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Notes and Discussions

The Effect of Temperature on Growth and Ammonia Excretion of the Manila Clam *Tapes japonica*[a]

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The effect of sustained temperatures of 12, 14, 16 and 18 °C on the growth and ammonia excretion of the Manila clam, *Tapes japonica* (Deshayes, 1853) were measured over a 77-day period. Meat growth was highest at 12 °C, less at 14 and 16 °C, and the lowest at 18 °C. No significant differences were noted in shell growth at the four temperatures. Ammonia excretion decreased from 12 to 14 °C but increased markedly at 16 and 18 °C. It is suggested that increased ammonia excretion is related to a change in predominant respiratory substrate concurrent with gonad production.

Introduction

The waste-recycling aquaculture system proposed by Ryther *et al.* (1972) removes dissolved inorganic nutrients, predominantly nitrogen and phosphorus, from secondary sewage effluent with an accompanying production of animal protein in the form of edible bivalve molluscs. The combination of such a system with a thermal effluent source presents the prospect of year round culture of bivalve species that are usually environmentally restricted to only seasonal growth. However, the optimization of temperature regimes in such a situation is problematic in that cycles of growth, gonad production and spawning in bivalves are directly related to temperature. Thus any chosen temperature regime might compromise the individual goals of promoting meat growth, minimizing protein catabolism and supressing gonad production. The present study reports data on one aspect of this problem, namely the effect of sustained elevated temperature on growth and ammonia excretion of the Manila clam, *Tapes japonica*,[c] which in recent unpublished studies has exhibited considerable promise as a candidate for culture in waste-recycling systems.

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[c]*Tapes japonica* (Deshayes, 1853) has a junior synonym, *T. semidecussata* (Reeve, 1859). The species has been confused with *T. philippinarum*. Other (incorrect) synonyms used include *Venerupis semideccusata*, *V. japonica*, *V. philippinarum*, *Paphia philippinarum* and *Venus philippinarum*. These alternatives are discussed by Cahn (1951) and Ohba (1959). *T. japonica* has been used in the present text except in reference to the work of other authors where their own nomenclature has been followed.

Methods

Growth experiment

Experimental animals were obtained from International Shellfish Enterprises, Moss Landing, California in November 1974 as 3 mm (14 mg) juveniles and grown in the waste-recycling aquaculture system at Woods Hole Oceanographic Institution (Ryther *et al.*, 1975) until the present study commenced in February 1976. Throughout the intervening period animals were fed on cultures of marine phytoplankton, predominantly the diatom, *Phaeodactylum tricornutum* grown in outdoor ponds (120 000 liters) in seawater enriched with either secondary sewage effluent or inorganic nutrients. Water temperature was maintained at 20 °C throughout the winter months and at ambient when this exceeded 20 °C during the summer months.

For the present study 100 animals, of 2·8 g mean live weight, were selected from the parent stock. These were divided at random into five groups of 20 individuals. One group was sacrificed to estimate initial experimental values for dry meat and dry shell weight following drying for 24 h at 100 °C. The remaining groups were placed in mesh-bottomed, wooden boxes in each of four fibreglass trays (57×45×9 cm). Each tray was supplied with sand-filtered seawater at a flow rate of 200 ml min^{-1} at an initial temperature of 20 °C. The seawater was enriched with a continuous supply of mixed phytoplankton culture, obtained from the outdoor mass cultures described above, to a mean concentration of 80 cells μl^{-1}. The seawater temperature in the experimental trays was decreased at a rate of 1 °C day^{-1} by mixing with sand-filtered seawater of ambient temperature (2–4 °C), to obtain experimental temperatures of 18, 16, 14 and 12 °C in the four respective trays. These temperatures were held constant (±1 °C) for the remainder of the growth experiment (77 days).

At the end of the experiment live weight, dry meat weight and dry shell weight were recorded for each group. A dry meat condition index (*Ci*) was calculated for each group by the following equation:

$$Ci = \frac{\text{dry meat weight} \times 1000}{\text{dry shell weight}}.$$

Ammonia excretion

Dissolved ammonia was measured by the colorimetric method of Solorzano (1969). Static systems were used in these assays, in which ammonia accumulation was monitored in beakers of sand filtered or Gelman GF–A (1 μm) filtered seawater containing the experimental animals. A preliminary study indicated that a linear increase in dissolved ammonia concentration with time ($r=0{\cdot}97$, 7 d.o.f. $P<0{\cdot}001$) was recorded over a period of 5 h at 20 °C in beakers containing 1, 3 or 5 animals plus 200 ml of water per animal. For the present study incubations were of 3 h duration in 250- or 800-ml beakers containing either one or three animals plus 150 ml of water per animal. Control beakers without animals were included in all incubations. During incubation beakers were immersed in the relevant growth tray to ensure temperature control.

No assays of ammonia excretion were attempted during the first 2 weeks of the growth experiment to allow the animals to acclimate to the experimental temperatures. Following this period assays were carried out at frequent but irregular intervals throughout the course of the experiment, a minimum of 16 assays per temperature being completed. During the final 7 days of the growth period assays were carried out at all the experimental temperatures. For this latter period animals were numbered individually and subsequently sacrificed thus allowing calculation of excretion rate on both live weight and dry meat weight bases.

TABLE 1. The growth of *Tapes japonica* at various temperatures for a period of 77 days. Initial values are the mean of a pooled sample of 20 individuals. Final values at each temperature give the mean ± one standard deviation

	Initial value	12 °C	14 °C	16 °C	18 °C
Live weight (g)	2·80	3·94±0·08	3·88±0·17	4·09±0·31	4·00±0·36
Dry meat (mg)	106·0	337·5 ±8·70	263·3 ±23·3	277·7 ±27·3	221·2 ±20·5
Dry shell (g)	1·30	1·89±0·05	1·85±0·09	2·16±0·19	2·07±0·20
Condition index, *Ci*	81·54	179·4 ±4·4	140·6 ±9·2	129·2 ±9·4	107·3 ±5·1

TABLE 2. Ammonia excretion in *Tapes japonica* grown at various temperatures for a period of 77 days. Data for (A) was collected during days 15–77 inclusive. Data for (B) was collected during days 70–77 inclusive. A mean value ± one standard deviation is given for each temperature, the accompanying bracketed number giving the number of assays completed

	12 °C	14 °C	16 °C	18 °C
(A) μg NH_4-N g^{-1} live wt h^{-1}	1·47±0·06 (52)	1·16±0·06 (18)	2·20±0·24 (36)	3·01±0·15 (52)
(B) μg NH_4-N g^{-1} dry meat h^{-1}	19·11±0·69 (16)	14·21±1·48 (18)	32·09±3·57 (18)	29·48±4·10 (16)

Results

Growth

All initial values were recorded on pooled samples; final values were recorded on individual animals. Both are summarized in Table 1.

Similar mean final values were recorded at all temperatures for both live weight and dry shell weight ($P<0.05$), these being consistently higher than the mean initial values (as initial values were taken from pooled, rather than individual samples it is not possible to effect a statistical comparison of the initial and final mean values). A final mean dry meat weight of 337·5 mg was recorded at 12 °C, greater than that at all other temperatures ($P<0.05$) and indicating a threefold increase in mean dry weight over the experimental period. Similar final dry meat weights were recorded at 14 and 16 °C ($P<0.05$), the value for 16 °C being greater than that for 18 °C ($P<0.05$). Decreasing final mean values of condition index, *Ci*, are evident with increasing experimental water temperature.

Ammonia excretion

No significant differences were recorded in excretion rate (μg NH_4–N g^{-1} live weight h^{-1}) throughout the time course of the experiment within any one of the experimental groups, although differences did occur between the groups. Values obtained for static systems using either sand filtered or 1 μm filtered seawater were similar for any one group of animals. Thus values collected throughout the experimental period have been pooled for each temperature and are given in Table 2, together with values recorded on a dry meat basis (μg NH_4–N g^{-1} dry meat h^{-1}) during the final 7 days of the experiment.

Ammonia excretion rate was higher at 12 °C than at 14 °C ($P<0.01$ on both live weight and dry meat basis). Similarly the ammonia excretion rate at 16 °C was higher than at 12 °C

($P<0.01$). Maximum excretion rate was recorded at 18 °C when data are expressed on a live weight basis ($P<0.01$) although this is not significant on a dry meat basis. Mean values for the present study vary in the range 1·16–3·01 μg NH_4–N g^{-1} live weight h^{-1} (27·84–72·24 μg NH_4–N g^{-1} live weight day^{-1}). These compare well with values of 9·6–67·2 μg g^{-1} live weight day^{-1} recorded by Bayne (1973) for *Mytilus edulis*, and with values of 42 μg g^{-1} live weight day^{-1} recorded for *Modiolus demissus* (Lum & Hammen, 1964) and 25 μg g^{-1} live weight for *Crassostrea virginica* (Hammen *et al.*, 1966).

Discussion

The temperature related seasonal cycles of growth, gonad production and spawning in bivalves are usually accompanied by distinct cycles of accumulation and utilization of gross biochemical components (Ansell, 1972; Ansell *et al.*, 1964; Ansell & Trevallion, 1967; Masumoto *et al.*, 1934; Walne & Mann, 1975). The rapid production of gonad material in the warmer months is often accompanied by a depletion of metabolic reserves, usually in the form of glycogen, with a subsequent shift in the predominant respiratory substrate. The use of protein as a substrate is indicated by a quantitative increase in ammonia excretion (see Bayne, 1973). The use of sustained elevated temperatures in any proposed bivalve culture facility will obviously affect this seasonal cycle, but gametogenesis and spawning is possible even at constant high temperatures. Such a situation is to be avoided in that animals containing ripe gonad are often unsuitable for marketing and, during growth, are predominantly catabolizing the protein substrate that such aquaculture facilities were designed to produce.

Meat production in the present study was enhanced at the lower experimental temperature. At temperatures above 14 °C a marked increase in ammonia excretion occurs. Holland & Chew (1974) report that specimens of *Venerupis japonica* from Hood Canal, Washington have ripe gonad in May–June when water temperatures rise above 14 °C, and subsequently spawn in July–October. Ohba (1959) summarizes much Japanese work on the biology of *Tapes japonica*: two annual periods of spawning are reported in spring and late autumn coincident with a minimum water temperature of 14 °C.

Thus results from the literature and the present study suggest that the marked increase in ammonia excretion rate and gametogenic activity above 14 °C are related, and that this temperature coincides with a change in emphasis in *Tapes japonica* from a metabolism concerned with accumulation of reserve material to reproductive activity.

In the case of species with distinct seasonal growth cycles further investigations are required as to their physiological responses to a non-seasonal temperature regime. In bivalve molluscs such investigations must include certain basic parameters; oxygen consumption and nitrogen excretion as indices of physiological rate and stress, glycogen content as an index of available reserve material for use under stress conditions, an histological quantification of gonad production and the relative production of meat and shell for assessment of economic potential.

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