and Pasific oyster (*Crassostrea gigas*) larvae and spat. *Aquaculture*, 68: 39—44.

Parke, M. 1949. Studies on marine flagellates. *J. mar. boil. Ass.* U.K., 28: 255—286.

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.

Sokal, R.R. and F.J. Rohlf. 1981. Biomentry. 2nd ed., W. H. Freeman Co., San Fransisco, 859 pp.

Stein, J.R. (ed.). 1973. Handbook of phycological methods. Cambridge University Press, 448 pp.

Ukeles, R. 1961. The effect of temperature on the growth and survival of several marine algal species. *Biol. Bull.*, 120: 255—264.

Ukeles, R. 1976. Cultivation of plants. I. Unicellular. *In*: Marine Ecology, vol. 3, ed. O. Kinne. Willey & Sons, Chichester, p. 367—466.

Ver, L.M.M. 1986. Early development of *Crassostrea iredalei* (Faustino, 1932) (Bivalvia: Ostreidae), with notes on the structure of the larval hinge. *Veliger*, 29(1): 78—85.

Volkman, J.K., S.W. Jeffrey, P.D. Nichols, G.I. Rogers, and C.D. Garland. 1989. Fatty acid and lipid composisition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.*, 128: 219—240.

Walne, P.R. 1963. Observations on the food value of seven species of algae to the larvae of *Ostrea edulis*. I. Feeding experiments. *J. mar. boil. Ass.* U.K., 43: 767—784.

Walne, P.R. 1965. Observations on the influence of food supply and temperature on the feeding and growth of larvae of *Ostrea edulis* L. *Fishery Invest., Lond. Ser.* 2, 24(1): 1—45.

Walne, P.R. 1966. Experiments in the large-scale culture of the larvae of *Ostrea edulis* L. *Fishery Invest., Lond. Ser.* 2, 25(4): 1—53.

Walne, P.R. 1970. Studies on food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria* and *Mytilus*. *Fishery Invest., Lond. Ser.* 2, 26(5), 62 pp.

Walne, P.R. 1974. Culture of bivalve molluscs. 50 Years' experience at Conwy. Fishing News Books, Farnham, 189 pp.

Walne P.R. 1981. Culture of shellfish. *In:* Aquarium Systems, ed. A.D. Hawkins, Academic Press, p. 347—371.

Walne P.R. and B.E. Spencer. 1968. Some factors affecting the growth of the larvae of *Ostrea edulis*. ICES, C.M. 1968 (K15), 7 pp (mimeo).

Wilson, J.H. 1978. The food value of Phaeodactylum tricornutum Bohlin to the larvae of Ostrea edulis L. and Crassostrea gigas Thurnberg. Aquaculture, 13: 313—323.