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SOME BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS OF GROWTH AND GAMETOGENESIS IN *CRASSOSTREA GIGAS* AND *OSTREA EDULIS* GROWN AT SUSTAINED ELEVATED TEMPERATURES*

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(Figs. 1-5)

Crassostrea gigas (Thunberg) and *Ostrea edulis* L. were grown at sustained temperatures of 12°, 15°, 18° and 21 °C for a period of 19 weeks. Regular assays of weight specific ammonia excretion rate were made, following which animals were sacrificed for estimation of dry meat weight, dry shell weight, biochemical composition (percentage carbon, nitrogen, carbohydrate, ash) and gonadal development (histological assessment). *Crassostrea gigas* grew from an initial live weight of 5.2 g to values of 23.5, 28.2, 34.6 and 38.7 g at 12°, 15°, 18° and 21 °C respectively. However, dry meat weight was not positively related to temperature: from an initial value of 88.7 mg, final values of 1736, 1253, 1322 and 1219 mg were recorded at 12°, 15°, 18° and 21 °C respectively. *Ostrea edulis* exhibited increases in both live weight and dry meat weight which were proportional to experimental temperature. Live weight increased from an initial value of 4.1 g to 10.6, 13.6, 19.3 and 22.3 g at 12°, 15°, 18° and 21 °C respectively. Dry meat weight increased from an initial value of 95.0 mg to 444.5, 566.0, 617.0 and 756.0 mg at 12°, 15°, 18° and 21 °C respectively.

An initial increase in percentage carbohydrate was observed at all temperatures in both species. In *C. gigas* this was followed by a decrease in carbohydrate content which was closely related to the late active stage of gametogenesis. The transition from an increase to a decrease in percentage carbohydrate occurred earlier at higher temperatures. In *O. edulis* no marked decrease was noted in the percentage carbohydrate content despite marked gametogenic activity. No evidence of hermaphroditism was found in *C. gigas*. Twenty-seven per cent of *O. edulis* examined contained sex products of both sexes at various stages of development. Ripe gonads developed in both species at all temperatures. However, *C. gigas* spawned only at 18° and 21 °C, and *O. edulis* spawned only at 15, 18° and 21 °C. Ammonia excretion data exhibited an allometric relationship ($E = aW^b$) with weight prior to spawning in both species at all temperatures. After spawning an increase in weight specific excretion rate was observed.

INTRODUCTION

In recent years there has been an increasing interest in the use of thermal effluents for the intensive culture of marine bivalve molluscs. This has been based on the premise that elevated temperatures will stimulate feeding and growth with a concurrent reduction in the time required to produce a marketable product. However, it is well documented that gametogenesis and spawning in these organisms are closely related to seasonal changes in sea-water temperature (see reviews by Giese, 1959; Sastry, 1975). Similarly, elevated temperatures may result in a disproportionate increase in shell growth (Walne & Mann, 1975) and give rise to physiological stress that is not immediately evident from

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a superficial examination of natural populations. This latter point has been made particularly evident for the mussel *Mytilus edulis* by Bayne, Widdows & Worrall (1977). However, delineating the effect of temperature *per se* from natural population data is difficult in that not only does temperature vary continuously, but its effects are superimposed upon those of other environmental variables, notably short-term changes in salinity in estuarine situations, tidal exposure, and longer-term effects due to phytoplankton blooms.

A knowledge of the effect of temperature on growth and gametogenesis of commercially valuable bivalves is necessary if the constructive use of thermal effluents is to be made in culture facilities. This paper presents data on the effects of sustained temperatures of 12°, 15°, 18° and 21 °C on two commercially important species of oyster, namely the Japanese oyster, *Crassostrea gigas* (Thunberg), and the European oyster, *Ostrea edulis* L. The temperatures were chosen from literature values (Fujiya, 1970; Millar, 1963; Walne, 1958) to cover the observed natural temperature range from initiation of growth in the spring months to subsequent spawning.

MATERIALS AND METHODS

Experimental technique

All experiments were carried out in four wooden tanks ($3 \times 0.75 \times 0.75$ m). Each tank was supplied with sand-filtered sea-water (30‰ salinity or greater) at a rate of 8 l/min and a culture of the diatom *Skeletonema costatum* of mean cell density 2.74×10^5 cells/ml ($\pm 0.10 \times 10^5$ cells/ml S.D., $n = 82$) at a rate of 800 ml/min. (*Skeletonema costatum* was cultured out of doors in ponds of 10 m diameter and 1 m depth in a 15% mixture of secondary treated sewage effluent in sea water.) Both sea-water and diatom cultures were injected at one end of the tank to flow through ten plastic mesh trays ($60 \times 60 \times 6$ cm) containing the experimental animals and then to waste via a standpipe. Continuous gentle aeration effected mixing of the tank contents.

Juvenile *C. gigas* were obtained from Sea Salter Shellfish Ltd, Whitstable, England in June 1976. Juvenile *O. edulis* were obtained from International Shellfish Enterprises, Moss Landing, California during the same month. Both were held in concrete raceways ($12 \times 1.2 \times 1.5$ m), supplied with 200 l/min of natural sea water at ambient temperature, until the initiation of the present study in November 1976. Mortalities during this period were less than 1%.

In November 1976, 40 populations, each of 20 individual *C. gigas*, matched by live weight both for individual oysters (5.0 ± 1.0 g) and populations (100 ± 5 g), were selected. Similarly, 40 populations of 25 juvenile *O. edulis* were selected (individual weight 4.0 ± 1.0 g, population 100 ± 5 g). Four groups consisting of 10 populations of each species were made by random selection of populations within each species. Each group was placed into one of the four wooden tanks previously described such that each plastic tray held one population of each species. The temperature in the experimental tanks was raised from ambient (10 °C) at a rate of 1 °C/day to obtain temperatures in the four tanks of 12°, 15°, 18° and 21 °C, respectively. These water temperatures were maintained ± 1.0 °C for the duration of the experiment (19 weeks) from the day of attaining a water temperature of 12 °C in all four tanks (taken as Day 0). Throughout this period, treatment of all tanks was identical in every respect except for temperature. All tanks and pipes were cleaned at weekly intervals throughout the experiment.

Sampling programme

Estimates of growth and gonadal development were made by sacrificial sampling of individual populations. The initial sample is considered to be the population taken from 12 °C on Day 0. One week later a population was removed from each of the temperatures 15°, 18° and 21 °C. Thereafter populations from all temperatures were removed for estimation of growth and gonadal development every 2 weeks.

The oysters were washed clean of adherent material and blotted dry with a paper towel. They

were then weighed in a tared vessel on a Mettler top-loading balance and the mean individual weight calculated. Each population was then randomly divided into two further lots, the first of which was sacrificed for estimation of dry shell, dry meat and biochemical composition (*15 C. gigas* or *20 O. edulis*), and the second was shucked and fixed in Bouin's solution for subsequent histological analysis (five animals each of *C. gigas* or *O. edulis*).

No mortalities were evident throughout the study.

Biochemical analysis

Oysters were deep frozen to await analysis. Shucked meats were pooled for each sample and freeze-dried in a LabConCo Model 3 freeze drier. Dried meats were weighed in a tared vessel on a Mettler analytical balance, ground to a fine powder in an unglazed pestle and mortar, and stored under desiccation prior to analysis. Shells were dried at 100 °C for 24 h, cooled under desiccation and weighed on a Mettler top-loading balance.

Percentage carbon (C) and nitrogen (N) contents of the meats were assayed using a Perkin-Elmer CHN analyser. For carbohydrate, 5–10 mg of freeze-dried meat was weighed into a 15 ml centrifuge tube and extracted overnight at 4 °C in 5% (w/v) trichloroacetic acid. Following centrifugation for 10 min at 5000 rpm 0.5 ml aliquots of the supernatant were taken and carbohydrate assayed as anthrone reactive substances by the method of Strickland & Parsons (1968). Calibration was against glucose. Ash content was estimated as the percentage material remaining following ignition at 600 °C for 12 h.

Histological analysis

To facilitate handling only the dorsal part of the visceral mass, containing predominantly the digestive gland and overlaying gonadal material, was fixed. Other material was discarded. Following fixation in Bouin's solution, specimens were dehydrated in alcohol, cleared in two changes of xylene and embedded in paraffin wax. After sectioning at 8–10 µm, specimens were stained in Delafield's Hematoxylin and counterstained in Eosin Y following the methodology described by Humason (1962). Specimens were examined microscopically for evidence of gametogenesis and spawning. For *C. gigas* the guidelines used were based on those originally described for *C. virginica* by Kennedy & Battle (1964); for *O. edulis* the guidelines were based on the works of Cole (1942) and Loosanoff (1962). Specimens were classified as representing one of five categories on a 0–4 scale ranging from inactive (0) to spent (4). The population value was taken as the arithmetic mean of the individual values. A similar method has been used previously to quantify gonadal development in *Tapes japonica* by Holland & Chew (1974). In the present study, the stages are defined as follows:

Stage 0 (inactive). C. gigas and O. edulis

No evidence of the presence of follicles peripheral to the digestive gland. (This stage was observed in only two individuals of *C. gigas* in the study.)

Stage 1 (early active). C. gigas and O. edulis

Male. Many follicles; spermatogonia and spermatocytes numerous, no spermatozoa.

Female. Oogonia arising from stem cells along the follicle; no free oocytes.

Stage 2 (late active)

Male. C. gigas: follicle cells contain predominantly spermatids and spermatozoa; characteristic swirling pattern of spermatozoa, with tails toward follicle lumen, in centre of follicle. *O. edulis:* some sperm balls, characterized by the presence of tails of spermatozoa, present in the middle of the follicles; periphery of follicle occupied by spermatids and few spermatocytes.

Female. C. gigas and O. edulis: both free and attached oocytes present with distinct nuclei that stain lighter than cytoplasm.

Stage 3 (ripe)

Male. C. gigas: follicles filled with spermatozoa oriented with tails to follicle lumen forming characteristic swirling pattern that completely fills follicle. *O. edulis:* sperm balls fill follicles.

Female. C. gigas and O. edulis: predominantly free oocytes with distinct nucleus and nucleolus.

Stage 4 (spent)

Male. *C. gigas:* most follicles empty or partially so with sperm evident in sperm ducts in some individuals; some phagocytes present. *O. edulis:* follicles appear empty or broken or both, sperm balls present in ducts; some phagocytes present.

Female. *C. gigas* and *O. edulis:* follicle walls appear broken and follicles empty; ripe ova fill genital canal; some phagocytes present.

Loosanoff (1962) has advised caution in the staging of gonadal development in *O. edulis*: in this species different parts of the gonad may develop asynchronously with respect to one sex and also development of both sexual reproductive products may occur simultaneously (although not necessarily synchronously with respect to the different stages) in the same individual. This has been well documented during the male-female transition by Cole (1942). In the present study no large disparities in stages of one sex were evident in any one individual. Where individuals contained both stage 2 and stage 3 follicles of the same sex they were allotted a value of 2.5 (14 individuals in all). Where both sex products were present they were staged and treated as individual values.

Table 1. *Growth and biochemical composition of Crassostrea gigas at temperatures of 21°, 15°, 18° and 21 °C over a 19-week period*

All data are mean values ($n = 15$) on a per individual basis. W, live weight; M, dry meat weight; S, dry shell weight; C, carbon content of soft tissues; N, nitrogen content of soft tissues; CHO, carbohydrate content of soft tissues; ASH, ash content of soft tissues.

Temp. (°C)	Parameter	Weeks										
		0	1	3	5	7	9	11	13	15	17	19
12°	W (g)	5.2	—	6.2	7.2	8.0	9.4	10.4	12.8	14.3	16.5	23.5
	M (mg)	88.7	—	256.6	400.0	524.0	646.0	918.0	972.0	1141.0	1414.0	1736.0
	S (g)	2.4	—	3.1	3.6	3.9	5.4	5.8	6.8	7.1	8.1	11.4
	C (mg)	41.5	—	101.8	158.9	216.9	272.6	391.0	415.6	477.0	621.0	750.5
	N (mg)	10.8	—	18.9	30.2	40.6	47.9	52.1	61.6	70.5	96.9	113.0
	CHO (mg)	0.9	—	43.3	62.4	138.8	178.8	281.5	331.0	383.6	302.3	390.2
	ASH (mg)	27.1	—	38.4	72.0	58.0	46.7	86.2	87.8	86.4	106.8	118.2
15°	W (g)	—	5.6	6.2	8.1	11.2	10.8	12.5	18.8	21.3	31.2	28.2
	M (mg)	—	131.5	228.7	462.0	718.0	696.0	844.0	1266.3	1614.0	2211.0	1253.0
	S (g)	—	2.7	3.2	3.7	5.7	5.7	6.7	8.8	9.8	14.8	14.3
	C (mg)	—	43.2	88.1	196.1	310.4	259.3	366.6	525.6	665.0	969.3	530.5
	N (mg)	—	10.1	17.5	36.9	55.1	44.1	53.8	78.9	101.0	168.7	93.7
	CHO (mg)	—	4.9	37.9	110.6	178.2	183.8	241.5	363.3	443.0	331.0	199.0
	ASH (mg)	—	37.7	35.8	43.1	65.1	64.7	58.6	140.6	198.2	275.9	109.5
18°	W (g)	—	5.7	6.7	8.7	12.2	15.6	17.9	19.2	23.6	29.6	34.6
	M (mg)	—	94.0	270.0	491.0	641.7	851.0	751.0	888.0	1122.0	1425.0	1322.0
	S (g)	—	2.6	3.0	4.2	5.9	8.1	8.6	9.9	12.1	14.6	16.3
	C (mg)	—	32.6	110.0	202.4	273.6	341.2	305.1	352.9	442.6	593.2	525.9
	N (mg)	—	7.9	19.2	34.8	47.0	48.9	53.9	60.6	87.1	110.7	114.5
	CHO (mg)	—	4.9	39.9	60.3	166.3	200.7	144.7	167.3	178.4	186.7	133.1
	ASH (mg)	—	24.1	33.1	52.7	57.1	84.6	84.0	127.9	189.6	157.8	176.9
21°	W (g)	—	5.6	7.5	10.6	12.4	18.3	22.5	19.6	25.2	29.8	38.7
	M (mg)	—	134.0	374.0	570.6	742.0	1145.0	1054.7	703.6	1136.0	1286.0	1219.0
	S (g)	—	2.7	3.8	5.0	6.4	9.3	9.6	10.1	12.7	15.2	19.0
	C (mg)	—	44.5	157.2	238.5	319.7	456.5	399.6	258.1	450.8	510.9	478.2
	N (mg)	—	10.4	30.4	45.6	67.8	75.7	89.2	60.9	100.2	102.0	102.3
	CHO (mg)	—	5.8	78.5	117.5	137.1	190.2	150.6	99.3	92.0	184.5	129.2
	ASH (mg)	—	36.1	60.6	56.8	65.8	119.0	136.3	156.4	89.8	117.7	160.2

Ammonia excretion

Prior to sampling, 4–6 individual animals were assayed for ammonia excretion rate. Animals were immersed in beakers of 1 µm filtered sea water, maintained at the relevant experimental temperature, and ammonia levels assayed at time zero and after 3 h in both blank and experimental beakers by the method of Solorzano (1969). Volumes of 150, 300 or 500 ml of water were used dependent upon the size of the experimental animals. This method has been used previously for *Tapes japonica* by Mann & Glomb (1978) who showed that there was a linear increase in ammonia levels at 30 min intervals over a time course of 3 h, indicating that no reduction in excretion rate occurred as ambient ammonia levels rose.

Table 2. Growth and biochemical composition of *Ostrea edulis* at temperatures of 12°, 15°, 18° and 21 °C over a 19-week period

All data are mean values expressed ($n = 20$) on a per individual basis. Abbreviations – see Table 1.

Temp. (°C)	Parameter	Weeks										
		0	1	3	5	7	9	11	13	15	17	19
12°	W (g)	4.1	—	5.3	5.5	6.3	7.2	7.5	7.7	9.0	9.2	10.6
	M (mg)	95.0	—	171.0	201.0	241.5	304.0	334.0	347.0	442.0	404.0	444.5
	S (g)	2.7	—	3.5	3.6	4.3	4.4	5.0	5.3	6.1	6.5	7.3
	C (mg)	33.6	—	66.7	80.7	99.6	125.3	137.7	143.0	175.5	170.3	169.4
	N (mg)	7.8	—	12.9	15.0	16.3	21.3	20.2	21.8	28.8	24.9	28.1
	CHO (mg)	6.4	—	21.1	40.9	66.4	73.9	111.2	82.6	159.1	114.1	150.3
	ASH (mg)	20.8	—	22.3	23.9	22.7	26.9	26.7	27.3	32.6	28.0	33.8
15°	W (g)	—	5.9	4.9	8.1	7.5	8.4	8.6	10.2	11.2	12.3	13.6
	M (mg)	—	114.0	140.0	322.0	327.5	352.0	435.5	430.5	521.0	525.0	566.0
	S (g)	—	2.9	3.2	5.5	4.9	5.6	5.7	6.8	7.7	8.4	9.0
	C (mg)	—	44.5	55.2	131.3	134.4	144.9	179.0	177.1	224.1	215.4	209.0
	N (mg)	—	10.1	10.9	22.8	22.4	21.9	27.6	26.7	34.8	33.0	32.3
	CHO (mg)	—	10.0	17.9	76.3	89.7	87.6	130.6	106.3	112.0	159.6	161.8
	ASH (mg)	—	20.1	20.8	33.7	27.6	25.8	36.7	41.2	41.3	44.5	82.9
18°	W (g)	—	4.9	5.7	7.9	9.1	10.0	10.2	12.7	14.7	19.4	19.3
	M (mg)	—	116.5	189.5	321.5	419.4	448.0	412.0	442.5	529.0	650.0	617.0
	S (g)	—	3.1	3.5	5.3	5.9	6.6	6.9	8.3	9.7	10.8	12.3
	C (mg)	—	43.7	75.2	131.0	169.4	183.3	164.7	170.2	209.6	259.3	233.7
	N (mg)	—	9.4	13.3	21.2	27.4	32.3	28.6	32.6	35.6	42.1	38.9
	CHO (mg)	—	12.1	36.5	80.2	123.3	119.6	90.6	91.6	174.6	140.4	175.8
	ASH (mg)	—	21.8	23.2	34.1	46.6	40.6	39.6	67.1	68.5	72.7	75.6
21°	W (g)	—	5.3	6.2	8.1	9.8	11.4	12.5	13.8	15.8	19.9	22.3
	M (mg)	—	139.5	211.5	325.0	447.5	478.0	456.0	375.3	591.0	838.0	756.0
	S (g)	—	3.1	4.0	5.3	6.6	7.7	8.3	8.9	10.1	12.4	13.7
	C (mg)	—	51.7	83.8	132.7	186.3	183.0	177.3	136.9	233.6	330.2	301.4
	N (mg)	—	10.9	15.6	21.0	30.9	31.1	31.8	27.1	40.3	53.1	45.6
	CHO (mg)	—	13.2	40.6	79.3	117.7	99.9	115.4	70.2	164.9	274.6	247.1
	ASH (mg)	—	26.0	24.5	32.8	40.3	66.8	56.8	59.8	75.7	87.6	85.8

RESULTS*Growth and biochemical composition*

The absolute live weight increment over the experimental period was greater in *C. gigas* than *O. edulis* (Tables 1, 2). In both species terminal live weights increased with increasing temperature. *Crassostrea gigas* grew from an initial live weight of 5.2 g to values of 23.5, 28.2, 34.6 and 38.7 g at 12°, 15°, 18° and 21 °C, respectively. *Ostrea edulis*

grew from an initial live weight of 4.6 g to 10.6, 13.6, 19.3 and 22.3 g at 12°, 15°, 18° and 21 °C, respectively. Final dry meat weight was not related to temperature in *C. gigas* (Tables 1, 2): an initial value of 88.7 mg, and final values of 1736.0, 1253.0, 1322.0 and 1219.0 mg were recorded with increasing experimental temperature. However, *O. edulis* exhibited an increase in dry meat weight proportional to temperature: an initial value of 95.0 mg and final values of 444.5, 566.0, 617.0 and 756.0 mg were recorded at 12°, 15°, 18° and 21 °C respectively.

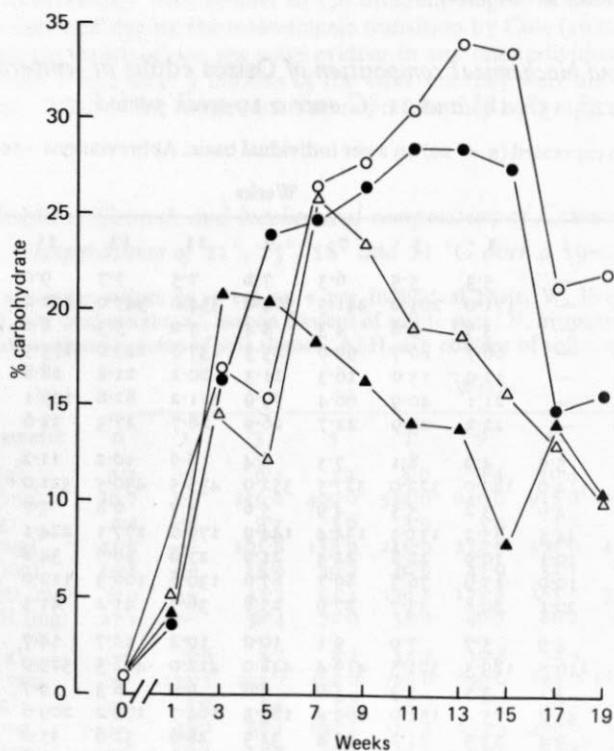


Fig. 1. *Crassostrea gigas*. Percentage carbohydrate content maintained at 12° (○), 15° (●), 18° (△) and 21 °C (▲) for a 19-week duration.

Carbon and nitrogen contents (Tables 1, 2) generally followed the trend previously described for dry meat weight in both species.

In growth experiments the absolute content of individual biochemical constituents increases with time. However, it is often the relative changes in the constituents that are of interest. To compare the relative increase in carbohydrate content during the experimental period with increase in dry meat weight, data on carbohydrate content of *C. gigas* (Table 1) have been recalculated as percentage content and plotted in Fig. 1. It is notable that, at first, there was a period of increasing carbohydrate content, followed by a period in which the carbohydrate content declined, the transition from the first to the second period occurred successively earlier as the experimental temperature increased. Furthermore, the carbohydrate content was higher at the transition point at 12° and 15 °C than

at 18° and 21 °C, irrespective of whether the carbohydrate content is expressed on an absolute or percentage basis. By contrast, no such temperature effect is evident in *O. edulis* (Fig. 2). The percentage carbohydrate content rose steadily during Weeks 0–7 at all temperatures, following which a fluctuation in values is evident at all temperatures. However, no marked decrease in percentage carbohydrate content was observed at any of the experimental temperatures by the completion of the study.

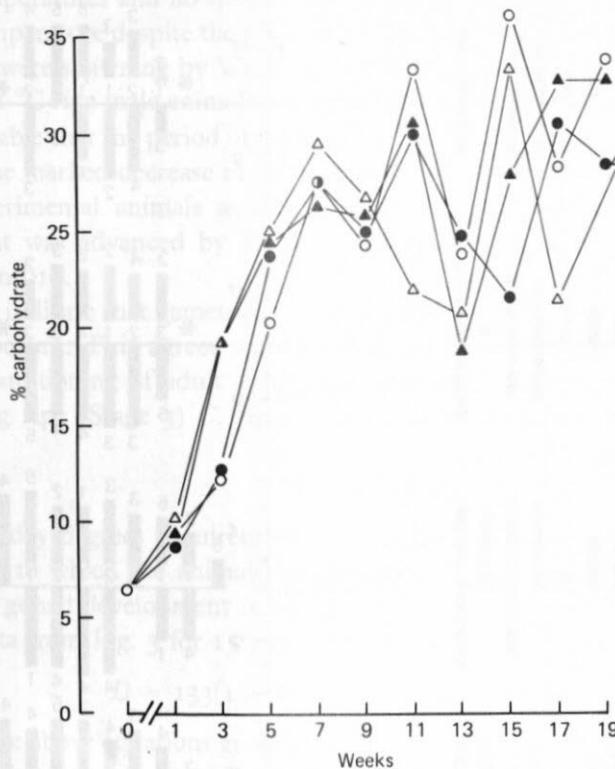


Fig. 2. *Ostrea edulis*. Percentage carbohydrate content maintained at 12°, 15°, 18° and 21 °C for a 19-week duration. Symbols as in Fig. 1.

Previously, Walne (1970) and Gabbott & Stephenson (1974) have described the high degree of correlation between dry weight condition index (dry meat (g) × 1000/internal shell volume) and glycogen content of adult *O. edulis*. Although no shell cavity data were collected in the present study, linear regressions were calculated of dry meat:dry shell condition index ($C_i = \text{dry meat} \times 1000/\text{dry shell}$) against percentage carbohydrate content (CHO) for each species. The following relationships were obtained:

$$C. gigas: \% \text{ CHO} = 0.1915 C_i - 1.832, \quad r = 0.811, \quad P < 0.001, \quad \text{D.F.} = 38.$$

$$O. edulis: \% \text{ CHO} = 0.564 C_i - 9.07, \quad r = 0.754, \quad P < 0.001, \quad \text{D.F.} = 38.$$

A significant correlation is evident between percentage carbohydrate content and condition index as calculated in both species.

During Weeks 0–7 the percentage ash content decreased from initial values of 30.6%

(*C. gigas*) and 21.9% (*O. edulis*) to approximately 10% of the dry meat weight. Subsequently, both *C. gigas* and *O. edulis* exhibited consistently low (6.6–9.4%) ash contents at 12 °C. As experimental temperatures increased, more variable and higher percentage ash contents were evident in *C. gigas* but in *O. edulis* both absolute and percentage ash contents increased consistently with increasing experimental temperature.

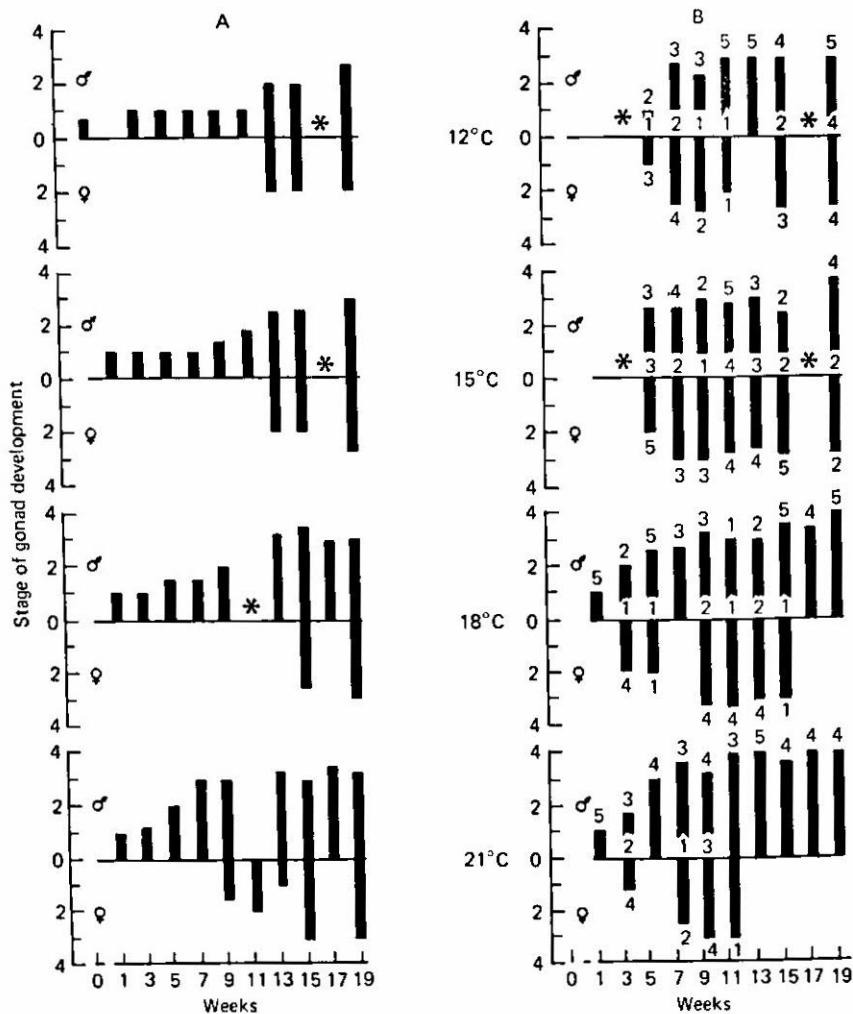


Fig. 3. Gonadal development in (A) *Crassostrea gigas* and (B) *Ostrea edulis* maintained at 12°, 15°, 18° and 21°C for a 19-week duration. Stages: 1, early active; 2, late active; 3, ripe; 4, spent (see text for explanation). In (B) numbers of each sex recorded are placed at the top (solid bar) and bottom (dashed bar) of the histogram bar; numbers on the line of origin indicate animals containing both sex products. Asterisk denotes sample damaged in preparation.

Gonadal development

Crassostrea gigas. The data is summarized in Fig. 3A. No previous history of gonadal development was available for the experimental stock prior to commencing the study. However, the combination of a low stage of gonadal development (Fig. 3) and the small

size of the oyster (Table 1) at Day 0 suggest that no previous maturation and spawning had occurred.

No hermaphrodites were found in the individuals examined. Data obtained at 18° and 21 °C (Fig. 3) suggest that *C. gigas* generally matures first as a male before subsequently alternating its functional sex. Some evidence of functional female development was evident at both 12° and 15 °C by Week 13. However, male development is very slow at these temperatures and no spawning was evident throughout the experimental period at either temperature despite the presence of ripe individuals by Week 19. At 18 °C some individuals were spawning by Week 13, this being recorded as a mean population stage of 3.2. At 21 °C ripe male animals were present by Week 9 and were spawning by Week 13. It is notable that the period of ripening and first spawning at 18° and 21 °C coincides well with the marked decrease in both mean dry meat weight and carbohydrate content of the experimental animals as described in the previous section. At 21 °C female development was advanced by Week 9 with fully ripe specimens being prevalent at Weeks 15 and 19.

The data indicate that gametogenesis is dependent on both absolute temperature and time of exposure. This agrees with the concept of 'day-degrees' used in commercial hatchery conditioning of adult oysters for spawning. The requirement in day-degrees for attaining ripe (Stage 3) *C. gigas* in the present study can be calculated using the formula:

$$D = d(t - t_0),$$

where D = day-degrees requirement, d = number of days to attain a ripe state, t = temperature to which the animals are exposed and t_0 = temperature below which no evidence of gonad development is found.

Using data from Fig. 3 for 15°, 18° and 21 °C:

$$D = 133(15 - t_0) = 91(18 - t_0) = 49(21 - t_0).$$

Solving the above equations gives a t_0 value of 10.55 and a D value of 592. These compare with values of $t_0 = 12.0$ °C and $D = 450$ for *C. virginica* (Price & Maurer, 1971).

The data also suggest that there is a minimum absolute temperature requirement to induce spawning (Fig. 3) and that this temperature is higher than that required to stimulate gametogenesis. Such a hypothesis correlates well with observations by the author that populations of *C. gigas* introduced into locations in British waters, where temperature rarely exceeds 18 °C, often attain maturity but rarely spawn. Similarly in British Columbian waters, spawnings of introduced *C. gigas* are rarely observed in cold locations (Bernard, 1974).

Ostrea edulis. Data are summarized in Fig. 3B. A large percentage of animals containing both sexual products were recorded in the present study. No marked preponderance of either sperm or ova was universally evident in these individuals: predominantly male, equal male-female, and predominantly female individuals were present, as well as totally male and totally female individuals. It seems probable that most, if not all, of the animals containing both sex products correspond to the male-female transition stage described previously by Cole (1942).

The preponderance of one sex during first maturation found in *C. gigas* was not ap-

parent in *O. edulis* although this observation may be somewhat biased as the interval between samples was large (2 weeks) compared to the relatively short time required to form mature gametes (Fig. 3). Previously, Cole (1942) had described initial maturation as male in *O. edulis*. Gametogenesis was more rapid in *O. edulis* than in *C. gigas* over the range of experimental temperatures. Ripe individuals were recorded by Week 11 at 12 °C but no histological evidence of spawning or brooding females was found in the

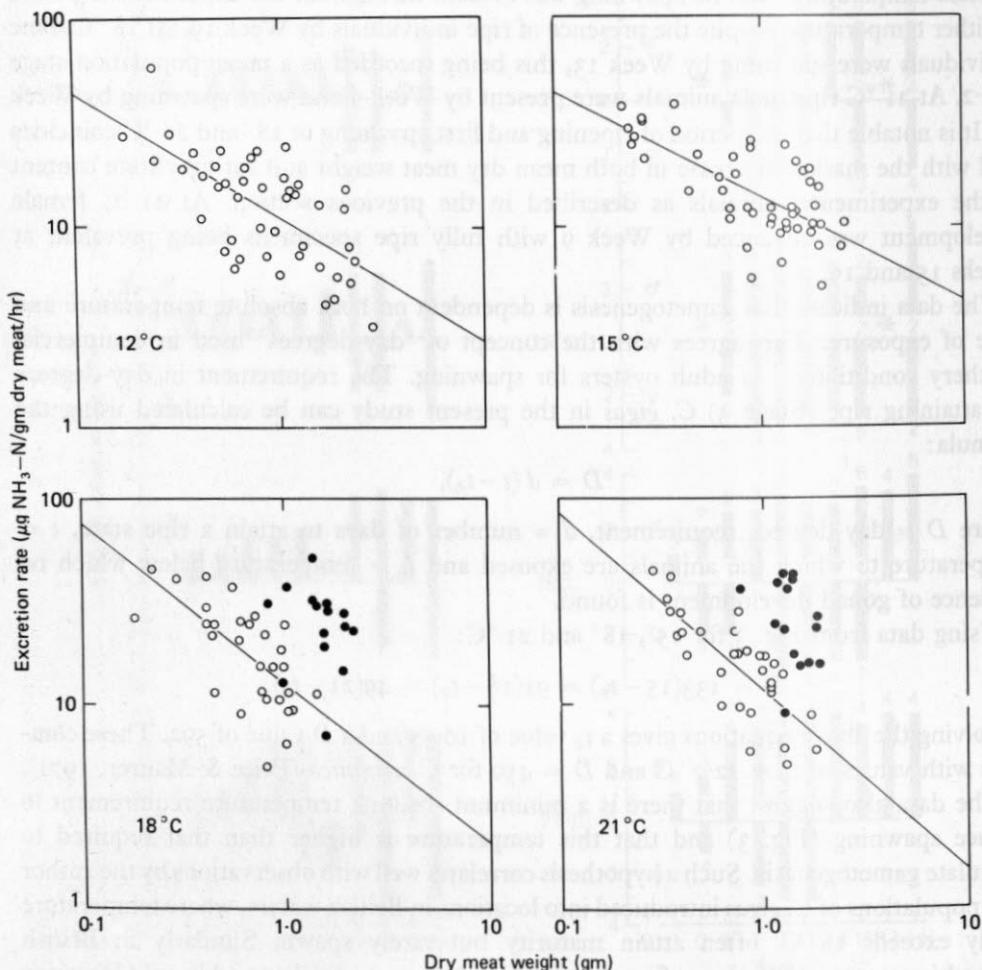


Fig. 4. *Crassostrea gigas*. Double logarithmic plots of weight specific ammonia excretion rate versus dry meat weight for individuals maintained at temperatures of 12°, 15°, 18° and 21 °C. Post-spawning values are shown as closed circles. Regressions are fitted to values recorded prior to spawning (open circles), details are as follows:

12 °C	$b = 0.51$	$a = 16.04$	$r = 0.514$	$n = 47$
15 °C	$b = 0.535$	$a = 8.53$	$r = 0.445$	$n = 46$
18 °C	$b = 0.47$	$a = 20.82$	$r = 0.61$	$n = 21$
21 °C	$b = 0.81$	$a = 14.39$	$r = 0.58$	$n = 21$

subsequent 8 weeks. This again suggests that the absolute temperature minima to stimulate gametogenesis and spawning are different. A mean stage of gonad development of 3.0 was recorded at 15 °C for females by Week 7 and at 15 °C for males by Week 9. Two individuals brooding larvae were found at Week 17 at 15 °C; however, these samples along with those at 12 °C for the same date were accidentally damaged during histological preparation and are therefore omitted from Fig. 3. Histological evidence indicates

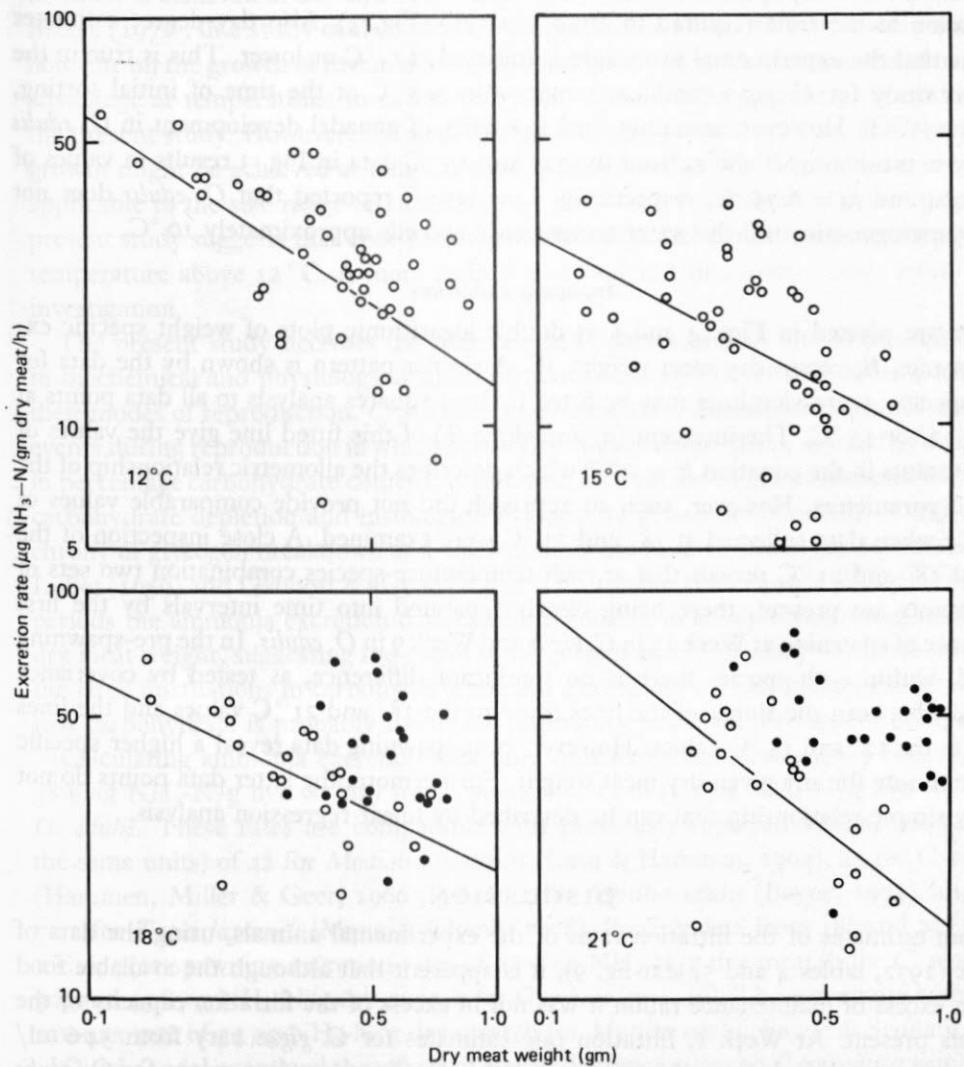


Fig. 5. *Ostrea edulis*. Double logarithmic plots of weight specific ammonia excretion rate versus dry meat weight for individuals maintained at 12°, 15°, 18° and 21 °C. Post-spawning values are shown as closed circles. Regressions are fitted to values recorded prior to spawning shown as open circles, details are as follows:

12 °C	$b = 0.601$	$a = 10.92$	$r = 0.664$	$n = 46$
15 °C	$b = 0.501$	$a = 13.88$	$r = 0.696$	$n = 48$
18 °C	$b = 0.796$	$a = 12.71$	$r = 0.657$	$n = 30$
21 °C	$b = 0.874$	$a = 11.73$	$r = 0.773$	$n = 31$

that spawning of both sexes occurred by Week 9 at 18 °C, and of males by Week 7 at 21 °C. A brooding female was evident at Week 9 at 21 °C. However, a mean population value of 3·0 was recorded due to the presence of a late active (Stage 2) female individual. As with *C. gigas*, the decrease in mean dry weight observed during Weeks 9–11 in *O. edulis* at 18° and 21 °C appears to be related to the onset of first spawning.

It is doubtful if accurate estimates of the values of D and t_0 for the 'day-degrees' requirement can be made for *O. edulis* as the 2-week interval between samples is large in relation to the time required to attain ripeness (Fig. 3). Also day-degree estimates assume that the experimental procedure is initiated at t_0 °C or lower. This is true in the present study for *C. gigas* (ambient temperature 9·5 °C at the time of initial sorting, $t_0 = 10\cdot55$ °C). However, assuming total inactivity of gonadal development in *O. edulis* at Day 0, estimating D and t_0 from the 12° and 15 °C data in Fig. 3 results in values of $D = 404$ and $t_0 = 6\cdot75$ °C, respectively. Cole (1942) reported that *O. edulis* does not start gametogenesis until the water temperature exceeds approximately 10 °C.

Ammonia excretion

Data are plotted in Figs. 4 and 5 as double logarithmic plots of weight specific excretion rate, E , versus dry meat weight, W . A similar pattern is shown by the data for both species; regression lines may be fitted by least squares analysis to all data points at either 12° or 15 °C. The intercept (a) and slope (b) of this fitted line give the values of the constants in the equation $E = aW^b$ which describes the allometric relationship of the plotted parameters. However such an approach did not provide comparable values of a and b when data collected at 18° and 21 °C were examined. A close inspection of the data at 18° and 21 °C reveals that at each temperature-species combination two sets of data points are present, these being clearly separated into time intervals by the first incidence of spawning at Week 13 in *C. gigas* and Week 9 in *O. edulis*. In the pre-spawning period, within each species there is no significant difference, as tested by covariance analysis, between the slopes of the lines fitted to the 18° and 21 °C values and the lines fitted to the 12° and 15 °C values. However, post-spawning data reveal a higher specific excretion rate for any given dry meat weight. Furthermore, the latter data points do not fit any simple relationship that can be described by linear regression analysis.

DISCUSSION

From estimates of the filtration rates of the experimental animals, using the data of Walne (1972, tables 4 and 5, text-fig. 9), it is apparent that although the available food was in excess of maintenance ration it was not in excess of the filtration capacity of the animals present. At Week 1, filtration rate estimates for *C. gigas* vary from 54·6 ml/oyster/min at 12 °C to 64·3 ml/oyster/min at 21 °C. Corresponding values for *O. edulis* are 39·9 and 63·9 ml/oyster/min at 12° and 21 °C respectively. At this time the product of the filtration rate times the number of animals present, 200 *C. gigas* and 250 *O. edulis*, exceeds the flow of water through the experimental tanks. Despite a decrease in the number of animals present due to sampling, this situation is still evident at Week 17 when 40 *C. gigas* and 50 *O. edulis* were present at each temperature. Estimates of filtration rate at this time vary from 132·9 ml/oyster/min to 123·5 ml/oyster/min for *C. gigas* at

12° and 21 °C respectively, and 54.6 ml/oyster/min to 108.9 ml/oyster/min for *O. edulis* at 12° and 21 °C respectively. It is only during Weeks 18 and 19 of the study that the water flow exceeds the filtration capacity of the animals present. Consequently, the observed growth may be viewed as an index of the energy balance of the experimental animals with respect to varying temperature but with a limited food supply.

Newell, Johnson & Kofoed (1977) showed that the maximum scope for growth in *O. edulis* is achieved at 20 °C, a result that agrees well with the present study. Malouf & Breese (1977), in a study of the seasonal changes in the effects of temperature and water flow rate on the growth of juvenile *C. gigas*, concluded that there was no consistent growth advantage at temperatures in excess of 15 °C. Again a good agreement is evident with the present study. However, the same authors suggest that at high food levels maximum growth might be achieved at temperatures approaching 20 °C. While this result may be applicable to the size range of animals (3–25 mm) used by Malouf & Breese (1977), the present study suggests that once gametogenesis is initiated growth may not increase with temperature above 12 °C. A more definitive answer to this problem must await further investigation.

The present study describes the time course of gametogenesis and associated changes in biochemical and physiological status of two species of oysters that differ markedly in their modes of reproduction. *Crassostrea gigas* exhibits a distinct sequence of metabolic events during reproduction in which a period of storage metabolism, marked by an increase in percentage carbohydrate content, is followed by a period of gametogenesis, marked by carbohydrate depletion and histological evidence of gonad development. A similar synchrony of glycogen breakdown and vitellogenesis has been recorded for *Mytilus edulis* by Lubet (1959) and Gabbott & Bayne (1973). Throughout both storage and gametogenesis periods the ammonia excretion data exhibit a good fit to an allometric relationship with dry meat weight, suggesting the use of some protein as a respiratory substrate. However, the large fluctuations in carbohydrate content during storage and gametogenesis indicate that carbohydrate is probably the major respiratory substrate throughout this period.

Calculating ammonia excretion data on a unit live weight basis gives ranges of 9.0–38.8 µg NH₃-N/g live wt/day for *C. gigas*, and 11.6–21.2 µg NH₃-N/g live wt/day for *O. edulis*. These rates are comparable with previously reported values, (expressed in the same units) of 42 for *Modiolus demissus* (Lum & Hammen, 1964), 25 for *C. virginica* (Hammen, Miller & Geer, 1966), 9.6–67.2 for *Mytilus edulis* (Bayne, 1973), and 27.8–72.2 for *Tapes japonica* (Mann & Glomb, 1978). Pooling data from 18° and 21 °C gives mean, post-spawning excretion rates of 25.9 µg NH₃-N/g dry meat/h for *C. gigas* (*n* = 30) and 45.8 µg NH₃-N/g dry meat/h for *O. edulis* (*n* = 49). These compare with a mid-summer rate of 34 µg NH₃-N/g dry meat/h for *Mytilus edulis* (Bayne & Scullard, 1977).

Quick (1971) examined the effects of elevated temperatures on *C. virginica* using short-term studies at intervals throughout the year. Considerable fluctuations in the glycogen: protein ratio were noted depending upon the time of year. However, during gametogenesis and spawning, a marked drop in glycogen content was noted, similar to that for carbohydrate in the present study.

It appears that the viviparous breeding system evolved by *O. edulis* is reflected in its lower fecundity compared to the oviparous *C. gigas* (see Cole, 1941). However, its lower

fecundity is certainly not the result of a lower carbohydrate content of the adults; in fact, the percentage carbohydrate content of *O. edulis* was comparable with, or higher than, the maximum values recorded for either species by Gabbott & Walker (1971), Holland & Hannant (1976), and Walne & Mann (1975). Also, unlike *C. gigas*, *O. edulis* appears to be able to maintain active gametogenic processes (Fig. 3), while increasing its absolute carbohydrate content (Table 2).

The possible involvement of neuroendocrine factors in the control of the reproductive cycles and glycogen metabolism of marine bivalve molluscs has been reviewed by Gabbott (1975). Nagabhushanam (1963a, b, 1964) has provided circumstantial evidence of the involvement of neurosecretory cells in the control of spawning of *Spisula solidissima*, *Crassostrea virginica* and *Modiolus modiolus*. In a series of papers, Lubet and his co-workers (see Lubet, 1973 for bibliography) have presented evidence for the control of gonad development in *Mytilus edulis* and *Mytilus galloprovincialis* by neurosecretions from the cerebral ganglia. Ropes (1968) states in reference to *Spisula solidissima* that 'the hormonal control of spawning can be overridden in surf clams. Nevertheless, a minimum temperature threshold for a spawning response is probably necessary'. His first statement appears to be based upon the work of Nagabhushanam (1963a) rather than direct evidence that hormonal control of spawning is indeed predominant in *S. solidissima*. The present author can find no evidence of investigations of the role of neurosecretion in gametogenesis and spawning of either *O. edulis* or *C. gigas*. It is notable, however, that the second statement of Ropes concerning a minimum temperature for spawning is applicable to both species of oyster.

In attempting to optimize culture regimes in aquacultural facilities, one of several end points may be important: a marketable size product in a minimum time, a maximum conversion efficiency of food to end product, maximum nitrogen (and hence protein) production, or culturing an organism that has a large store of metabolic reserves with an accompanying ability to withstand unforeseen environmental stress. A consideration of these options in bivalves indicates that certain of their combinations are often mutually exclusive. For example, a high temperature will favour the growth of large *C. gigas* (Table 1), but absolute meat production is higher at lower temperatures. Nitrogen production, however, is very similar at all the present experimental temperatures. Also, the importance of the ability to withstand stress cannot be underestimated since the death of cultured organisms can be a severe economic problem. In physiological and biochemical terms there is a requirement for oysters with high carbohydrate reserves. It is inevitable that some catabolism of protein (Figs. 4, 5) and losses of dissolved amino acids and metabolic faecal nitrogen will occur. For maximum protein production, culture regimes should be biased to reduce the role of protein as a respiratory substrate.

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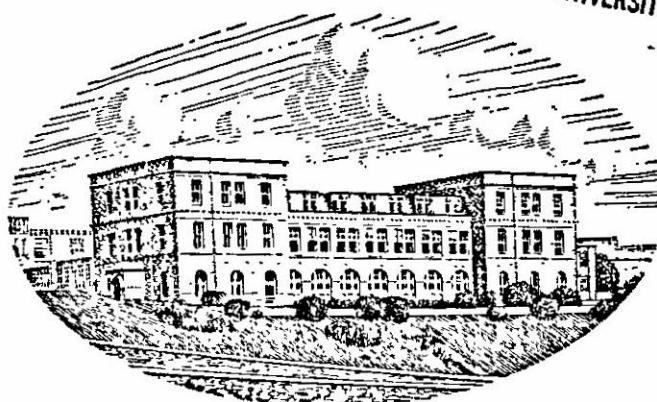
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