THE INFLUENCE OF ENVIRONMENT FACTORS ON JUVENILE *Tridacna gigas*

Thesis submitted by
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A Tridacna gigas juvenile (ca. 15 mm in length)

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S. S. M. Mingoa
1 November 1990

ABSTRACT

Giant clams (F. Tridacnidae) have recently been investigated for mariculture, in order to replenish diminishing reef populations and to satisfy commercial demand for clam meat and shell. Development of juvenile-rearing techniques has necessitated a better understanding of the effects of environmental factors on juveniles. This thesis addresses this need by providing physiological bases for clam responses (survival, growth) to various environmental conditions, using Tridacna gigas juveniles cultured at the Orpheus Island Research Station. The following factors were studied separately or in combination: light, temperature, salinity, seawater flowrate, stocking density, nutrient supplements (microalgae, dissolved inorganic nutrients), cleaning, and emersion.

Measurement of rates of oxygen production (photosynthesis) and consumption (respiration) in clams acclimatized to high and low light regimes revealed that photoadaptation occurs in juvenile T. gigas. Zooxanthellar contribution to clam respiration (CZAR) was significantly greater for clams acclimatized to unshaded light conditions (CZAR = 92% at 32% Translocation, T), than those acclimatized to a 90% shaded light regime (CZAR = 72%, T = 32%). Appreciable clam growth was measured in suboptimal light intensities, although tissue condition indices (wet tissue weight/shell length; dry tissue weight/shell length) revealed reduced tissue growth in low light and negative growth in darkness. The irradiance level for maximum photosynthesis rate was significantly greater in high than in low light acclimatized clams (i.e. 206 and 150 $uE.m^{-2}.s^{-1}$, respectively).

Studies on upper temperature tolerance of juveniles using the direct transfer method showed better survival at 26, 28 and 29°C than at 33 and 37°C. Whole clam respiration measurements showed that respiration rate was

detrimentally affected at temperatures below 19°C and beyond 33°C. Q_{10} values for temperature intervals between 12°C and 33°C ranged from 1.42 to 3.07, whereas for temperature intervals between 33°C and 40°C, Q_{10} values were about 1.0.

Salinity tolerance studies also using the direct transfer method showed better survival at 26, 33 and 36 ppt than at salinities 22-0 ppt and 40-45 ppt. Studies on osmotic adaptation to varied salinities, based on comparisons of osmolal concentrations of their extracellular fluids and mantle cavity fluid against external salinities, indicate that Tridacna gigas is an osmoconformer, with its extracellular fluids slightly hyperosmotic to the ambient salinity. Maximum shell growth over a 6 week period occurred at about 35 ppt salinity. Studies on salinity-induced regulation of intracellular free amino acids by High Performance Liquid Chomatography analysis demonstrated that non-essential free amino acids are more important than essential free amino acids in intracellular osmotic pressure regulation.

The effects of seawater flowrate, stocking density, nutrient supplements and cleaning were separately assessed in terms of survival and growth. Studies on nutrient supplements and cleaning employed a 2-Factor experimental design. Clam survival was dependent on stocking density, whereas growth was related to flowrate, specifically flowrate per clam. Supplements and cleaning did not influence survival nor growth, due to confounding factors.

The effects of emersion on juvenile clams were investigated according to their needs for water and energy conservation, by measuring rates of water loss, respiration, excretion and photosynthesis during or after air exposure. Respiration and photosynthesis in air were both demonstrated in T. gigas, although at reduced rates. Respiration measurements on clams exposed to anoxic atmospheres indicated some tolerance of anoxia for an exposure period of 3 h; clams after a 9 h anoxic exposure

manifested an oxygen debt 61% greater than that acquired after a 3 h exposure. Water loss after 27 h exposure to desiccating, oxygen-saturated atmospheres was minimal (5%). The rate of ammonia excretion, determined by the phenolhypochlorite method, was dependent on the duration of air exposure.

Overall, this study showed that a change in the environment of juvenile Tridacna gigas elicits corresponding physiological changes: 1) light modifies the photosynthetic capacity and efficiency of the algal endosymbionts, thus clam phototrophic capability, 2) temperature influences clam respiration rate, hence its metabolic rate, 3) salinity affects both extra- and intracellular osmotic balance in juvenile clams, and 4) emersion reduces rates of certain metabolic functions, i.e. respiration, excretion and photosynthesis. This study also showed that conditions of seawater flowrate and stocking density may be controlled to optimize clam growth and survival in culture. Juveniles must therefore be reared in environmental conditions that are non-lethal and not deleterious to physiological processes. Juvenile clams must be grown where light intensities are greater than 200 $uE.m^{-2}.s^{-1}$, in water temperatures from 26°C but not exceeding 32°C, in salinities near 35 ppt. Manipulation of clam stocking density and seawater flowrate may either increase water turnover time or flowrate per clam to enhance survival and growth. Juveniles can withstand periods of emersion of up to 27 h in oxygen-saturated atmospheres, and are capable of aerial photosynthesis.

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THE INFLUENCE OF ENVIRONMENTAL FACTORS ON JUVENILE *Tridacna gigas*

1. INTRODUCTION

Tridacnids are an important component of coral reefs in the tropical Indo-West Pacific regions. By virtue of their size and numbers, they may comprise a significant portion of the total reef biomass (Salvat 1971, Yonge 1974). Various studies have been done on their anatomy and morphology (see Rosewater 1965, Stasek 1962, Yonge 1936), their distribution (see Munro and Heslinga 1983), general ecology (e.g. Alcala 1981, Jaubert 1977), biology (e.g. Stasek 1962, Yonge 1975, 1980), algal symbiosis (e.g. Fankboner 1971, Yonge 1936), and evolution (see Yonge 1980). Tridacnids are among the few groups of bivalves harboring endosymbiotic dinoflagellates (see Kawaguti 1950, 1983), known as *Symbiodinium microadriaticum*, and commonly called zooxanthellae. These zooxanthellae are the same species found in symbiosis with hermatypic corals (Taylor 1969, but see Blank and Trench 1985).

Tridacnids are also economically important bivalves (see Munro and Heslinga 1983, Rosewater 1965). They are popular for their large shells and colorful mantles. Indo-Pacific peoples have traditionally harvested giant clams for food; while the valves are used for a variety of purposes, for instance, as washbasins, material for tools, or as ornaments (Rosewater 1965). In Southeast Asia, e.g. in Taiwan and Hongkong, there is strong demand by the exclusive restaurant trade for the adductor muscle (Dawson 1988) which is considered a delicacy. The adductor muscle is often processed dried or preserved, and is eaten either steamed or as a flavor enhancer in soups. The adductor muscle is known to contain high levels of inosine monophosphate to which may be attributed its flavor-enchancing properties (Gwyther and Munro 1981).

As the demand for clam meat is greater than the supply (Crawford et al. 1987), giant clam meat fetches a high price. For instance, illicit trading in Taiwan demands 100 tonnes of dried adductor muscle annually, with a dockside price of $7.50-$21.25/kg wet weight (Dawson 1988). In Hongkong, the retail price of dried adductor muscle reaches over US$100/kg (Crawford et al. 1987). The ASEAN region has an annual demand conservatively estimated at 3000 tonnes (live weight), which is equivalent to US$160 million (Heslinga and Watson 1985).

All tridacnids are now listed as endangered species (IUCN 1983). Both local and overseas demands for giant clams have led to overfishing, adversely affecting breeding populations (Braley 1987). For instance, Tridacna gigas has only recently become extinct in Micronesia, the Marianas, Guam, Tuvalu, Vanuatu, New Caledonia, and Fiji (Heslinga et al. 1984; Munro, in press), and is now considered rare in Philippine (Crawford et al. 1987) and Indonesian reefs (Brown and Muskanofola 1985, Usher 1984). Contrary to what Rosewater (1965) believed, the large reproductive potential among tridacnids is not sufficient to ensure a lasting resource. Clearly, proper management procedures are required. In addition, a program to study breeding in tridacnids may provide seed clams to replenish the diminishing stocks, re-introduce species to areas where they have become extinct, and possibly initiate a giant clam mariculture industry to provide the valued clam products on a stable basis (Copland and Lucas 1988).

1.1. Tridacnid Cultivation

Studies on the breeding of giant clams began over 50 years ago. Among the early investigators were Stephenson (1934) and Wada (1954) on clam reproduction and spawning, and La Barbera (1974, 1975), Jameson (1976) and Beckvar (1981), elaborating on larval and post-larval development.

Their cultivation potential was first recognized by Beckvar (1981) and Munro and Gwyther (1981). Tridacnid

clams are considered good mariculture species for the following reasons: high fecundity, a short planktonic larval life, hardiness and autotrophic capability (Beckvar 1981). Tridacnid culture has 5 phases (see Crawford et al. 1987, Heslinga et al. 1984):

(1) spawning - the selection of sexually mature broodstock, spawning induction, and fertilization of eggs,

(2) larval hatchery - larval rearing throughout the trochophore and veliger stages until settlement and metamorphosis as juvenile clams,

(3) landbased nursery - rearing of juveniles from 0.2 mm to 20 mm in shell length in outdoor tanks,

(4) ocean nursery - rearing of juveniles, 20 mm in shell length or greater, in exclusion mesh cages until they are predator-free, and

(5) grow-out - the "release" of the clams unprotected on the reef.

1.2. This Study

Juvenile Tridacna gigas (onshore and offshore rearing sizes) was the focus of the present research. "Juvenile", as used in this study, refers to that period from the onset of the benthic form (about 200 um shell length), following metamorphosis from a pediveliger (the final larval stage), until the onset of reproductive development (sub-adult male). Having acquired zooxanthellae as a pediveliger, the early juvenile is already phototrophic (Fitt and Trench 1981). Tridacnids are simultaneous protandric hermaphrodites, first developing the male and later the female gonads (Wada 1952), and becoming fully sexually mature adults after about 5 years (Heslinga and Fitt 1987). By the above definition, the juvenile stage is distinguished from both larval and sub-adult phases.

Although their reproductive potential is high, survival among juvenile tridacnids at metamorphosis and after is extremely low under culture conditions, about 1% (Heslinga et al. 1984). This observation is probably also applicable to natural field conditions, where juvenile

recruitment is usually very low (Yamaguchi 1977). While the low survival rates in culture may be attributed to culture techniques (Crawford et al. 1986), causes of mortality under natural conditions are difficult to identify and have been little studied.

Cultivation studies have shown that rates of survival and growth in juvenile Tridacna gigas are influenced by settlement substrate (Braley 1987, Crawford et al. 1986), wave action (Crawford et al. 1988), air exposure, predation (Lucas et al. 1989), and sedimentation (Belda et al. 1988). Physiological studies have demonstrated the importance of light (e.g. Trench et al. 1981a).

This study investigated the influence of certain environmental factors on juveniles: light, temperature, salinity, seawater flowrate, stocking density, microalgal food, inorganic nutrients, and air exposure. Different aspects of juvenile physiology were studied to provide a better understanding of the tridacnid's response to changes in its environment. Most of the experiments were conducted under controlled experimental conditions in order to reduce, if not eliminate, the influence of unknown factors.

The overall aim of this study was to determine the conditions that will enhance growth and survival of T. gigas juveniles during land-based nursery and ocean culture. A review of literature on juvenile tridacnid physiology is provided in the Chapter Introductions. Where information is lacking on certain aspects of juvenile physiology, studies and examples from other bivalve groups have been incorporated to provide some basis of comparison.

2. LIGHT

2.1. Introduction

The dependence of tridacnids on the photosynthesis of their endosymbionts restricts their distribution to shallow sunlit waters (Jaubert 1977). In the absence of light, tridacnids lose their zooxanthellae, become aposymbiotic, and eventually perish (Fankboner 1971).

Tridacnids are obligate phototrophs (Lucas et al. 1989), hence they are unique among cultured bivalves. They derive from their endosymbiotic algae soluble organic molecules which account for a major portion of the clam's metabolic carbon requirements. In Tridacna gigas, zooxanthellae are known to translocate in vivo glucose and oligosaccharides which comprise 32% of host respiratory requirements (Griffiths and Streamer 1988). Isolated algae from T. crocea, in the presence of host animal tissue homogenate, release up to 40% of labelled algal photosynthates, mainly as glycerol (Muscatine 1967); while in T. maxima, the algal contribution amounts to 39-45% (Trench et al. 1981b). These photosynthates become part of the building blocks of major centers of metabolic activities, specifically in mucus production, secretion of the style, and calcification (Goreau et al. 1973). The pathways involved in the transfer of algal photosynthates to the tridacnid host still remain to be elucidated.

Isotope studies on other invertebrate-algal symbioses have shown that a large part of host metabolism is fuelled by photosynthetically-fixed carbon (known as CZAR, which is the contribution of zooxanthellae to animal respiration). For instance, under favorable light conditions, CZAR in the reef coral Stylophora pistillata reaches 143%, indicating that coral metabolism is fully autotrophic. In shade conditions, however, this value decreases to 58%, indicating that a portion of their respiratory carbon demand is met by heterotrophic feeding (Muscatine et al. 1984).

Light utilization by photosynthetic cells is characterized by parameters of the light saturation curve (also called the Photosynthesis-Irradiance, P-I, curve) (Rabinowitch 1951). These parameters are alpha and P_m. Alpha (also called photosynthetic efficiency) refers to the initial slope of the curve. P_m (or P_{max}) refers to the photosynthetic capacity which is the photosynthetic rate at saturating light intensities (Chalker and Taylor 1978, Jassby and Platt 1976). Alpha and P_m have been correlated with environmental factors such as depth and irradiance in several symbiotic corals, the sea anemone Aiptasia pulchella, and the giant clam Tridacna maxima (Muscatine 1980, Chalker 1981, Chang et al. 1983).

Interpretation of photosynthesis rates measured from intact associations is complicated by the fact that such measurements, while indicating net photosynthesis rates, cannot distinguish between host respiration and that of the endosymbionts (see Trench 1987). In hermatypic corals, host respiration rates and algal photosynthesis rates can vary with depth (Chalker and Dunlap 1983, Muscatine et al. 1981, Wethey and Porter 1976). Additionally, algal respiration rates may vary with ambient light conditions; that is, higher rates for zooxanthellae adapted to high light conditions than for those adapted to low light (Falkowski and Owens 1980, cited in Falkowski and Dubinsky 1981).

Attenuation of light and modification of its spectral quality with depth (Jerlov 1976) have caused algal symbionts to evolve adaptive mechanisms to promote photosynthesis in sub-optimal light conditions. Photoadaptation (see Prezelin 1976) in symbiotic dinoflagellates is achieved by: 1) adjusting the algal densities in animal tissue (in hermatypic corals, e.g. Stylophora pistillata (Falkowski and Dubinsky 1981); in the sea anemone A. pulchella (Muller-Parker 1984, 1985)); 2) modifying the light-capturing apparatus of the chloroplasts (called the photosynthetic unit, PSU (Gaffron and Wohl 1936), cited in Falkowski 1981); and 3) changing

the rates of dark reactions in photosynthesis (see Trench 1987). Different mechanisms have been associated with different symbiotic associations. It was found that changes in PSU and in photosynthetic dark reaction rates may be achieved by: 1) changing the number of PSU through variations in pigment concentrations, but maintaining constant pigment ratios (hermatypic corals: Redalje 1976, Titlyanov et al. 1980; the sea anemone Aiptasia pulchella: Chang et al. 1983); 2) increasing the size of the PSU (hermatypic corals: Falkowski and Dubinsky 1981, Zvalinskii et al. 1980; Tridacna maxima: Chang et al. 1983); 3) altering the activities of the carbon dioxide fixing enzymes or the electron transport system (the hermatypic coral Montipora verrucosa: Chang et al. 1983).

Photoadaptation has been demonstrated in T. gigas in relation to clam size. As the clam grows, and the mantle tissue thickens, shading among zooxanthellae occurs. Hence, in small individuals (about 10 mm shell length) with thinner mantle tissue, zooxanthellae experience less shading compared to those in large individuals. Shading is compensated for in the latter by increasing chlorophyll a content per algal cell (Fisher et al. 1985).

Small-sized juvenile T. gigas are amenable to photoadaptation studies because of a reduced mantle-shading effect. Fisher et al. (1985) have shown that 10 mm (shell length) individuals require low light-saturation levels (about 500 $\mu E.m^{-2}.s^{-1}$) to achieve maximum photosynthesis rates. Whether or not photosynthetic efficiency varies with light adaptation is not known. Studies on other shell-secreting organisms have shown that calcification processes are also affected by ambient light conditions. Hermatypic corals exhibit higher calcification rates than ahermatypic species and aposymbiotic forms (Kawaguti and Sakumoto 1948, Goreau 1959, Goreau and Goreau 1959). Higher calcification rates during illumination have been found to be a function of zooxanthellar photosynthesis (Crossland and Barnes 1974, Goreau et al. 1979, Simkiss 1964b, Vandermeulen et al.

1972). Further studies showed that the daily rhythm in calcification is correlated with the rhythm in photosynthetic capacity of their isolated algal symbionts, specifically shown for the hermatypic coral Acropora cervicornis (Chalker and Taylor 1978).

In non-symbiotic bivalves, the effect of light on calcification is manifested by variations in shell texture and pigmentation. The oyster Crassostrea virginica grown in an unshaded environment produces a firm shell with green radial markings; whereas, in shaded conditions, the shell is chalky and its pigmentation changes to a brownish hue (Medcoff and Kerswill 1965). Stromgren (1976a) obtained light and dark shells when mussels were grown in shaded and unshaded conditions, respectively. Stromgren attributes this difference to the presence of mantle pigments which shield sensitive tissue from the harmful effects of the infra-red portion of natural daylight.

The aims of this study were to investigate the effects of light on the physiology of juvenile Tridacna gigas, by determining:

1) the survival of juveniles in the absence of light;
2) changes in tissue condition during exposure to darkness;
3) rates of shell and tissue growth upon acclimatization to different light regimes;
4) the photoadaptive capability of juveniles, and the mechanisms involved in photoadaptation;
5) the effects of different light regimes on the clam-algae association, in terms of the ratio of photosynthesis to respiration rates (P:R) and the contribution of symbiotic zooxanthellae to animal respiration (CZAR).

2.2. Materials and Methods

2.2.1. Survival and tissue condition in the absence of light

Experiments were conducted at Orpheus Island Research Station (OIRS). Eleven-month old Tridacna gigas (mean shell length (SL) ± s.d. of a representative sample = 17.52 mm ± 1.00 mm) were randomly grouped into 5 batches of 20 clams. Four groups ("dark clams") were each placed in 10-l aquaria which were covered with black plastic to totally exclude light. The remaining group, serving as controls ("light clams"), was placed in a 10-l aquarium with access to natural daylight. All aquaria had constantly flowing seawater. Mortality was determined from one batch of dark clams every three days.

For determination of tissue condition index, clams from the same cohort (SL = 16.98 mm ± 0.86 mm, representative sample) were placed in the dark and in the light. Each day for six days, one batch (30 clams) of dark clams was dissected and the tissue dried at 65°C. The light clams were dissected simultaneous with the last batch of dark clams. Tissue condition index (CI) was expressed as dry tissue weight/shell length. SLs were measured with a vernier caliper (± 0.02 mm).

2.2.2. Growth at different light regimes

Four month-old T. gigas juveniles (initial SL=5.75 mm ± 20 mm) were reared for 10 weeks at different light regimes: unshaded, 50% shaded and 90% shaded ambient light. The clams were randomly divided into nine groups of 50 clams; each treatment being replicated three times. For convenience, "light clams" refers to clams grown in unshaded conditions, "50% shade clams" and "90% shade clams" to those grown under 50% and 90% shade, respectively. Groups of clams were placed in 0.8-l plastic containers. All were aerated and had 1 um filtered and ultraviolet-treated seawater running at a flowrate of 1.5 $l.h^{-1}$. The experiments were maintained in

a bath of running water to minimize fluctuations in water temperature. Shading was achieved by shade screens. Measurements of irradiance at noon on several clear days (using a Li-Cor light meter and a submersible sensor) showed that attenuation of light by shade screens approximated the desired light levels. SLs were measured using a dissecting microscope with a micrometer eyepiece.

2.2.3. Acclimatization of juveniles to high and low light regimes for oxygen flux measurements

Eight-month old juveniles (SL = 11.78 mm ± 2.09 mm) were reared under high and low light environments for 53 days at OIRS. Forty-five clams were reared under 90% shaded ambient light, and the same number of clams without shade; the clams being referred to as "90% shade clams" and "light clams", respectively. Changes in irradiance over 12 hours of daylight on a clear day were determined for both unshaded and 90% shaded treatments with a Li-Cor light meter and a submersible sensor. Both treatments were maintained in constantly flowing, 1 um filtered seawater (FSW) with adequate aeration. The growth of filamentous algae along with the clams necessitated periodic cleaning by siphoning. The clams were minimally disturbed during cleaning.

After 53 days, the acclimatized clams were transferred to the Australian Institute of Marine Science (AIMS) seawater system for oxygen flux measurements (Section 2.2.4). The light levels (ambient light and 90% shade) were still maintained, and measurements with the Li-Cor light meter and a submersible sensor showed close approximation to desired light levels.

2.2.4. Photosynthesis and respiration rates of intact clams

The rates of oxygen production and consumption by juveniles were measured after epibionts adhering to the shell were removed. The clams were allowed to recover overnight in constantly flowing seawater.

A 4-ml respiration chamber was used to measure oxygen production and consumption by the intact clam (see Fig. 1). A single clam lying on its side was placed on a platform in the chamber. The chamber was filled with 0.45 µm FSW, then sealed with a rubber bung which held the glass oxygen electrode (Radiometer E5046-0) in place through a central bore. A stirrer bar maintained the water circulation around the oxygen electrode constant. The chamber was submerged in a water bath (MGW Lauda RC6) at 27.8°C ± 0.1°C. The numbers of clams used from 90% shaded and unshaded treatments were 4 and 5, respectively.

Illumination was provided by an Elmo slide projector. Irradiance levels ranging from 10-700 $\mu E.m^{-2}.s^{-1}$ were achieved by moving the light source away from or towards the chamber. Irradiance was measured at the chamber surface by a Li-Cor light meter and a non-submersible sensor.

Oxygen flux was measured with an oxygen electrode connected to a Radiometer PHM72 Mk2 Digital Acid-Base Analyzer. Continuous recordings were obtained using a chart recorder (REC80 Servograph with a REA 112 High-sensitivity Unit). Irradiance was altered at random to avoid any conditioned response. Rates of oxygen production (gross photosynthesis) and consumption (respiration) were expressed in terms of wet flesh weight, chlorophyll a per alga and number of zooxanthellae per g clam WTW (see Forstner 1983). Respiration rates were measured in the dark by covering the chamber with black plastic. All measurements were conducted between 10:00 a.m. and 7:00 p.m.

2.2.5. Isolation of zooxanthellae

After oxygen flux measurements, the clam was dissected, the flesh removed from the shell, blotted briefly and weighed (see Fig. 2). The inner mantle fold was excised and cut up finely on a glass slide. Zooxanthellae were then extracted from the mantle by maceration using a Teflon tube homogenizer. The

Fig. 1. Diagram of experimental set-up for photosynthesis and respiration measurements. (See Chapter 6, Fig. 2A for photograph of similar set-up.)

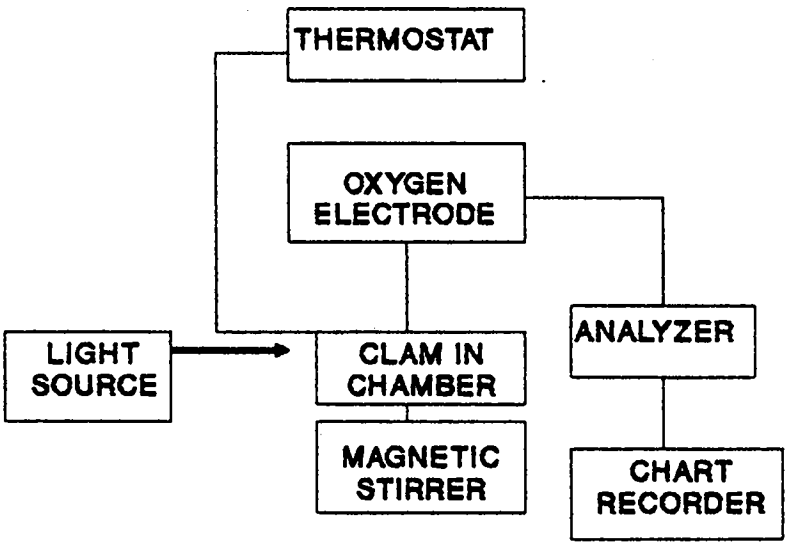
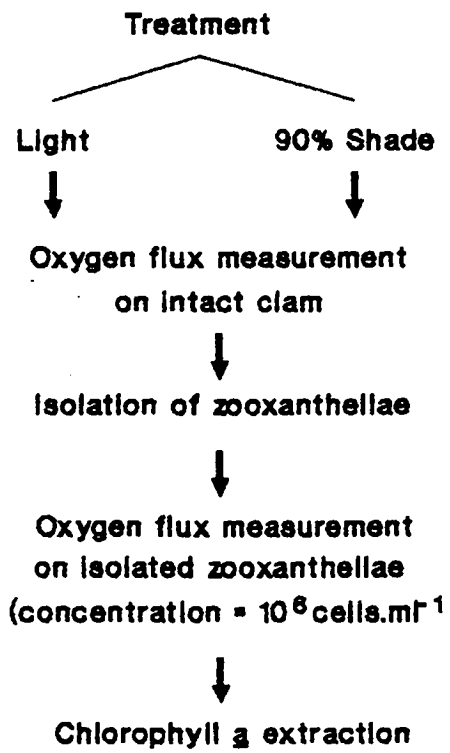


Fig. 2. Outline of methodology to obtain P-I curves for intact clam and isolated zooxanthellae.



homogenate was filtered through three layers of cheesecloth to remove animal tissue debris and obtain a yellow-brown filtrate containing zooxanthellae. The filtrate was centrifuged repeatedly at about 1000 rpm until the supernatant was clear, discarding the supernatant and resuspending the pellet with FSW.

Number of zooxanthellae was determined with a hemocytometer. Preliminary counts showed that a clam 20 mm in length or smaller contained less than 10^6 algal cells. Hence zooxanthellae samples from clams from each light regime treatment were pooled and the algal cell concentration adjusted to get a final concentration of 10^6 algal cells per ml. However, number of zooxanthellae was not counted for each clam prior to pooling the zooxanthellae sample (see Section 2.2.8).

2.2.6. Photosynthesis and respiration rates of isolated zooxanthellae

Photosynthesis and respiration rates of isolated zooxanthellae from light and 90% shade clams were determined in the same manner as for the intact clam, using pooled zooxanthellae sample (Section 2.2.5) at a concentration of 10^6 cells per ml. Only one algal subsample was measured for all irradiances because of the limited sample available. Rates of gross photosynthesis and respiration were expressed in terms of the chlorophyll *a* content of this subsample.

2.2.7. Quantification of chlorophyll *a*

The zooxanthellae subsamples (10^6 cells.ml^{-1}) used in Section 2.2.6 was centrifuged at 3000 rpm for 2 minutes to sediment the cells. The pellet was resuspended in 3 ml of 100% acetone and a drop of magnesium carbonate solution added to prevent pigment degradation (Prezelin 1976). The sample was covered with Parafilm and kept in the dark at 0°C overnight for pigment extraction. The chlorophyll extract was then centrifuged at 3000 rpm for 2 minutes. Absorbance of the supernatant was measured at 630 and 663 nm with a Hitachi U-3200 spectrophotometer, and the

amount of chlorophyll *a* determined (Jeffrey and Humphrey 1975).

2.2.8. Zooxanthellae density at different light regimes

This experiment was conducted to provide data on number of zooxanthellae per clam. Juveniles (about 10 mm SL) were acclimatized to 90% and 50% shade, and unshaded ambient light regimes for 30 days at OIRS. Each treatment had 10 clams, with three replicates each. For convenience, reference to the treated clams is as in Section 2.2.2. After 30 days, zooxanthellae for each clam were isolated as in Section 2.2.5. Number of zooxanthellae was counted per clam and standardized to the wet flesh weight of the clam. Final SLs were determined with a vernier caliper ($\pm$ 0.02 mm).

2.2.9. Data analysis

The rate of gross photosynthesis was obtained from the sum of the rates of net photosynthesis and respiration. Gross photosynthesis was plotted against light intensity, and the curve estimated by the hyperbolic tangent function (see Jassby and Platt 1976, Chalker 1980):

$$P = P_m \tanh (I/Ik)$$

where P is the rate of gross photosynthesis at a specific irradiance (I), P_m (also known as P_{max}: Muscatine 1980) is the rate of gross photosynthesis at the level of saturating irradiance, and Ik is the irradiance at which alpha (the initial slope of the curve) intersects P_m. The statistical program used was BMDP3R (Health Sciences Computing Facility, University of California, Los Angeles). Alpha was computed from derived estimates:

$$\text{alpha} = P_m/Ik$$

2.3. Results

2.3.1. Survival rates and tissue condition index

Dark clams showed heavy mortality after 6 days in contrast to light clams with 100% survival (Fig. 3). The percentage survival of dark clams decreased to 95% on day 6, 35% on day 9, and 5% on day 12. Condition index (CI) decreased by about 13% at day 6; on the other hand, the CI of light clams increased slightly. Loss of condition indicated that the clams were starving in the absence of light, which accounts for the mortality in darkness.

2.3.2. Growth at different light regimes

Fig. 4 shows shell growth rates of light, 50% shade and 90% shade clams over 10 weeks. Final SLs (mean $\pm$ s.d.) were: 8.62 mm $\pm$ 0.49 mm for light clams, 8.91 mm $\pm$ 0.32 mm for 50% shade clams, and 8.62 mm $\pm$ 0.14 mm for 90% shade clams. There was no significant difference between SLs after 10 weeks of rearing at different light regimes (One-Way ANOVA, with replication: $F = 0.72$, $df = 2,6$, $P>0.50$). From pooled values, SLs increased at an average rate of 0.042 mm per day.

Log-transformed values of shell length and dry shell weight were plotted as shown in Fig. 5. The correlation coefficient was high for all treatments. An Analysis of Covariance (ANCOVA, Snedecor and Cochran 1980) showed that the regression lines had similar slopes ($F = 0.24$, $df = 2,216$, $P>0.25$), but the elevations of the intercepts were significantly different ($F = 43.94$, $df = 2,218$, $P<<0.0005$). This means that the clams differed significantly in the rate of shell deposition, with lowest rates at 90% shade, maximum under ambient light, and intermediate values at 50% shade. Although there was no significant difference between the light treatments in shell length, there was greater shell deposition, and hence shell thickness or density, with greater light regime. This was confirmed by the observation that the

Fig. 3. Percentage survival and condition index (CI, mg dry tissue weight per mm shell length) of clams kept in darkness and in the light. (Vertical bar=s.d.)

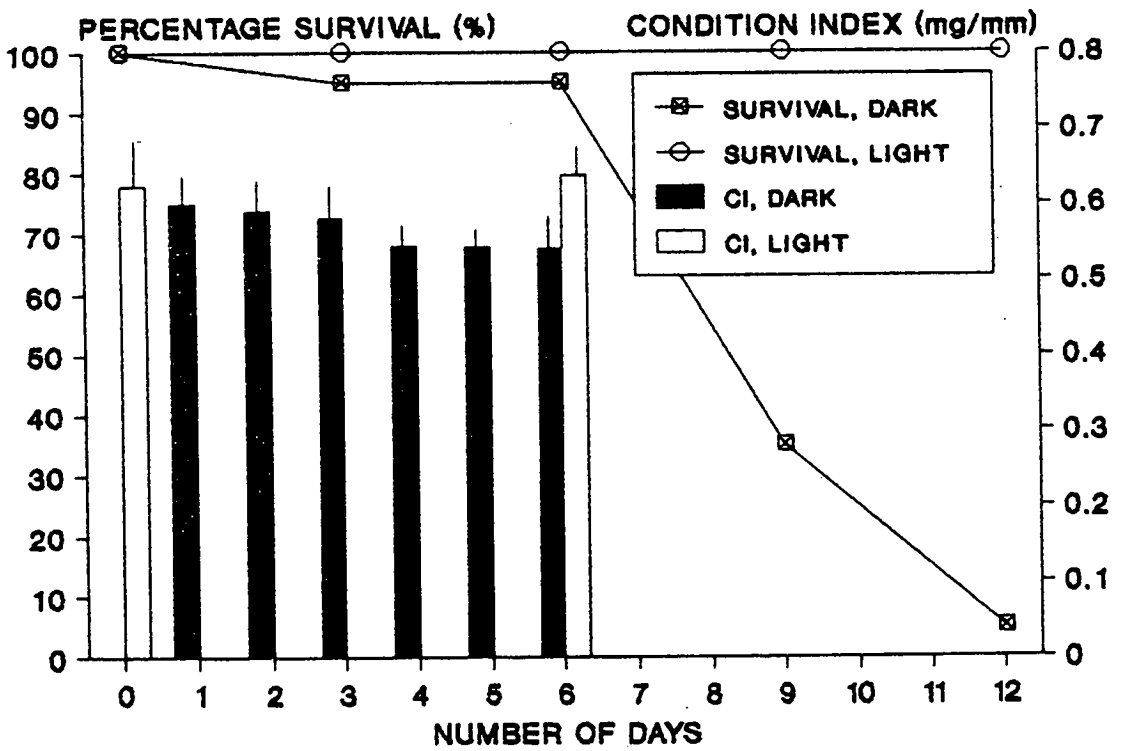


Fig. 4. Growth of juveniles over 10 weeks under 3 light regimes: in the light, 50% shade and 90% shade. (Vertical bar=s.d., other s.d. too small to plot)

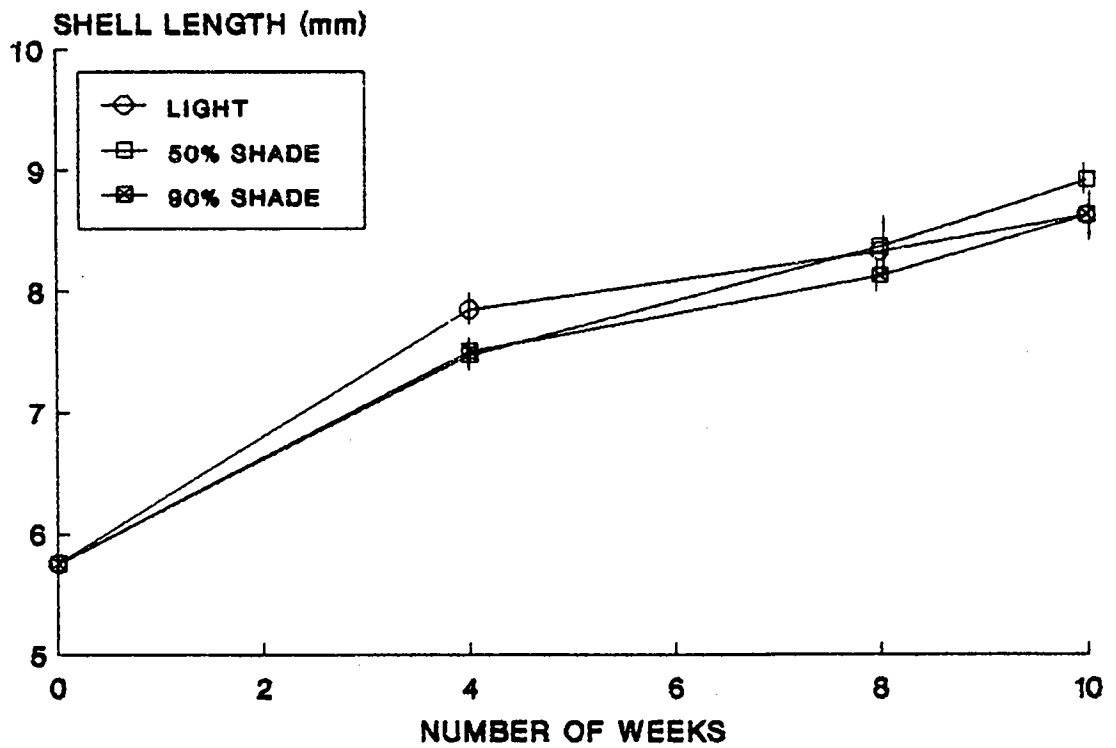
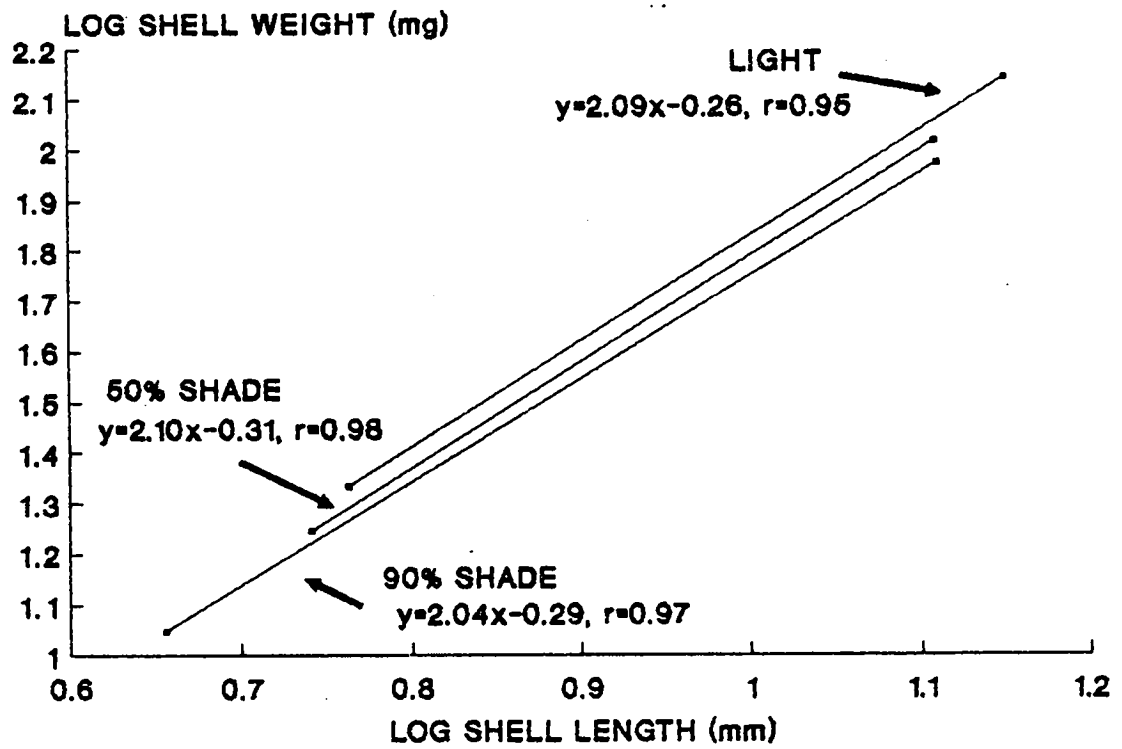


Fig. 5. Linearized regressions of dry shell weight against shell length of clams grown under 3 light regimes: in the light , 50% shade and 90% shade.



opacities of the shells of light, 50% shade and 90% shade clams were markedly different (Fig. 6).

2.3.3. Photosynthesis and respiration rates of intact clams and isolated zooxanthellae

Variations in underwater light intensity measured on a clear day in tanks under ambient light and 90% shade screen are shown in Fig. 7. Light clams received a maximum irradiance of 2500 $\mu E.m^{-2}.s^{-1}$, whereas 90% shade clams only about 250 $\mu E.m^{-2}.s^{-1}$ maximum.

Rates of gross photosynthesis at different light intensities of light and 90% shade clams are shown in Fig. 8. The curves show the characteristic shape of light-saturation curves, i.e., a gradual increase in oxygen production with increasing irradiance developing into an asymptote at saturating irradiance levels.

On a wet flesh weight basis (Fig. 8, Table 1), alpha and P_m values of 90% shade clams were higher than those obtained for light clams, indicating higher gross oxygen production by the former at both subsaturating and saturating irradiance levels. However, when these values were standardized to chlorophyll a content per alga (see Table 2), there was a reversal of trends (Fig. 9): gross photosynthesis rates of light clams were higher than those of 90% shade clams at subsaturating irradiances, with P_m for light clams being attained at about 200 $\mu E.m^{-2}.s^{-1}$ and that for 90% shade clams at about 150 $\mu E.m^{-2}.s^{-1}$ (Table 3A). This indicates a higher gross oxygen production at subsaturating and saturating light intensities for chlorophyll a of light clams than 90% shade clams.

The reversal in trends was attributed to differences between values of wet flesh weight and chlorophyll a per alga for light and 90% shade clams. A comparison of wet flesh weight/shell length indices (Table 2) showed that 90% shade clams had significantly less tissue than light clams (Two-sample t test, $t = 4.19$, $v = 7$, $P<0.005$), despite their similarity in sizes (Two-sample t test, $t = 1.13$, $v = 7$, $P>0.20$). Whereas chlorophyll a measurements

Fig. 6. Shells of clams reared under (A) no shade, (B) 50% shade and (C) 90% shade.

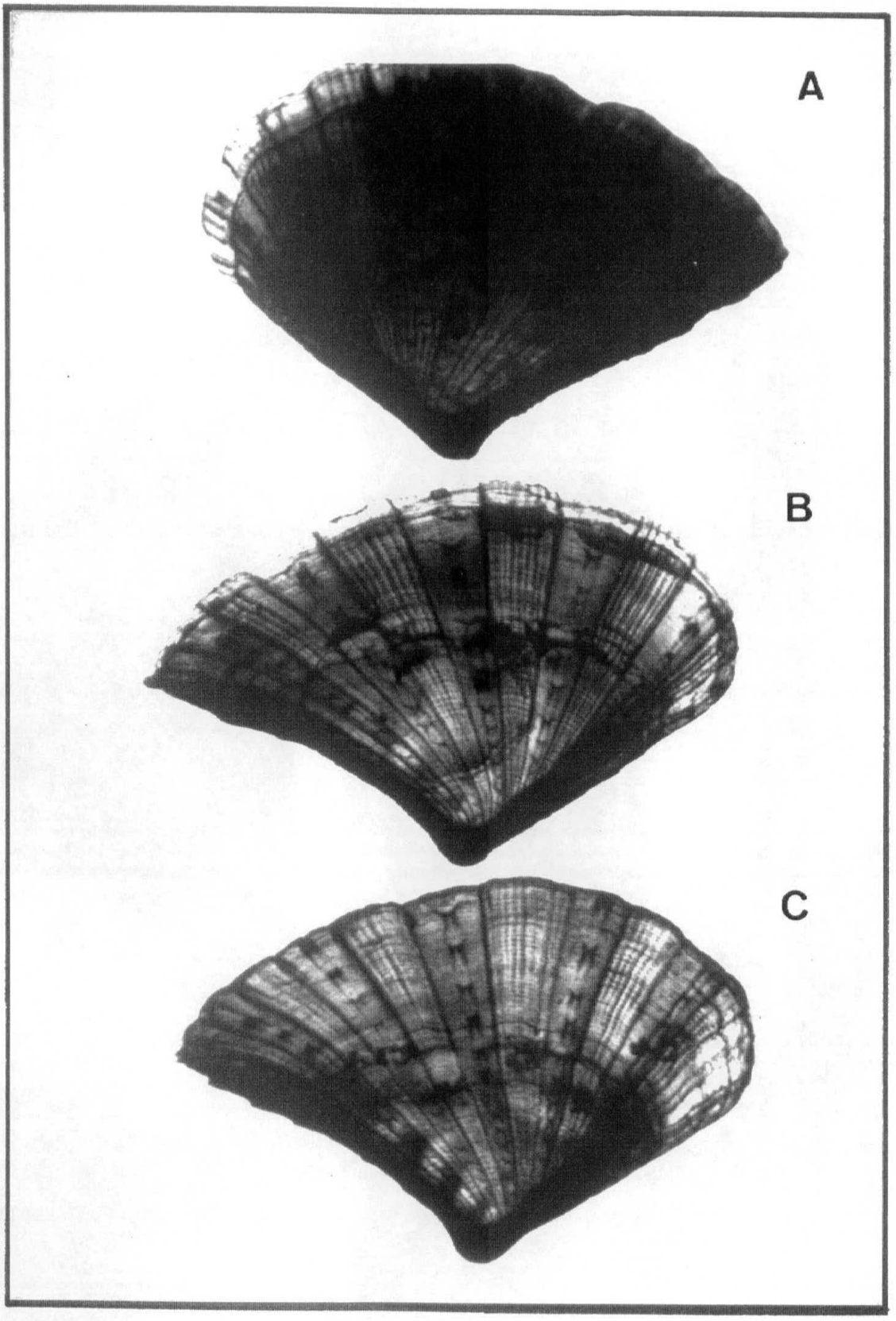

A
B
C

Fig. 7. Variations in underwater light intensity (measured in tanks) on a clear day at Orpheus Island Research Station.

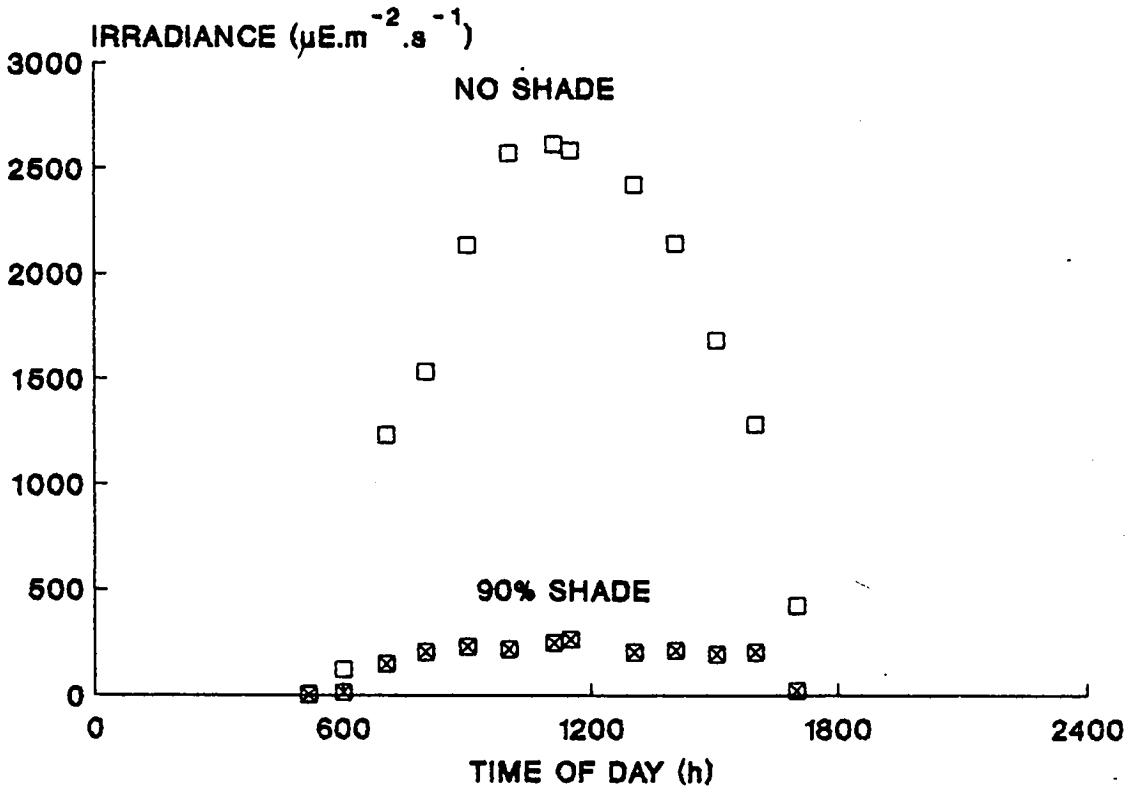


Fig. 8. Rates of gross photosynthesis of intact clams, based on wet flesh weight (WTW). Pairs of curves show minimum and maximum P-I curves for clams reared under 90% shade and in ambient light.

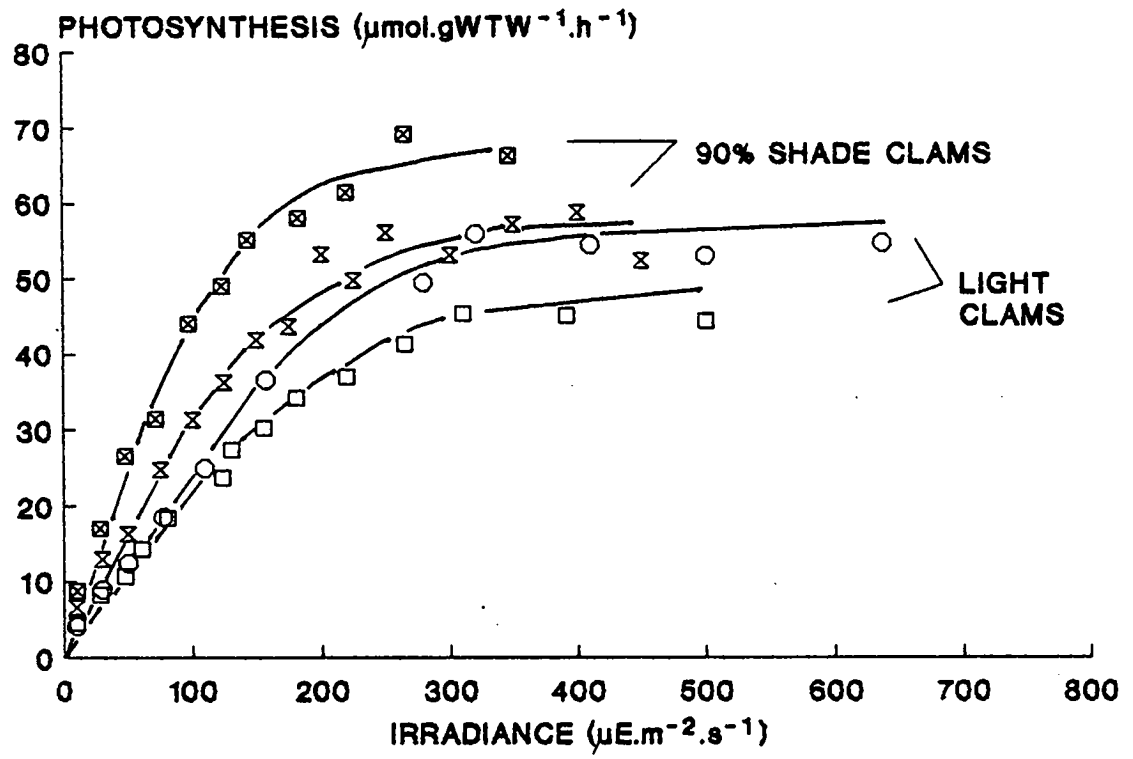


Table 1. Photosynthetic parameters from P-I curves for intact clams reared under ambient light and 90% shade, based on clam wet flesh weight (WTW) (see Fig. 8).

AMBIENT LIGHT

	P_m [a]	Ik [b]	α (P_m/Ik)	RESPIRATION RATE [a]	P:R	CZAR [c] T=32%	CZAR [c] T=95%
	33.47	193.80	0.17	9.43 ± 0.03	2.77	88.64	263.15
	50.64	204.19	0.25	8.58 ± 0.05	3.33	106.67	316.35
	46.94	196.96	0.24	11.40 ± 0.08	2.31	73.84	219.45
	54.65	228.64	0.24	10.08 ± 0.05	3.19	102.13	303.05
	56.27	207.80	0.27	11.47 ± 0.13	2.76	88.29	262.20
MEAN	48.39	206.28	0.23	10.19	2.87	91.91	272.84
S.D.	9.10	13.69	0.04	± 1.25	0.40	12.97	38.29

90% SHADE

	57.36	160.99	0.63	10.02 ± 0.05	2.66	85.05	252.70
	62.85	180.12	0.35	10.81 ± 0.04	2.41	77.13	228.95
	67.26	127.21	0.53	13.81 ± 0.03	2.13	68.22	202.35
	65.91	133.21	0.49	14.21 ± 0.03	1.91	61.19	181.45
MEAN	63.35	150.38	0.50	12.21	2.28	72.90	216.36
S.D.	4.40	24.69	0.12	± 2.11	0.33	10.40	31.06

[a] In μmol oxygen.gWTW $^{-1}$.h $^{-1}$

[b] In μE.m $^{-2}$.s $^{-1}$

[c] See Sections 2.3.4 and 2.4 for discussion.

Table 2. Clam tissue and algal parameters (values are mean ± s.d.) of juveniles reared under ambient light, 50% shade and 90% shade. WTW=wet tissue weight, SL=shell length, ZC=zooxanthellar cells

	LIGHT	50% SHADE	90% SHADE	CLAM SIZE (mm)
Chl. a/alga (μg chl.a.alga^{-1})	0.38 [a]	---	1.03 [b]	16.46 ± 2.10 n = 9
WTW/SL (mg.mm^{-1})	6.09 ± 0.99	---	3.45 ± 0.85	-ditto-
No. of ZC per gWTW (algae.gWTW^{-1})	35 ± 3.7 x 10^6	34 ± 6.3 x 10^6	31 ± 4 x 10^6	11.73 ± 1.74 n = 8

[a] Pooled algal sample from 5 clams.

[b] Pooled algal sample from 4 clams.

Fig. 9. Rates of gross photosynthesis per unit chl. a of intact clams. Pairs of curves show minimum and maximum P-I curves for clams reared under ambient light and 90% shade.

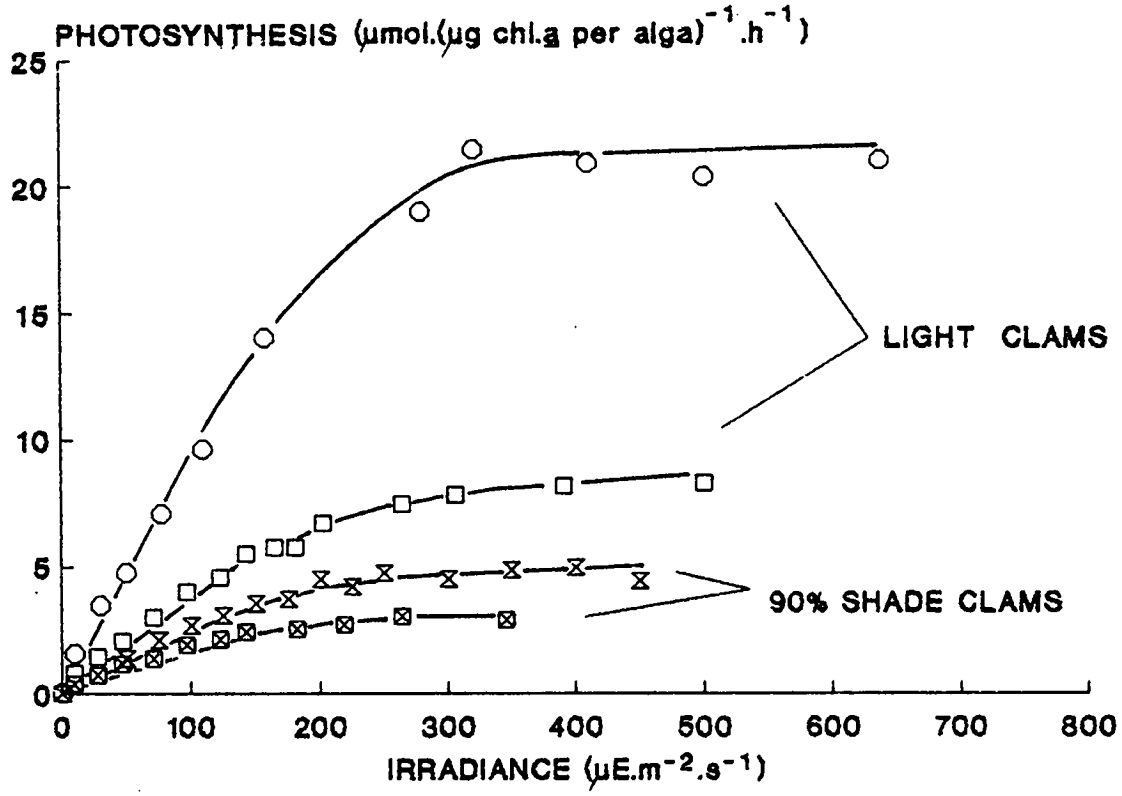


Table 3. Photosynthetic parameters from P-I curves for (A) intact clams reared under ambient light and 90% shade (see Fig. 9) and (B) pooled zooxanthellae isolated from these clams (see Fig. 11), both based on chl. a per alga. (C) Photosynthetic parameters from P-I curves of intact clams reared under ambient light and 90% shade (see Fig. 10), based on number of zooxanthellae per g clam WTW (see Table 2).

A. INTACT CLAM [a]

	LIGHT			90% SHADE		
	P_m	Ik [b]	α P_m/Ik	P_m	Ik [b]	α P_m/Ik
	8.44	194.56	0.043	4.86	161.14	0.030
	13.41	202.19	0.066	3.33	180.71	0.018
	9.54	196.86	0.048	2.94	127.34	0.023
	16.66	227.58	0.073	2.24	132.82	0.017
	21.61	207.79	0.104			
MEAN	13.93	205.80	0.067	3.34	150.50	0.022
S.D.	5.39	13.20	0.024	1.11	25.00	0.006

B. ISOLATED ZOOXANTHELLAE (POOLED SAMPLE) [c]

	6.15	80	0.077	2.4	80	0.03

C. INTACT CLAM [d, e]

	9.31	210.12	4.431	15.81	170.23	9.287
	14.22	261.01	5.448	10.40	192.78	5.395
	10.30	232.89	4.423	9,82	155.31	6.323
	18.01	248.99	7.233	7.35	144.89	5.073
	23.21	236.23	9,825			
MEAN	15.01	237.85	6.272	10.85	165.80	6.520
S.D.	5.73	19.08	2.293	3.56	20.78	1.920

[a] P_m in units of μmol.(μg chl.a per alga)$^{-1}$.h^{-1}

[b] In units of μE.m^{-2}.s^{-1}

[c] P_m in units of μmol.μg chl.a^{-1}.h^{-1}

[d] P_m in units of μmol.(alga per gWTW)$^{-1}$.h^{-1}, x 10^{-6}

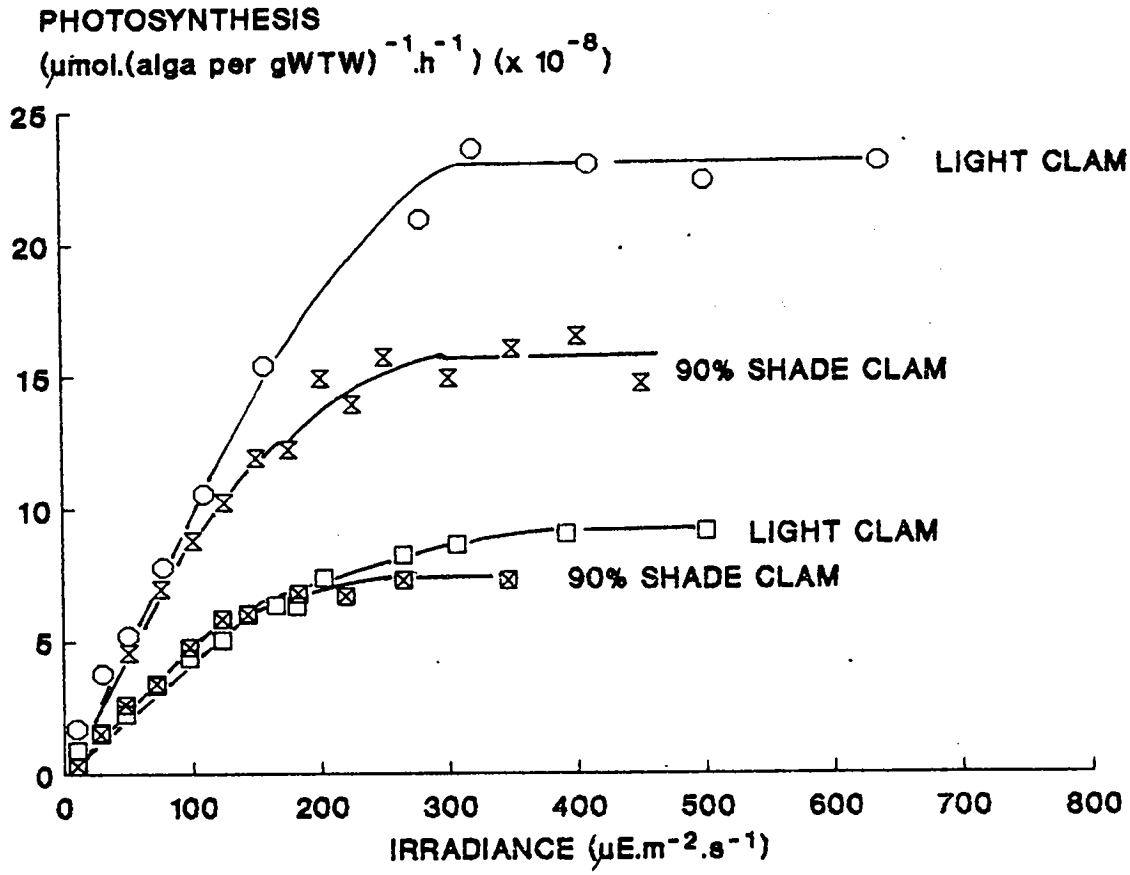
[e] α values x 10^{-10}

(Table 2) showed that 90% shade clams had a higher chlorophyll content per alga than light clams. In effect, rates of oxygen production standardized to a lower weight value would yield higher values for photosynthetic rates than when using a higher weight value, and vice-versa.

However, the gross photosynthesis rates shown in Fig. 9 were based on chlorophyll a per alga and not on total chlorophyll a per clam. In order to ascertain that the difference in photosynthesis rates of light and 90% shade clams resulted from the response of chlorophyll a to the light regime, gross photosynthesis rates must be based on total chlorophyll a, i.e. for the whole clam. This takes into account the number of zooxanthellae per clam. As mentioned in Section 2.2.5, zooxanthellae isolated from clams measured for oxygen flux (Sections 2.2.3 and 2.2.4) were not counted for each clam. Therefore, number of zooxanthellae per clam was determined using clams from another acclimatization experiment (Section 2.2.8). From this set of data, gross photosynthesis rates were thence based on number of zooxanthellae per g clam WTW (=alga per gWTW).

Maximum and minimum P-I curves for gross photosynthesis rates based on number of zooxanthellae per g clam WTW are presented in Fig. 10. Graphically, there is an overlap between these two sets of curves, indicating that differences in photosynthesis rates of light and 90% shade clams cannot be attributed to differences in number of zooxanthellae per clam. Furthermore, comparison of photosynthetic parameters (Table 3C) derived from Fig. 10 showed no difference in values of P_m (Two-sample t test, $t = 1.261$, $v = 7$, $P>0.20$) and alpha (Two-sample t test, $t = -0.172$, $v = 7$, $P>0.50$) for light and 90% shade clams, but a significant difference in Ik values (Two-sample t test, $t = 5.417$, $v = 7$, $P<0.001$). These results support the findings presented in Fig. 9, and confirm that the light clams contained higher chlorophyll a than the 90% shade clams.

Fig. 10. Rates of gross photosynthesis of intact clams, based on number of zooxanthellae per g clam WTW. Pairs of curves show minimum and maximum curves for clams reared under ambient light and 90% shade.



P-I curves generated from pooled zooxanthellae isolated from light and from 90% shade clams are shown in Fig. 11, with gross photosynthesis based on chlorophyll *a*. Zooxanthellae isolated from light clams had higher alpha and P_m than zooxanthellae isolated from 90% shade clams, but both have the same Ik (Fig. 11, Table 3B). Comparing the P-I curves of the intact clam association and their isolated zooxanthellae, P_m of zooxanthellae from light clams was lower than the lowest P_m values of intact clams; for zooxanthellae isolated from 90% shade clams, P_m value was close to the lowest value obtained from intact clams (Table 3A, B). Zooxanthellae isolated from light and 90% shade clams yielded maximum oxygen production (P_m) at a much lower Ik (80 $\mu E.m^{-2}.s^{-1}$) compared to *in vivo* measurements. Alpha for zooxanthellae isolated from light and from 90% shade clams did not vary greatly from values obtained from intact clams (Table 3A, B).

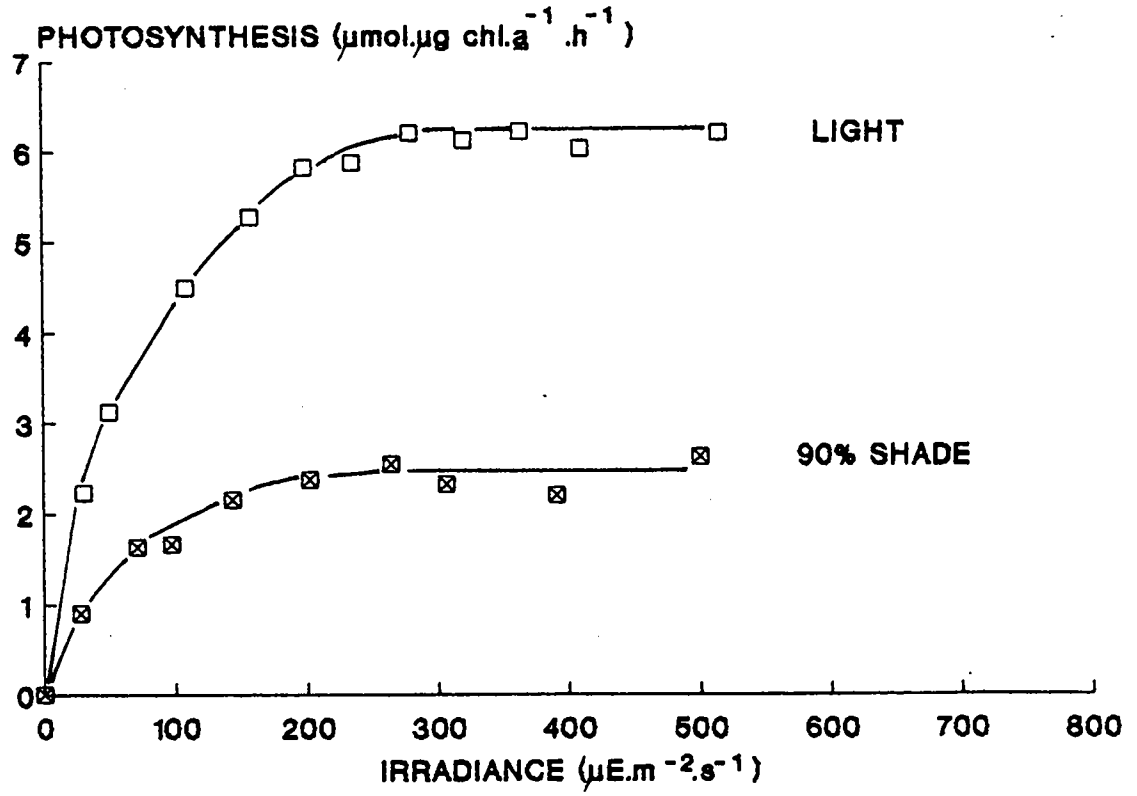
2.3.4. P:R ratios and CZAR

Ratios of net zooxanthellae photosynthesis to clam respiration over 24 hours (P:R) and the algal contribution of photosynthetically-fixed carbon to clam respiration (CZAR) were determined for each clam (Table 1) using the equation (Muscatine et al. 1981):

$$P/R = \frac{(P^O \times tday)(0.375PQ^{-1})-(1-\beta)(R^O \times 24)(0.375RQ)}{(\beta)(R^O \times 24)(0.375RQ)}$$

with the following assumptions: 1) the rates of daytime and nighttime respiration are equal; 2) respiration continues through a 24-hour period, 3) PQ is the photosynthetic quotient and is equal to 1.1; 4) RQ is the respiratory quotient and is equal to 0.8 (Muscatine et al. 1981); and 5) total respiration rate is composed of 5% algal respiration and 95% animal respiration (Trench et al. 1981). In the equation, P^O is gross oxygen production at saturating light intensity, tday is the period during which the clams were under saturating light intensity, R^O is the average respiration rate of the intact clam, $(1-\beta)$

Fig. 11. Rates of gross photosynthesis of isolated zooxanthellae based on chlorophyll a content per cell of juveniles reared in the light and under 90% shade.



is the algal contribution to total respiration, and B is the host contribution to total respiration.

Calculated P:R values varied significantly between light and 90% shade clams (mean $\pm$ s.d. = 2.87 $\pm$ 0.40, and 2.28 $\pm$ 0.33, respectively (Table 1)) (Two-Sample t test, t = 2.38, v = 7, $P<0.05$). All values were higher than 1.0, indicating the potential of photosynthetically-fixed carbon as a major source for the tridacnid host's metabolic carbon requirements.

CZAR was determined using derived estimates of the P:R ratio and a Translocation factor (Muscatine et al. 1981): CZAR = P/R x T%. The Translocation factor, T, refers to the percentage of photosynthetically fixed carbon that is released by the algae to the host. Griffiths and Streamer (1988) reported a T value of 32% from ^{14}C studies on Tridacna gigas juveniles (25-30 mm SL) that were cultured at OIRS, same as the clams used in the present study (see Section 2.4 for further discussion).

For comparison, CZAR was also calculated using a T value of 95%, as was done by Fisher et al. (1985). This T value was determined by Muscatine et al. (1984) for coral colonies of Stylophora pistillata using the so-called growth rate method, which does not use ^{14}C. The growth rate method assumes that, of the total carbon fixed by zooxanthellae per day, only a fraction is used for their growth and maintenance, and the rest translocated (Muscatine et al. 1984).

CZAR values (using T=32%) varied significantly (Two-Sample t test, t = 2.38, v = 7, $P<0.05$) with an average of 91.9% for light clams and 72.9% for 90% shade clams (Table 1, the clams used were about 1 cm SL). Thus, under high light acclimation, zooxanthellae produce and translocate more carbon products to the clam than under low light acclimation conditions.

Using a 95% Translocation factor, the average CZAR for light clams increased to about 273%, while that of shade clams increased to about 216%.

2.4. Discussion

The importance of light for algae-animal symbiotic relationships depends on the ability of the algae to adapt to short- or long-term changes in their photic environment, and on the ability of the animal to tolerate oscillations between relatively autotrophic and heterotrophic conditions.

Zooxanthellae densities in Tridacna gigas juveniles were dependent on irradiance, with densities increasing under a high light regime, and decreasing under low light. Similar observations have been reported for symbiotic corals (Redalje 1976).

Photoadaptation by zooxanthellae in T. gigas juveniles was manifested by an increase in algal chlorophyll a pigment concentration under low light intensities. Chlorophyll a concentration of zooxanthellae from 90% shade juveniles was more than twice that of light clams. Variations in the P-I curve parameters of 90% shade clams, based on chlorophyll a per alga (Table 3A) were lower alpha, P_m and Ik, similar to the results of Porter et al. (1984) for Stylophora pistillata. The implications of the variations in alpha, P_m and Ik are the following:

1) Although the rate of photosynthesis in clams acclimated to a low light regime (for convenience, referred to as "low light clams") is linearly related to light intensity at subsaturating light levels, their photosythetic rate at a specific light intensity is lower than those obtained from clams acclimated to a high light regime (for convenience, referred to as "high light clams"). This means that the photosynthetic efficiency of low light clams in subsaturating light intensities is lower than that of high light clams.

2) At saturating light intensity, low light clams also have lower maximum photosynthetic rates than high light clams, indicating a lower productivity for low light clams.

3) Low light clams reach maximum photosynthetic rates at a lower saturating light intensity than high light clams. This implies that the former requires lower light levels to attain maximum productivity.

Except for alpha, these trends were also similar to those obtained from Tridacna maxima zooxanthellae grown in culture at different light intensities (Chang et al. 1983). The disparity in alpha response between T. maxima and T. gigas zooxanthellae may be caused by: 1) differences between zooxanthellae strains (Chang et al. 1983); 2) difference in light acclimation histories; 3) other photoadaptive mechanisms not yet identified (Chang et al. 1983), or 4) unknown factors in the host which depress the light-harvesting capacity of chlorophyll a molecules in subsaturating irradiance.

Zooxanthellae freshly isolated from T. gigas had lower rates of maximum gross photosynthesis (P_m) than when in vivo, as similarly reported by Fisher et al. (1985). They enumerated the following possible causes: 1) physical damage to zooxanthellae during the isolation procedure resulting in partial inhibition of photosynthesis; 2) zooxanthellae may produce superoxide anions giving rise to hydrogen peroxide which cannot be detected by the oxygen electrode (Dykens and Shick 1982); and 3) other 'factors' in the host which could enhance photosynthesis in zooxanthellae (Deane and O'Brien 1980).

The calculated values of CZAR and their subsequent interpretation depend on the assumptions underlying the calculations, for instance, in the Translocation factor value. In a study on T. gigas, Griffiths and Streamer (1988) reported a T value of 32% which was used in the present study to calculate CZAR. For comparison, further CZAR calculations were made using the T value of 95%, which was determined by Muscatine et al. (1984) from the coral Stylophora pistillata, and used by Fisher et al. (1985) to calculate CZAR for T. gigas, in addition to using 40%, the T value reported by Trench et al. (1981)

for T. maxima.

For the present study, it seemed appropriate to use 32% for the Translocation factor for the following reasons:

1) Griffiths and Streamer (1988) have determined the Translocation factor (32%) using the same giant clam species, T. gigas, and the same cohort of clams as the ones used in the present study, both having been cultured at OIRS.

2) The T value of 32% has been estimated by Griffiths and Streamer (1988) using conventional ^{14}C labelling techniques, with the methodology much improved since the work of Muscatine (1967) on T. crocea, and Goreau et al. (1973) on T. maxima.

3) Studies that have quantified algal translocation in the tridacnid symbiosis are very few, and only Griffiths and Streamer (1988) have investigated this for T. gigas. Their Translocation value of 32% is close to 40% which has been reported by Trench et al. (1981) for T. maxima.

For the present study, regardless of the Translocation factor used, the average CZAR in shade clams was significantly lower than that in light clams. The effect of decreased CZAR was manifested in the lower condition index (wet tissue weight/shell length) of 90% shade clams, compared to light clams. The condition index of clams also decreased in the absence of light. These results affirm the dependence of clams on light. Although heterotrophic nutrition (plankton, and particulate matter and dissolved organic matter) by the host was not studied here, the lowering of condition in clams subjected to low light intensities suggests that a large part of the respiratory requirement of tridacnids must be derived from phototrophy, particularly for small T. gigas juveniles. A reduction in light intensity would reduce the amount of carbon fixed by zooxanthellae, but still be enough for maintenance, for some growth and for translocation. However, clam nutrition is complex in that there are

several ways by which tridacnids can obtain nourishment. There is a need to further holistically elucidate these mechanisms or paths, and to determine their importance relative to each other, as well as their significance relative to clam growth.

Although P:R ratios of 90% shade clams exceeded 1.0, these values were lower than those obtained from light clams, indicating potentially less carbon that is available for translocation to the host. This not unexpected effect of light on growth does not occur in all algal symbioses. In the case of the sea anemone, Aiptasia pulchella, final protein biomass was greatest for clones grown under the lowest experimental irradiance (45 $uE.m^{-2}.s^{-1}$), indicating that sea anemone growth cannot be directly related to zooxanthellar productivity (Muller-Parker 1985). Additionally, Muller-Parker (1985) showed that the relative contribution of zooxanthellae to host respiration depends not only on irradiance but also on the external food supply available to the host. This is because growth of zooxanthellae may also depend on growth rates of the host (Muller-Parker 1987).

Shell length (SL) growth of small Tridacna gigas clams (about 5 mm SL) was not affected by different light intensities. This finding does not conform with some previous observations for nonsymbiotic bivalves. For instance, Medcof and Kerswill (1965) reported that linear shell growth in Crassostrea virginica and Mytilus edulis increased by approximately 1.5 times when grown in reduced light. The results of the present study also differ from those of Lucas et al. (1989), who reported slow growth in large-sized T. gigas (about 40 mm SL) reared under 90% shade (about 10% increase in shell length after three months), in contrast to fast growth observed in clams of the same size under 50% shade and under ambient light (about 54% increase in shell length after three months). However, the effect of different light intensities on shell growth may not be seen in small-sized clams as those

used in the present study. The reason for this is that small clams saturate at low light intensities (Fisher et al. 1985), whereas large clams as those used by Lucas et al. (1989) require high light intensities to reach saturation to offset clam mantle shading of endosymbiotic zooxanthellae (Fisher et al. 1985). These results may also reflect the inadequacy of simple shell parameters like shell length as indicators for growth in T. gigas when light is a predominating influence.

Low light intensities modified shell growth in juvenile T. gigas, producing shells that were thinner and less opaque. This observation may be considered in terms of observations of nonsymbiotic bivalves and from other symbiotic algae-invertebrate symbioses. Generally, calcification in bivalves involves cellular processes of ion transport, protein synthesis and secretion, and also physicochemical processes resulting in the nucleation, orientation and growth of calcium carbonate crystals together with the secretion of an organic matrix (Clark 1974, Digby 1968, Wilbur and Saleuddin 1983).

It has been shown that exposure of nonsymbiotic bivalves to different levels of irradiance results in changes in the quality of shell deposited. Seed (1969) observed that when Mytilus edulis was kept in darkness, the shell was thinner compared to when exposed to light. For his studies on Modiolus modiolus, M. americanus and Mytilus edulis, Stromgren (1976b, 1976c, 1976d) proposed that biochemically active pigments present in the periostracum, which are capable of absorbing light energy, may influence certain physicochemical processes. The role of periostracal pigments in calcification in juvenile Tridacna gigas need to be investigated.

Similarly for symbiotic corals, the mechanism of calcification is not yet completely understood. The rate of calcification in symbiotic corals is higher in the light than in the dark (Goreau 1959, Goreau and Goreau 1959, Roth et al. 1982). Furthermore, the presence of

zooxanthellae enhances the rate of calcification in the light, calcification being directly related to algal photosynthesis (Vandermeulen et al. 1972). However, the role of zooxanthellae in coral skeleton formation has not yet been fully defined. Different mechanisms have been proposed to explain the role of zooxanthellae in coral calcification:

1) In carbon dioxide fixation. It is known that zooxanthellar fixation of carbon dioxide increases the carbonate ions in the polyp cells, thus raising the intracellular pH to become alkaline. The cellular pH is restored by the precipitation of excess carbonate ions as insoluble calcium carbonate (Goreau 1959, Goreau et al. 1979).

2) By removing phosphates that act as crystal poisons from sites of calcification (Simkiss 1964a, 1964b; see Chapter 5, Section 5.1.3).

3) Through their photosynthetic products (Pearse and Muscatine 1971). Zooxanthellae translocate to the coral host organic photosynthetic products such as glycolate, fumarate, glutamate and succinate (Trench 1971a, 1971b) which stimulate calcification (Crossland and Barnes 1974).

4) By increasing the amount of free energy for the active transport of calcium ions to the calcification site (Fang et al. 1989, Goreau et al. 1979, Pearse and Muscatine 1971).

5) By production of oxygen. Rinkevich and Loya (1984) found that in Stylophora pistillata, high oxygen production stimulates coral metabolism, which in turn is responsible for the higher rates of calcification.

The relevance of these mechanisms to calcification in tridacnids have not yet been studied. Studies dealing with the effect of high or low light regimes on calcification rates in symbiotic corals are few. From their studies on Pocillopora damicornis regarding calcification rates at saturating light intensities, Roth et al. (1982) found extremely high variability in the rate

of calcification at saturating light intensities. They proposed that, for P. damicornis, the calcification rates that they obtained at light saturation may be associated with the coral's light acclimation history. They based their hypothesis on investigations by Wethey and Porter (1976) on Pavona praetorta, exhibiting algal photoadaptation, which accounted for the photosynthetic maximum observed at light saturation.

These results are relevant to mariculture, in the selection of light regimes for optimum growth of giant clams. For example, in pilot studies of tridacnid juvenile rearing in landbased tanks, shade screens were used to control filamentous algae overgrowing the small juvenile clams (Lucas et al. 1989). This present study demonstrated the deleterious effects of heavy shading on the physiology of Tridacna gigas.

3. TEMPERATURE

3.1. Introduction

Tridacnids inhabit the warm tropical marine waters of the Indo-West Pacific region. Specifically, Tridacna gigas naturally ranged from the southern islands of Japan in the north, to New Caledonia in the south, and from the island of Sumatra, Indonesia, in the west, to Fiji in the east (Lucas 1988, Munro and Heslinga 1983). Latitudinal surveys on the distribution of T. gigas in the Great Barrier Reef show higher population densities further north, suggesting that the lower temperature of the southern Great Barrier Reef may adversely influence some phase or phases of the life-cycle of T. gigas (Braley 1987).

Tridacnids are reported to thrive in temperatures within the range of 26° to 31°C (Estacion and Braley 1988, Gomez and Belda 1988, Heslinga et al. 1984, Trinidad-Roa 1988). Clams subjected to fluctuating temperatures that go beyond the upper limit of this range display mantle bleaching which may lead to death, as observed with T. gigas about 40 mm SL in water temperatures reaching 35°C and above (Estacion and Braley 1988, Lucas et al. 1988).

The few studies dealing with temperature effects relate to clam survival upon aerial exposure. Lopez and Heslinga (1985) found that a temperature range of 24-28°C yields better survival rates for juvenile T. derasa (10-50 mm size); low temperature (11°C) produced high mortality. Temperature tolerance during emersion depends on clam size, as found with T. gigas. Juveniles 1-2 years old, having a small volume to surface area ratio suffer from heat mortality to a greater extent than large juveniles (more than 2 years old) (Nash 1988).

Heat mortality has also been reported for other tidally-exposed bivalve species. Potter and Hill (1982) reported a 23°C rise in the body temperature of Saccostrea (Crasostrea) commercialis after 90 minutes of solar exposure. Such physiological changes may have

debilitating consequences. They have suggested that successive exposures of oysters to high temperatures may reduce their heat tolerance, causing mass mortalities. Akberali and Trueman (1985) discussed the consequences of environmental conditions producing sublethal effects. Sublethal conditions may predispose organisms to low tolerance of other mortality-causing agents, for instance, predation and disease. In Pinctada maxima, high mortalities associated with sublethal low temperatures have been attributed to disease. At low temperature, animals lose vigor and succumb to high concentrations of bacteria (Pass et al. 1987).

It has been shown that growth in juvenile bivalves is temperature dependent (e.g. Crassostrea gigas, Brown and Hartwick 1988; Tridacna gigas, Estacion and Braley 1988; Argopecten irradians, Kirby-Smith and Barber 1974). Temperatures about 30°C have been recommended for the rearing of juvenile T. gigas (Lucas et al. 1988). Growth studies on Hippopus hippopus show enhanced growth rates at temperatures between 27 and 34°C (Alcala et al. 1986). Generally, the effect of temperature is on metabolic rate, which increases with increase in temperature. However, metabolic rate declines at high temperatures, apparently a result of the inhibiting effect of high temperatures on the activity of proteins, particularly enzymes.

Generally, in bivalved molluscs, as in ectotherms/poikilotherms, physiological functions and activity level (or physical activity) increase with elevation in temperature (see Kinne 1963, Newell and Branch 1980). The physiological rates, often used as a measure of metabolic rate, typically approximately double for every 10°C increase in temperature, so that their temperature quotient (Q_{10}) is about 2.

Respiration rate with temperature has not been studied in tridacnids. The aims of this study were, therefore, to: 1) estimate the upper range of temperature tolerance of juvenile T. gigas, and 2) determine the relationship between respiration rate and temperature.

3.2. Materials and Methods

3.2.1. Upper temperature tolerance

Experiments were conducted at Orpheus Island Research Station in December 1986. Tridacna gigas juveniles of 15-25 mm shell length (SL) were subjected to high temperature stress by direct transfer method. The temperature treatments were 28, 29, 33 and 37°C, with the control at 26°C (ambient temperature). Temperature level was maintained by aquarium heaters (± 1°C), and measured with a laboratory thermometer (± 0.05°C). Twenty clams were used per treatment. They were placed in 8 l glass aquaria filled with aerated 10 um filtered seawater at a constant flowrate of 0.5 $l.h^{-1}$. All tanks were under 50% shaded natural daylight. Light levels, measured at 12 noon with a Licor light meter and a submersible sensor, averaged 1581 $uE.m^{-2}.s^{-1}$ (± 534 $uE.m^{-2}.s^{-1}$). Temperature tolerance experiments were conducted in the summer month of December 1986.

Clams were checked for mortalities every 12 hours. Criteria for death were wide gaping, retracted mantle and lack of response to gentle prodding. Dead animals were collected at each observation. The experiment was terminated after 5 days.

3.2.2. Determination of respiration rate/temperature relationship

This experiment was conducted at James Cook University between October and November 1989. Rates of respiration of juveniles (90-105 mm SL) were measured at different temperature. A clam was placed in a respiration chamber (737.1 ml chamber volume) filled with 0.45 um FSW. Other details of the experimental set-up and methodology were as described in Chapter 2, Section 2.2.4. For ease of measurement of respiration rate, temperature was modified in one direction, either towards increasing or decreasing levels. Water temperatures below 25°C were regulated by a combination of heating and cooling coils

immersed in the recirculating water bath. Seawater within the chamber was replaced with fresh, temperature-equilibrated FSW prior to each measurement. Measurements lasted 30 minutes on average. Behavior was also recorded. Experiments were conducted between 7 a.m. and 7 p.m.

The influence of temperature on respiration rate was quantified by calculating the temperature quotient (Q_{10}) according to the equation (Eckert 1988):

$$Q_{10} = (n_2/n_1)^{10/(t_2 - t_1)}$$

where n_1 and n_2 are respiration rates at temperatures t_1 and t_2, respectively.

3.3. Results

3.3.1. Upper temperature tolerance

The survival of Tridacna gigas at various temperatures is shown in Fig. 1. No mortality occurred at 26, 28 and 29°C. In contrast, no clams survived for a day at 37°C. At 33°C, 100% mortality occurred in less than 3 days after clam transfer.

The median survival time (MT50), or the level of response time beyond which 50% of the experimental sample cannot live for an indefinite time (see Sprague 1969), was 0.27 (or 6.5 h) and 2.33 days at 37°C and 33°C, respectively.

3.3.2. Respiration rate and behavior

Clam respiration rates are plotted against temperature in Fig. 2. Respiration rates increased with increase in temperature from 11.7°C to 32.9°C and then declined.

Q_{10} values derived from Fig. 2A are presented in Table 1A. There was an abrupt change in Q_{10} values about 19°C. Between 20° and 33°C, Q_{10} values were increasing, ranging between 1.37 and 3.07. Above 33°C, there was a drop in Q_{10} values to 0.67 and 1.09. To obtain average values of Q_{10} below and above 19°C, and above 33°C, respiration rates were regressed against temperature (Fig. 2B, and regression equations calculated (Zar 1984) and shown in Table 2B. Below 19°C, the average Q_{10} value is less than 1.0, while above 19°C, this value lies between 1.0 and 2.0. Greater than 33°C, average Q_{10} value is close to 1.0 (Table 2B).

The behavior of clams changed with temperature. Clams tended to retract the mantle and shell gaping was somewhat restricted at low temperatures, particularly below 22°C. Additionally, the opening of the inhalant siphon was slight, while the exhalant siphon aperture was more often constricted than dilated. In contrast, at high temperatures, the mantle was fully expanded with valves

gaping widely. At temperatures above 33°C, the mantle, while still fully expanded, became flaccid and began to droop over the shell edge. Inhalant and exhalant siphons were both open, with the sizes of the apertures increasing at higher temperatures.

Fig. 1. Survival of juvenile Tridacna gigas at 5 temperatures. Temperature (°C) is indicated beside the curves. There were 20 clams per treatment.

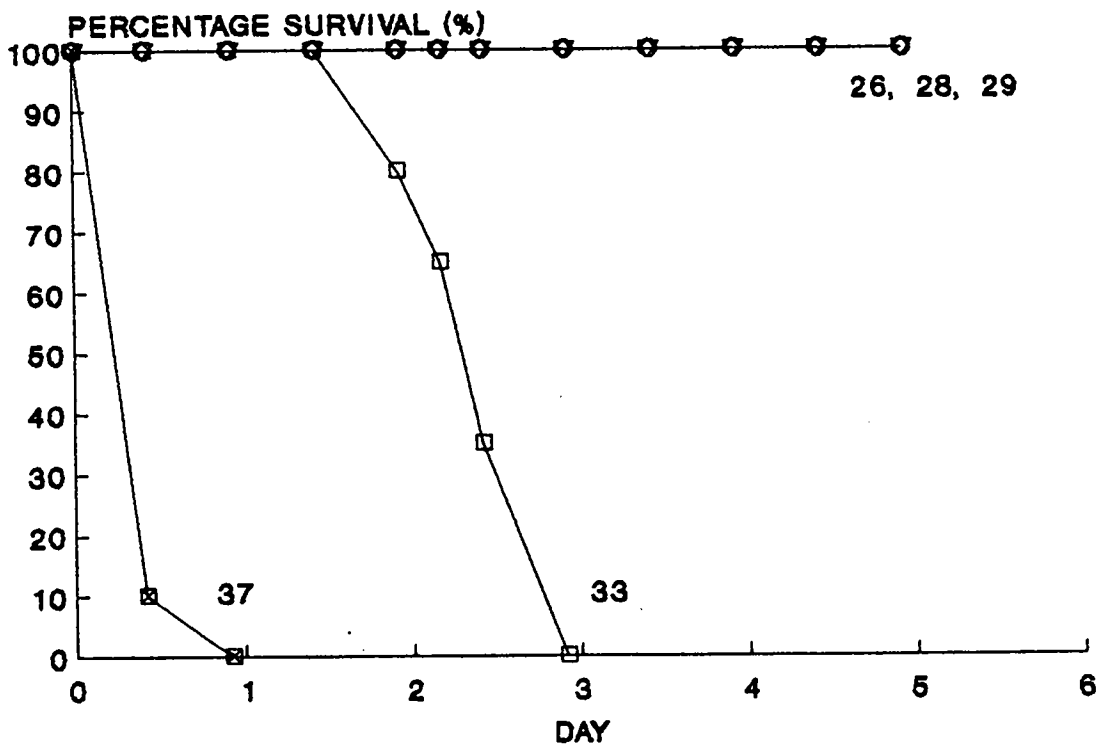
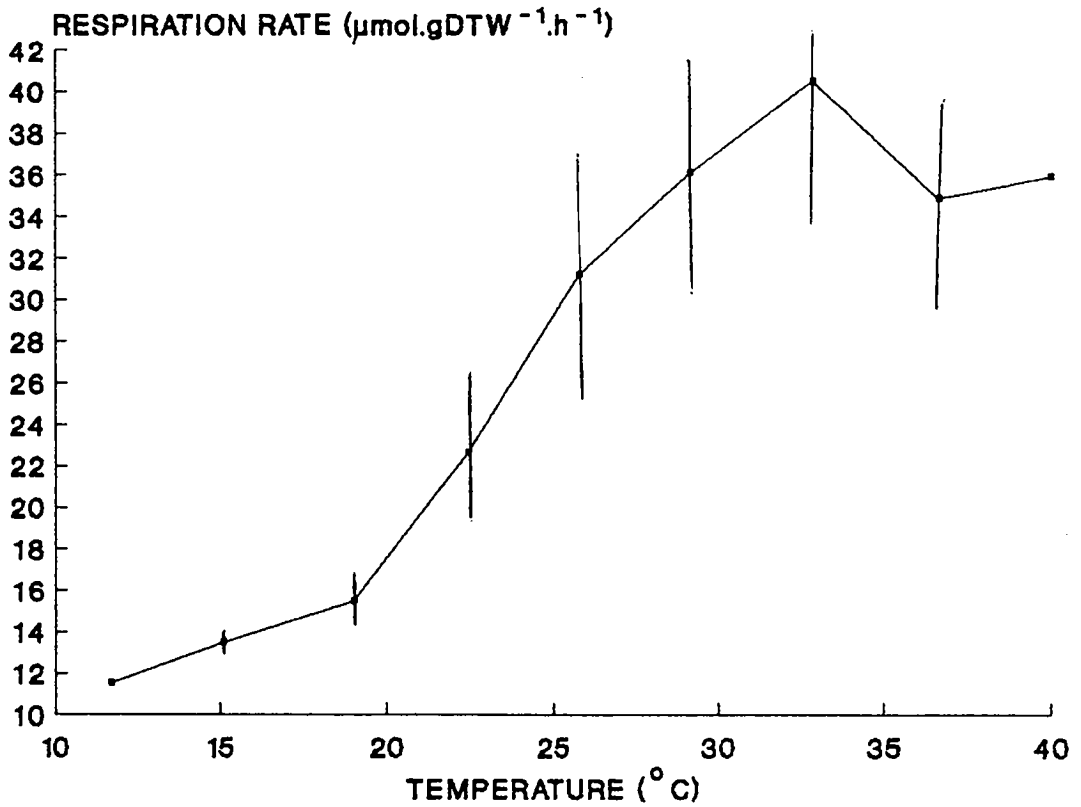


Fig. 2A. Effect of temperature on mean respiration rate of juvenile Tridacna gigas. (n=7; vertical bar=s.d., no s.d.= single value)

Fig. 2B. Effect of temperature on respiration rates of juvenile Tridacna gigas. Respiration rate was regressed against temperature for temperatures below and above 19° C, and above 33° C. See Table 1 for regression equations and regression analyses.


RESPIRATION RATE (µmol.gDTW⁻¹.h⁻¹)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
10
15
20
25
30
35
40
TEMPERATURE (° C)

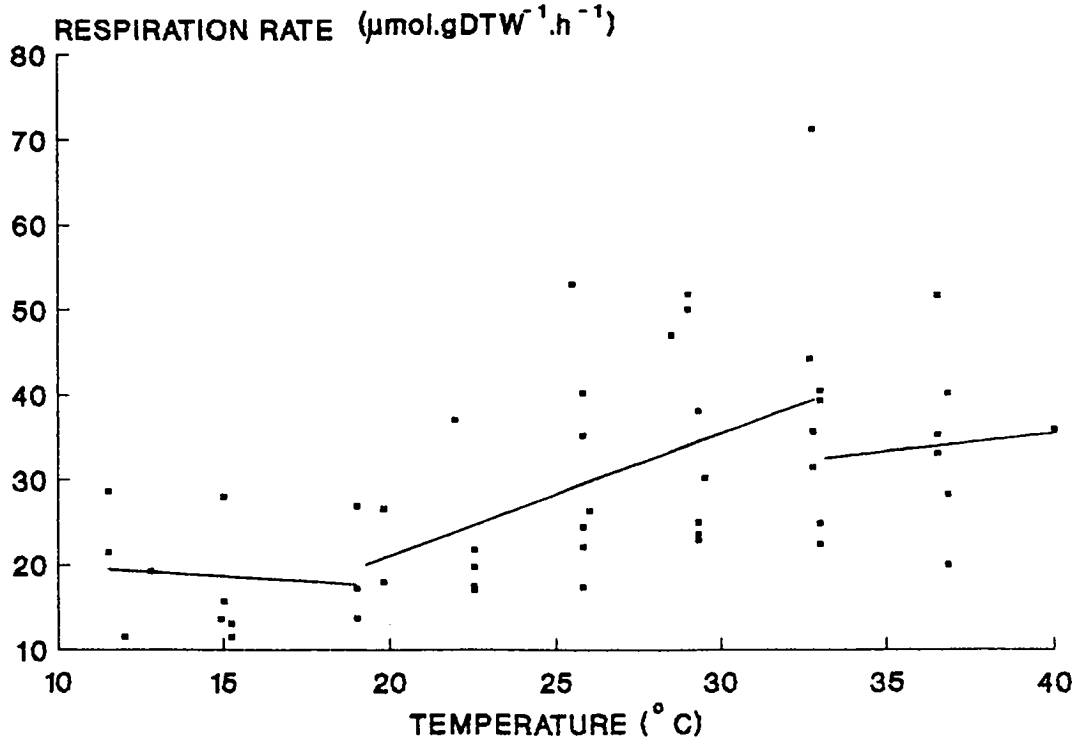

RESPIRATION RATE (µmol.gDTW⁻¹.h⁻¹)
80
70
60
50
40
30
20
10
10
15
20
25
30
35
40
TEMPERATURE (° C)

Table 1A. Q_{10} values for mean respiration rates of juvenile Tridacna gigas at specific temperature ranges (see Fig. 2A).

Table 1B. Regression equations and Q_{10} values for temperatures below and above 19 °C, and above 33 ° C. (P = probability) (See Fig. 2B)

TEMPERATURE RANGE (°C)	Q_{10}
11.7-15.1	1.60
15.1-19.0	1.42
19.0-22.4	3.07
22.4-25.8	2.57
25.8-29.2	1.54
29.2-32.9	1.37
32.9-36.7	0.67
36.7-40.0	1.09

TEMPERATURE RANGE (°C)	REGRESSION EQUATION	r^2	Q_{10}
11.7 - 19.0	y = 21.60 - 0.22x	0.01	0.89
19.0 - 32.8	y = -6.02 + 1.39x	0.25	1.61
32.8 - 40.0	y = 12.70 + 0.59x	0.02	1.19

3.4. Discussion

The results on upper thermal tolerance of juvenile Tridacna gigas, i.e. high mortality at temperatures 33°C and greater, agree with field studies by Estacion and Braley (1988) showing increasing rates of mortality as water temperatures increased during summer (reaching a maximum of 35°C).

Before discussion of temperature effects on respiration rate, it must be noted that values for respiration rate obtained in this study (specifically between 25° and 30°C) (see Fig. 2) were higher than those obtained from clams about the same size at 28°C (see Chapter 6, Fig. 5, specifically pre-exposure respiration rates). This may be attributed to different temperature acclimation histories between clams used here and those used in respiration measurements in Chapter 6. Temperature experiments were conducted in October to November 1989, hence high values for respiration obtained here may reflect the effect of summer acclimation on clam respiration rate. On the other hand, respiration rates of clams used for respiration experiments in Chapter 6 reflect the effect of winter acclimation on respiration rate of those clams, as rate measurements were conducted during winter, June to July 1989.

Temperature effects on respiration rates of juvenile T. gigas (about 90 mm SL) did not vary from the general trends obtained from other studies in the past. Molluscan respiration rate varies directly with temperature within limits of the tolerance range (e.g. Ostrea edulis, Buxton et al. 1981; Haliotis tuberculata, Gaty and Wilson 1986; Mulinia lateralis, Shumway 1983). In T. gigas, although the relationship between respiration rate and temperature was depressed between 12° and 19°C, then increased with rise in temperature up to 33°C. Above 33°C, respiration rates declined, with Q_{10} approaching 1.0.

The abrupt change in Q_{10} values below 19°C and the reduction in oxygen consumption at greater than 33°C are indicative of adverse and sublethal temperatures affecting clam metabolic rates. Continual exposure to sublethal temperatures does not necessarily kill giant clam juveniles, but may interfere with some of the their metabolic processes. Sublethal temperatures may weaken the clam's resistance to disease, as has been observed by Pass et al. (1987) on other bivalves, specifically Pinctada maxima. The immediate transfer of tridacnid clams to water temperatures to which they are not accustomed may kill them, although in nature this does not necessarily occur. However, for mariculture studies, it is important to know how survival rates are affected by immediate water temperature changes since transfer of clams from one mariculture facility to another requires rapid temperature acclimatization.

These results provide the physiological basis for previous findings on growth related to temperature. Lucas et al. (1988) reported that relative growth rate (based on shell length) is dependent on temperature (up to 32°C), with growth virtually ceasing at 19°C. Nash (1988) also observed that the increase in relative growth rates in Tridacna gigas is associated with an increase in water temperature (up to 30°C). As temperature dropped (down to about 23°C), relative growth rates also declined.

4. SALINITY

4.1. Introduction

Most studies on salinity effects on bivalves have focused on commercially important species, e.g. on cockles (Cerastoderma), oysters (Crassostrea), mussels (Modiolus, Mytilus), soft clams (Mya) and scallops (Pecten, Pinctada) (Funakoshi et al. 1988, Nell and Holliday 1988, Nossier 1986, Pierce 1970, Shumway 1977). The need to know the salinity requirements of such bivalves is obvious. Most mariculture operations, especially those utilizing nearshore coastal areas, may involve movement of bivalves (either as spat, seed or adult) from one locality to another for holding or rearing. Salinities at these localities may not be similar and salinities at a locality may vary seasonally. It is, therefore, important the physiological capacity of the maricultured species be known.

Information on salinity effects related to tridacnids in general and Tridacna gigas in particular is scarce. From their distribution in nature, tridacnids are generally believed to be stenohaline. However, some tridacnids may also be found, although not so frequently, in waters deviating from 34 to 35 ppt salinities. Giant clams like T. crocea and T. maxima are also found in intertidal pools where tidal exposure causes evaporation of seawater, thus increasing salinity, simultaneous with increases in water temperatures. In nature, their tolerance for salinity changes may not be narrow, depending on their acclimation history.

Giant clam mariculture may involve abrupt salinity changes, as would be the case when clams are transferred between mariculture facilities. Furthermore, knowing the range of tolerance of clam juveniles for abrupt salinity changes opens up another aspect of tridacnid mariculture that can later be developed. For example, culture facilities or clam farms may develop a type of pond culture that veers away from conventional coastal areas

that have continuous access to seawater (see Estacion and Braley 1988). Hence the effects of salinity on survival of clam juveniles must be studied.

Even if clams have acclimatized to the new environmental salinity, changes in clam physiology need to be studied, in order to assess their physiological capability, for instance, for osmotic adaptation and regulation, to (1) adjust to such a change, (2) maintain normal metabolic functions and (3) still allow for growth.

For this literature review, for lack of research on salinity effects done on tridacnids, studies on other bivalves have been used. Responses, in terms of growth (long-term response), and behavior and physiology (short-term response) to change in external salinity are considered.

4.1.1. Growth and survival

It has been well demonstrated that bivalve growth is influenced by environmental salinity. For instance, Cerastoderma edule transplanted to sites defined by a salinity gradient show increasing growth with increasing salinity (Vrins 1978, cited in Essink and Bos 1985). While bivalves living in saline waters characterized by some freshwater input, or those that experience freshwater inundation, show reduced growth (Eisma et al. 1976, Essink and Bos 1985). There exists a critical salinity level for Mytilus edulis, below which shell growth becomes negligible (Bohle 1972, Gruffydd et al. 1984). Additionally, the maximum age of shells decreased withsalinity, particularly for Cardium edule (Eisma 1965) and Macoma balthica (van der Bij 1973, cited in Eisma et al. 1976).

Reduction or cessation in growth may be attributed to the effect of sub-optimal salinity on important metabolic functions. For instance, Matthiessen (1960) observed that in Mya arenaria, pumping rate (hence feeding) is sharply depressed at low salinities. With a further decrease in salinity, pumping activity ceases (Matthiessen 1960).

Studies by Vernberg et al. (1963) on isolated gills of Aequipecten irradians, Modiolus demissus and Crassostrea virginica further show that ctenidial cilia stop beating at reduced salinity, which may explain the cessation of pumping observed by Matthiessen (1960) in Mya arenaria. Therefore, unless the bivalve is behaviorally and physiologically capable of coping with salinity stress, severe changes in salinity (as from heavy rainfall) may detrimentally affect its capacity for growth and survival (Cerastoderma edule, Essink and Bos 1985, Gemma gemma, Weinberg 1985).

4.1.2. Bivalve body fluids during osmotic equilibrium

Marine bivalves are generally osmoconfomers. Some bivalves show osmoconformity down to a certain salinity level, below which they show hyperregulation of blood osmotic concentrations (e.g. Rangia cuneata, Bedford and Anderson 1972, Otto and Pierce 1981b). The extracellular fluid (ECF) of most osmoconforming bivalves is usually isosmotic with the external seawater (e.g. Mytilus edulis, Davenport 1979), although slight hyperosmoticity has also been observed (e.g. Noetia ponderosa, Amende and Pierce 1980). Ionic concentrations of the ECF, however, may be different from the levels present in seawater. For instance, concentrations of potassium, calcium and inorganic phosphates in the hemolymph of Tridacna maxima were higher than those of seawater, while concentrations of sodium, chloride and sulfate were lower (Deane and O'Brien 1980).

Hyperosmotic ECF has been attributed to different causes. Studies on four species of Modiolus suggest that passive equilibrium, rather than active regulation, is responsible for such hyperosmoticity (Pierce 1970). Permeability of body surfaces, therefore, determines the solute composition and concentration of the ECF, particularly inorganic ions (Shumway 1977). The Donnan equilibrium may also be in effect, by the presence of non-diffusing anionic proteins in the ECF contributing to the

ECF ionic composition. This condition leads to an unequal distribution of bound ions between the ECF and the environment. On the other hand, the presence of certain inorganic ions is also known to influence permeability of surface membranes (Anderson and Bedford 1973). In addition, Burton (1983) stated that the hyperosmoticity observed in some bivalves may sometimes reflect an increased solute concentration in the gut during digestion.

The intracellular fluid (ICF) is usually isosmotic with the ECF. The active regulation of osmotic concentrations of the ICF is called isosmotic intracellular regulation, which is important in the regulation of cellular volume (Florkin and Schoffeniels (1969). Although intracelullar ions have a role in volume regulation (Otto and Pierce 1981a, Willmer 1978), volume regulation mostly relies on varying the concentration of organic solutes such as free amino acids (FAA) (Otto and Pierce 1981b).

4.1.3. Behavioral and physiological responses of bivalves to external osmotic change

An intertidal bivalve exposed to a change in external salinity usually displays initial behavioral responses, like shell closure (e.g. Mytilus edulis, Funakoshi et al. 1988, Shumway 1977), or siphonal withdrawal or constriction (e.g. Mya arenaria, Shumway 1977). Shell closure serves to defer equilibration of the water in their mantle cavity, and hence ECF and ICF, with the external environment. ICF equilibration requires energy, and is viewed more as a response to long-term salinity changes than to short-term changes such as when change in salinity tends to be cyclical (Shumway 1977) (e.g. caused by fluctuation of tides and freshwater run-off from land). There are some consequences of such behavioral responses; for instance, shell closure results in hypoxia, often leading to anoxia and thence the accumulation of anaerobic metabolic end-products (see Chapter 6, Section 6.1.2 for

discussion).

Little is known about osmotic adaptation in tridacnids. Generally, the response of osmoconforming marine bivalves to salinity stress is the alteration of extracellular osmotic concentrations by movements of osmotically obligated water and salts across body surfaces (Funakoshi et al 1988). For the present study, only total osmotic concentrations, rather than specific changes in ion concentrations, were investigated.

A change in extracellular osmotic concentrations incites a change in osmotic concentrations within the cell. Thus, in response to changes in the ECF, the size of the FAA pool within the cell decreases when salinity is lowered, and increases when salinity is elevated. This, together with further osmotic movement of cellular water and regulation of intracellular inorganic ions, leads to the regulation of cell volume in bivalves when under osmotic stress (Pierce 1982).

The regulation of intracellular FAA concentrations is dependent on FAA metabolism (e.g. Matsushima et al. 1987). Several mechanisms have been implicated or proposed to account for changes in their FAA concentrations. A bivalve exposed to reduced salinities loses some of its intracellular FAA by:

1) efflux to the hemolymph (three species of Modiolus, Pierce and Greenberg 1972) where FAA are then catabolized, thus producing ammonia (Modiolus demissus, Bartberger and Pierce 1976; Crassostrea virginica, Heavers and Hammen 1985; Mercenaria mercenaria, Rice and Stephens 1988a); and

2) incorporation into macromolecules, probably proteins (M. mercenaria, Rice and Stephens 1988a).

On the other hand, bivalves subjected to elevated salinities gain additional FAA by:

1) Protein catabolism. Studies by Baginski (1978) on Modiolus demissus suggest that certain FAA may directly arise from cellular protein. This contradicts an earlier

study on M. d. demissus (Baginski and Pierce 1975) showing a definite time course for accumulation of certain amino acids. However, unless unique proteins are involved, protein catabolism should give rise to the simultaneous accumulation of a variety of amino acids (Baginski and Pierce 1975). There is still no strong evidence to support protein depolymerization as a pathway in volume regulation. So far, it has been shown that FAA, particularly proline and alanine, can be derived from glucogenic precursor molecules through transaminase-linked pathways (Greenwalt and Bishop 1980).

2) Anaerobic metabolism. Metabolic shifts from the tricarboxylic acid (TCA) cycle at the level of phosphoenolpyruvate may occur to give rise to alanine (M. demissus demissus, Baginski and Pierce 1975).

3) Action of the enzyme glutamate dehydrogenase (GDH). The GDH pathway related to salinity stress has mainly been described in crustaceans (e.g. Gilles 1974). Although GDH has been detected in bivalves (Mytilus edulis, Addink and Veenhof 1975; Corbicula japonica, Matsushima and Kado 1983; Modiolus demissus, Reiss et al. 1977), its role in osmotic adjustment has not yet been established (Bishop et al. 1983).

4) Amino acid intake from seawater and from food. Bivalves can absorb amino acids from their surroundings (via the gills and epithelial tissue lining of the mantle cavity) even against a concentration gradient (Cerastoderma edule, Bamford and McCrea 1975; Mytilus edulis, Manahan et al. 1982; Crassostrea gigas, Rice and Stephens 1987). The high concentration gradients between intra- and extracellular and environmental FAA (Rice and Stephens 1988b) require carrier-mediated transport systems capable of "uphill" transport (Bamford and McCrea 1975, but see Bedford 1971).

In addition to cellular control over FAA regulation, the ECF, by regulating the concentrations of divalent

cations such as Ca^{2+} and Mg^{2+}, may exert control over cellular membrane permeability. For instance, in Rangia cuneata, the concentrations of these ionic species have to be maintained at certain levels or else FAA efflux increases dramatically and organic solutes will not accumulate intracellularly despite their synthesis (Otto and Pierce 1981a).

It is important to note that the nutritional status of a bivalve may influence its capacity for osmotic adaptation through, for instance, capacity for regulating FAA pool concentrations. Riley (1980) found that starved C. gigas utilize their carbohydrate resources to depletion. Protein is then catabolized, during which a decrease in protein-bound FAA (from whole body hydrolysates) is observed.

This effect could be important for tridacnids where zooxanthellar photosynthates (which include glucose, glutamate, alanine, aspartate and serine) comprise a significant portion of giant clam nutrition (Griffiths and Streamer 1988). Furthermore, it is generally known that salinity influences the rate of photosynthesis. To illustrate, photosynthesis and dark respiration are inhibited in continuous cultures of the marine diatom Skeletonema costatum exposed to low salinity, leading to reduced net carbon fixation and cell growth. Amino acid pools decrease while ammonium accumulates from osmoregulatory processes. Normal cellular functions in S. costatum resume upon exposure to increased salinity (Rijstenbil et al. 1989).

4.1.4. Aims of the study

The aims of this study were to determine in juvenile Tridacna gigas:

1) salinity tolerance;
2) the salinity levels optimum for growth;
3) variations in ECF osmolality with changes in salinity;
4) variations in concentrations of the intracellular free amino acid (FAA) pool at different salinities.

4.2. Materials and Methods

4.2.1. Survival at different salinities

Tolerance experiments were conducted at the Orpheus Island Research Station. All experiments were conducted under natural daylight. Juvenile Tridacna gigas (10-15 mm SL) from ambient salinity ca. 34 ppt were exposed to salinities ranging from 0 to 45 ppt. Ambient salinity was 36 ppt. Twenty clams were placed in an aquarium containing 9 l of 1 um filtered seawater (FSW) adjusted to the test salinity. Low salinities were achieved by dilution of seawater with rainwater, while high salinities were achieved by addition of artificial salts (Instant Ocean brand). Adequate aeration was provided to all aquaria, with daily seawater changes. All aquaria were placed in a large bath of running water to minimize water temperature fluctuations. Water temperature was monitored daily with a minimum-maximum thermometer.

Mortalities were recorded after 3, 6, 9, 12 and 24 hours of exposure to the test salinity. The frequency of subsequent observations was based on the clams' responses. The criteria for death were extensive shell-gaping, retracted mantle and lack of irritability (shell closure, mantle and foot retraction) to gentle prodding. The experiment was terminated after seven days.

4.2.2. Osmotic adaptation to salinity changes

The osmolar concentrations of the mantle cavity fluid and extracellular fluids (ECF) of T. gigas juveniles were investigated at different salinities. Experiments were conducted using the recirculating seawater system at James Cook University. Groups of eight tagged clams (50-80 mm SL) were each placed in an aquarium containing 18 l of 1-um FSW of the test salinity for six weeks. 50+ mm SL juveniles were used for ease of extraction. The experimental salinities were 23, 27, 30, 35, 39 and 42 ppt. The clams were acclimatized to the test salinity by adjusting the ambient salinity appoximately 3 ppt every third day towards the desired level. Salinities were

adjusted as in Section 4.2.1. All aquaria were adequately aerated, with water changes every third day. The entire set-up was maintained under natural daylight in a large bath of running seawater.

After six weeks, the mantle cavity fluid and ECF were extracted from each clam by hypodermic suction. Cavity fluid was obtained through the byssal orifice, while ECF was sucked from the middle mantle fold. Several attempts were required to obtain an ECF sample of more than 1 ml because extraction was often impeded by valve closure. Gentle pressure on the inner mantle lobe lining the byssal orifice induced the clam to relax its posterior adductor muscle, thereby causing the valves to gape. The valves were then obstructed to maintain the gape. Seawater samples were also obtained from each test salinity.

Each sample was repeatedly centrifuged at 3500 rpm for 2 minutes until the supernatant was clear. The supernatant was stored in screw-cap glass vials at 4^{o}C, and analyzed within 24 hours. Three replicates per sample were analyzed for osmolar concentrations using a freezing point depression osmometer (Osmomat model).

4.2.3. Free amino acid pool in osmotic regulation

Adductor muscles of clams used in Section 4.2.2. were dissected for free amino acid (FAA) extraction. A modified method of Shibko et al. (1967) for FAA extraction (P. C. Southgate, pers. comm.) was employed. 100 mg of the adductor muscle was weighed and homogenized in 3 ml distilled water for 5 minutes in an ice bath. 300 ul of cold perchloric acetic acid was added, and the sample allowed to stand for 15 minutes at 0^{o}C. The sample was then repeatedly centrifuged at 10,000 rpm until the supernatant was clear. The supernatant was transferred to a vial and neutralized with KOH solution (T. Piva, pers. comm.). FAA concentrations were determined by High Performance Liquid Chromatography (HPLC). Samples awaiting analysis were stored at -20^{o}C. HPLC analysis was

conducted at the Biochemistry Laboratory, Department of Chemistry and Biochemistry, James Cook University).

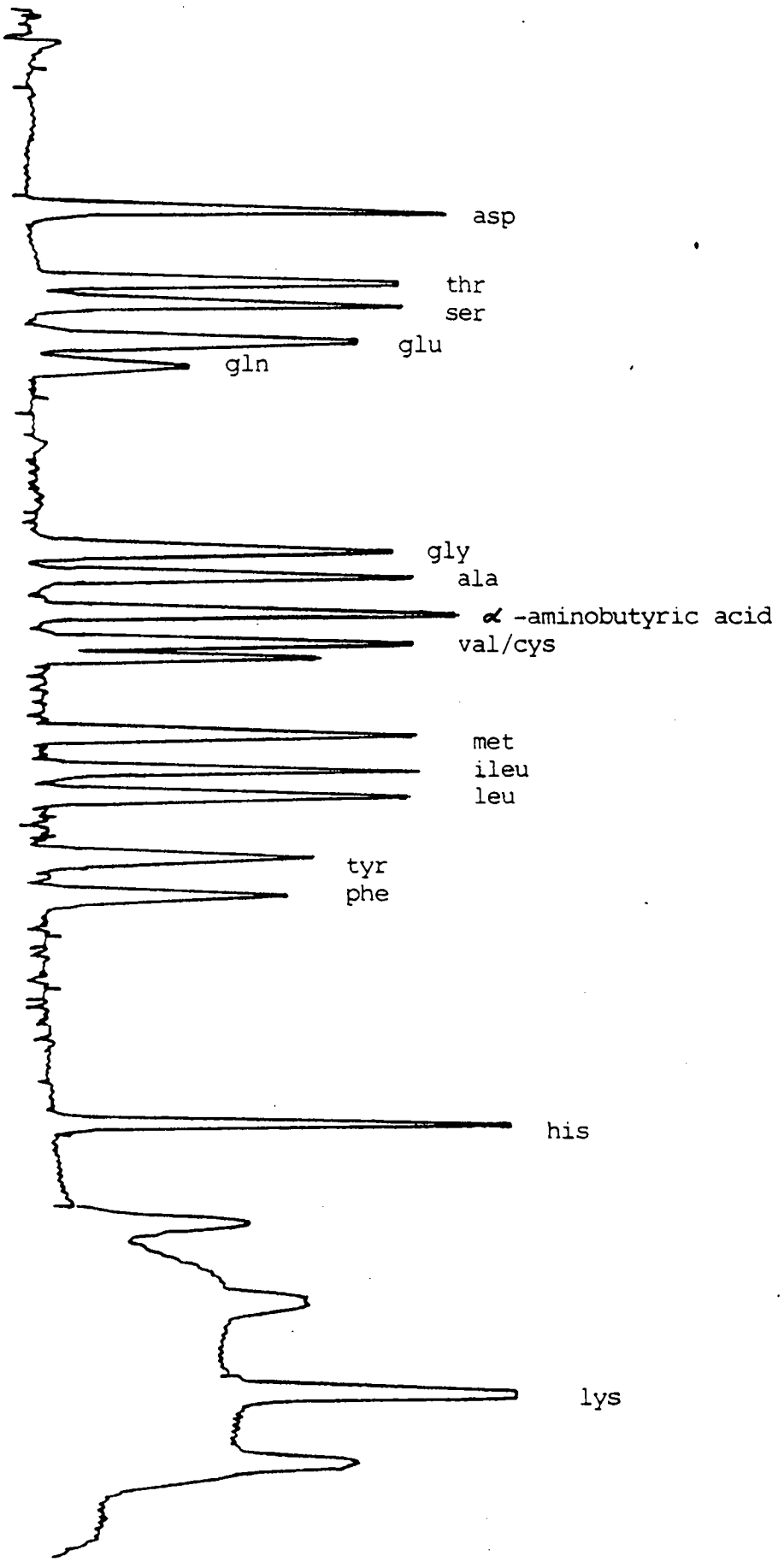
There were some problems in homogenizing adductor muscle tissue. A very small portion of the sample could not be recovered from the homogenizing bit of the tissue grinder (Ultra-Turrax T25 model). Thus, total FAA concentration for each sample could not be determined. FAA analysis was, therefore, based on relative proportions of different amino acids per test salinity.

The FAA were grouped into "essential" and "non-essential" amino acids based on the few available surveys on FAA present in marine molluscs. As with other metazoans, most bivalves require a dietary intake of essential amino acids (including arginine, histidine, lysine, threonine, phenylalanine, tryptophan, methionine, valine, leucine and isoleucine) (Allen and Kilgore 1975, Bishop et al. 1983, Harrison 1975, Potts 1958). Non-essential amino acids are those not derived from the diet, but synthesized by the organism. The HPLC standard calibration curve plotted in Fig. 1 shows the FAA analyzed in this study. This standard calibration does not have reference amino acids for some FAA also occurring in other bivalves, like taurine, proline, arginine (Allen 1961, Peterson and Duerr 1969, Pierce 1971), hence, these FAA were not included in this study.

4.2.4. Growth rates

Growth rates at different salinities were also determined for the experimental clams used in Section 4.2.2. Initial and final shell lengths were measured with a vernier caliper (± 0.02 mm). Clams were then drained of their mantle cavity water by inverting them for 15 minutes. Initial and final total wet weights were determined (± 0.01 g). Water bath temperatures ranged between 28° and 31°C.

Fig. 1. Standard calibration curve (using α -aminobutyric acid as internal standard) for HPLC analysis of free amino acids from adductor muscle homogenate obtained from juvenile Tridacna gigas. Some peaks could not be positively identified, hence were not labelled.


asp
thr
ser
glu
gln
gly
ala
∝ -aminobutyric acid
val/cys
met
ileu
leu
tyr
phe
his
lys

4.3. Results

4.3.1. Survival and growth at different salinities

The initial response of Tridacna gigas to abrupt changes in salinity was valve closure, deferring contact between the external medium and the clam's soft tissues. This protection was only of short duration, as the clams began to gape within an hour. Juveniles (10-15 mm SL) showed 75 to 100% survival for test salinities between 22 and 36 ppt over eight days (Fig. 2). On the other hand, there was total mortality at 0, 7 and 11 ppt within two days, and at 18 and 45 ppt over five days (Fig. 2).

Table 1 shows the median survival time (MT50) (derived from Fig. 2) of clams per test salinity. MT50 is the level of response time beyond which 50% of the experimental sample cannot live for an indefinite time (see Sprague 1969). For the salinities tested in this study, the lower limit of salinity tolerance in T. gigas was at 18 ppt with an MT50 of 2.9 days, while the upper tolerance limit was at 40 ppt with MT50 at 5.7 days.

Clams transferred to different salinities showed no obvious signs of stress during acclimatization. Their mantles remained moderately expanded, and the inhalant and exhalant siphons were continually opened.

A One-Way ANOVA testing for similarities between shell increments of clams grown at different salinities showed significant differences between shell increments per test salinity (Table 2A). Additionally, Bartlett's test for homogeneity of variances (Zar 1984) showed no significant difference between variances (B_C = 9.83, k = 3, P>0.10). Average shell increments are presented in Table 2B. The relationship between average shell increment and test salinity for T. gigas was determined by plotting these two variables as shown in Fig. 3. The relationship is defined by the quadratic regression equation (Zar 1984): $y = -14.98 + 1.09x - 0.02x^2$, with the maximum value of y (shell increment) being 4.36 mm at

Fig. 2. Survival of Tridacna gigas juveniles (size 10-15 mm) at different salinities (in ppt, indicated at end of each curve).

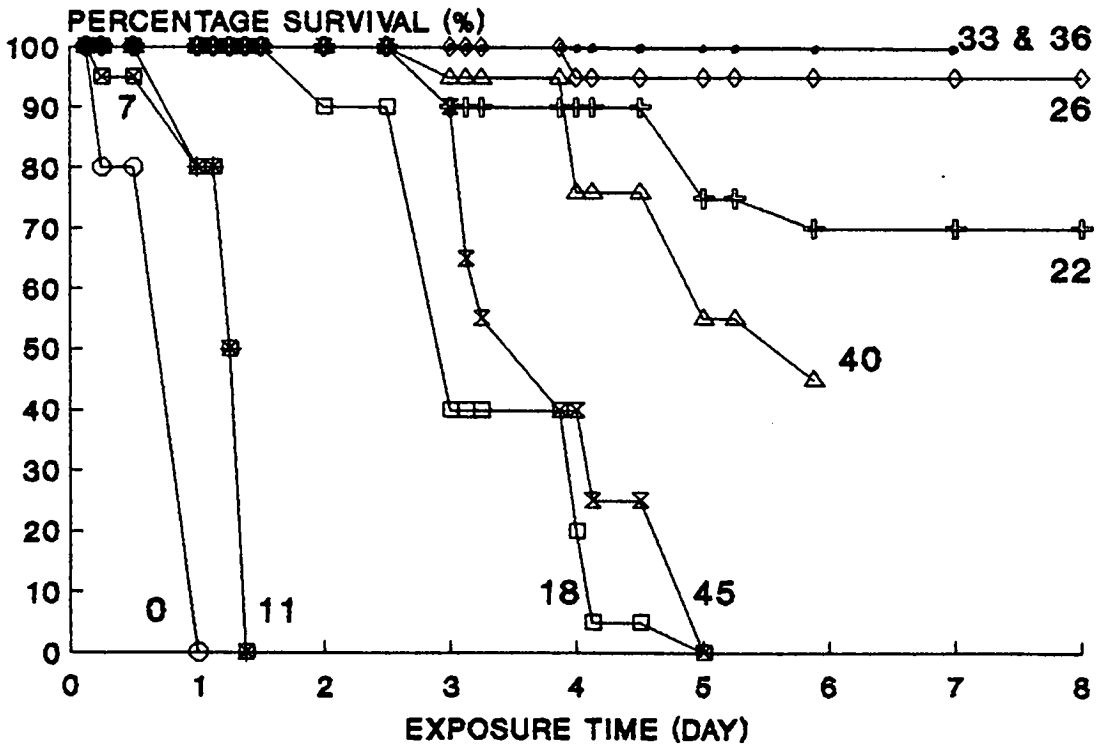


Table 1. Median survival time (MT50) of clams per test salinity.

SALINITY (PPT)	MT50 (DAY)
0	0.7
7	1.2
11	1.2
18	2.9
40	5.7
45	3.5

Table. 2. (A) 1-Way ANOVA of linear shell growth of clams reared at different salinities. (B) Mean shell growth (± s.d.) of clams per salinity treatment. (N=number of clams per treatment)

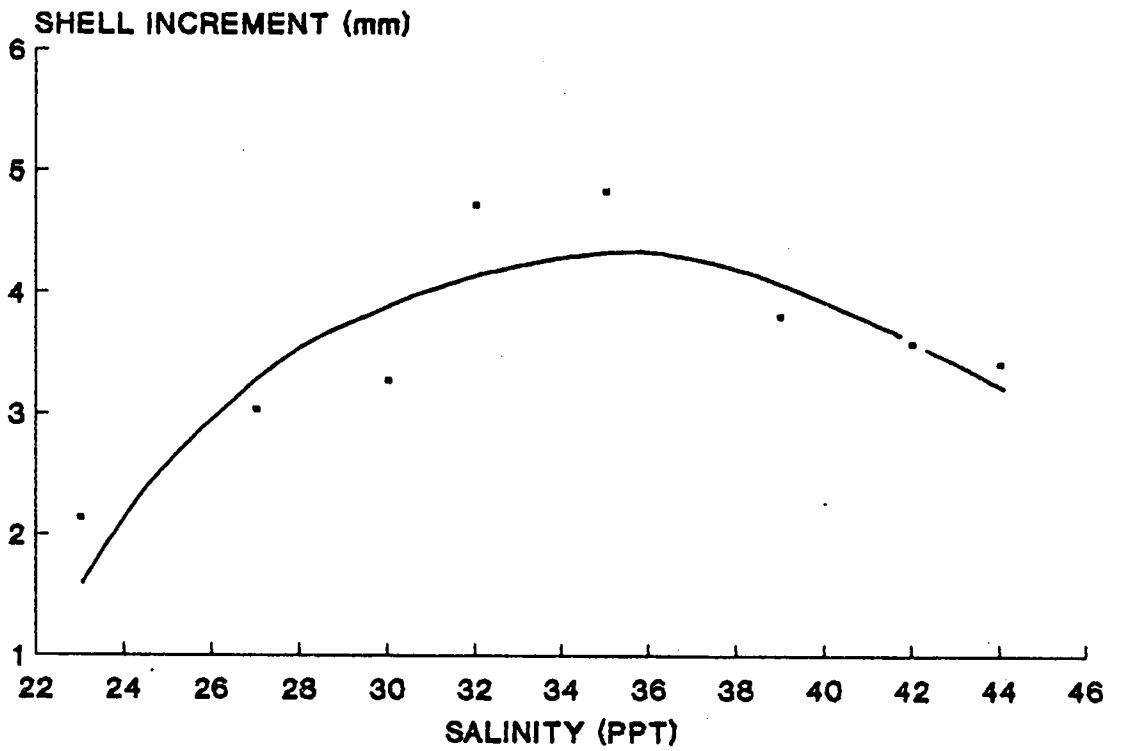
A. ONE-WAY ANOVA

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARES	F	P(LEVEL OF SIGNIFICANCE)
SALINITY	7	155.80	22.25	10.25•••	«0.001
WITHIN	52	112.90	2.17		
TOTAL	59	42.93			

B. SHELL GROWTH OVER SIX WEEKS AT DIFFERENT SALINITIES

SALINITY (PPT)	GROWTH (mm)	N
23	2.13 ± 1.57	8
27	3.03 ± 1.15	7
30	3.27 ± 1.59	8
32	4.72 ± 1.75	8
35	4.83 ± 2.11	8
39	3.80 ± 0.92	6
42	3.57 ± 1.23	8
44	3.41 ± 0.66	7

Fig. 3. Quadratic regression of average shell growth (data shown in Table 2B) against treatment salinities. $y = -14.98 + 1.09x - 0.02x^2$ Maximum value of y = 4.36 mm; x = 35.5 ppt.



x (salinity) of 35.5 ppt. For the salinities tested (23-44 ppt), growth was lowest at 23 ppt (Fig. 3A).

The gain in total wet weight (shell plus soft tissue) by clams grown at different salinities for 30 days was also determined (Appendix 3). However, Bartlett's test for homogeneity of variances showed that the variances of total weight gain per test salinity were heterogeneous (B_c = 17.23, k = 7, $P<0.02$), hence no further analysis was conducted. It would seem that there are still difficulties in the use of wet weight to assess clam growth, considering that clam wet weights were measured in this study as uniformly as possible for all treatments.

4.3.2. Adaptation to salinity changes

Changes in the osmotic concentrations of the extracellular fluids (ECF) and of the mantle cavity fluid relative to environmental levels are shown in Table 3, with regressions plotted in Fig. 4. ECF and cavity fluid osmotic concentrations varied with external osmotic pressure, both ECF and cavity fluid remaining slightly hyperosmotic to environmental levels. ECF osmolality was greater than that of the cavity fluid. Analysis of Covariance (ANCOVA, Snedecor and Cochran 1980) of the regressions indicates no significant differences between the slopes of the regressions ($F = 0.01$, df = 2,17, $P>0.50$), but significant differences between their elevations ($F = 5.17$, df = 2,19, $P<0.05$). This means that, relative to the ambient salinity, the osmolalities of the ECF and of the cavity fluid were significantly different from each other. Despite the slight difference in osmolality, the clams are clearly osmoconformers over the salinity range 23 to 44 ppt.

Most of the non-essential and essential FAA considered in this study were present at 30 and 35 ppt, (see Table 4). Alanine was absent at 30 and 35 ppt, and also glutamine from 35 ppt. At 23 and 27 ppt, only non-essential FAA were detected, specifically, aspartic acid, glutamic acid, glycine and alanine. At 39 and 42 ppt,

Table 3. Osmolality of the inhalant cavity fluid, extracellular fluids (ECF) and test salinity. 6-8 clams were used; values are mean and s.d.

SALINITY (PPT)	SEAWATER*	CAVITY FLUID*	ECF*
23	688	702 ± 1	700 ± 21
27	818	854 ± 14	868 ± 48
30	933	942 ± 16	978 ± 40
32	948	---	987 ± 12
35	1056	1064	1085 ± 22
39	1188	1195 ± 12	1230 ± 55
42	1278	1312 ± 15	1336 ± 41
44	1298	1314 ± 4	1315 ± 28

* units in $mOsm.kg^{-1}$ water.

Fig. 4. Regressions of osmotic concentrations of the test salinity, cavity fluid and extracellular fluids (ECF).

Regression equations: Test salinity: $y = 16.79 + 29.68x$, $r = 0.997$
cavity fluid: $y = 41.24 + 29.56x$, $r = 0.996$
ECF: $y = 41.39 + 30.03x$, $r = 0.991$

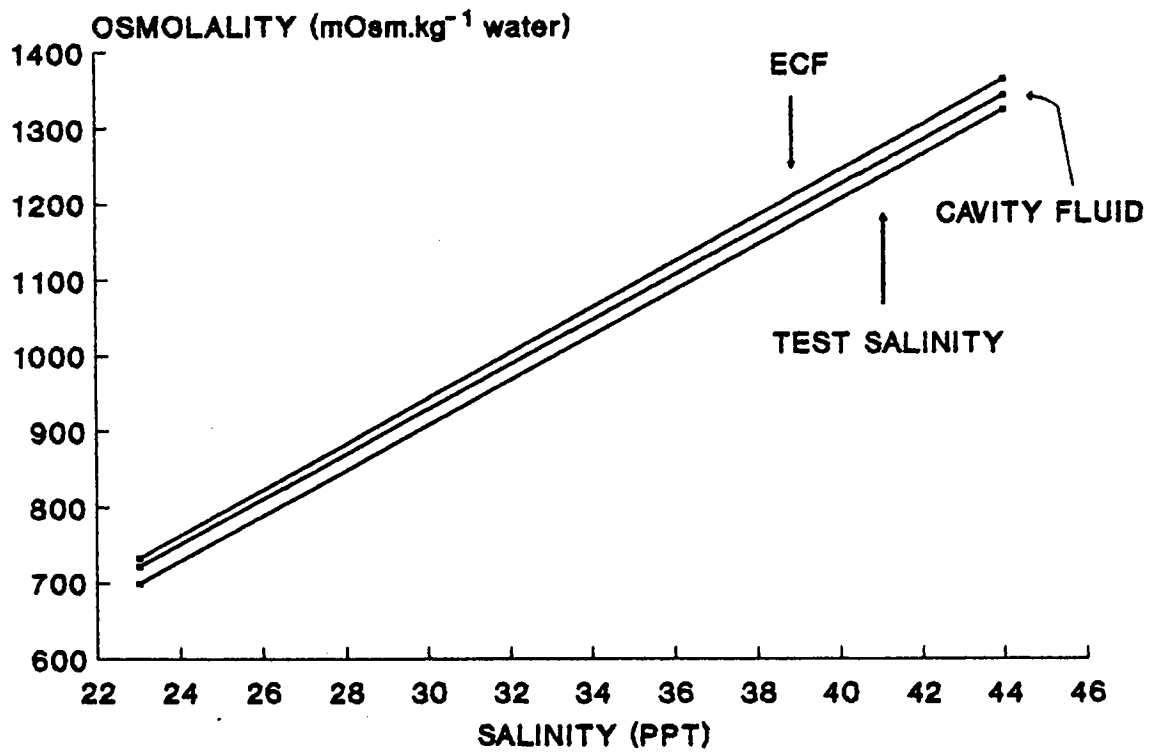


Table 4. Composition of intracellular FAA pool from adductor muscle of clams held at different salinities ('+' = present; 'n' = absent) All FAA listed below have been tested against a standard calibration curve (see Fig. 1).

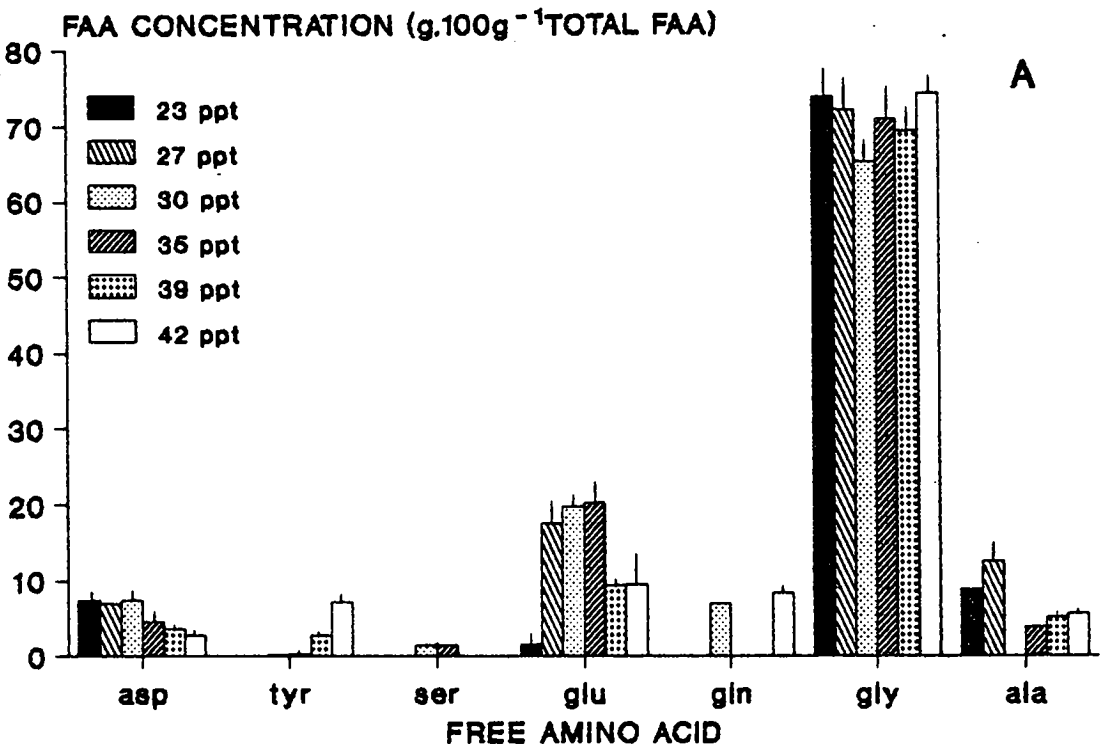
AMINO ACID	SALINITY (PPT)					
NON-ESSENTIAL*	23	27	30	35	39	42
asp	+	+	+	+	+	+
tyr	n	n	+	+	+	+
ser	n	n	+	+	n	n
glu	+	+	+	+	+	+
gln	n	n	+	n	n	+
gly	+	+	+	+	+	+
ala	+	+	n	n	+	+
ESSENTIAL*						
phe	n	n	+	+	+	+
lys	n	n	+	+	+	n
val/cys	n	n	+	+	n	n
met	n	n	+	+	n	n
lleu	n	n	+	+	n	n
leu	n	n	+	+	n	n
thr	n	n	+	+	n	n

*Classification into non-essential and essential amino acids based on Harrison (1975).

similar to the observations at 23 and 27 ppt, non-essential FAA were mostly present, with the addition of tyrosine and glutamine, and essential FAA namely phenylalanine and lysine.

The relative concentrations of the individual FAA (expressed in grams per 100 grams of total FAA) are presented in Fig. 5. Non-essential FAA constituted a much greater proportion of the total FAA (compare Figs. 5A and B). However, no clear pattern of change in FAA concentrations with variation of external salinity was observed, particularly for the major amino acids (Fig. 6). The major amino acids were glycine, glutamic acid, aspartic acid and alanine. Levels of glycine, the most abundant amino acid, did not manifest much variation from 23 to 42 ppt (Fig. 5A), its concentration ranging between 65 and 74 $g.100g^{-1}$ total FAA. On the other hand, glutamic acid levels increased with elevation in external salinity from 23 to 35 ppt and then decreased with further increase in salinity up to 41 ppt. Aspartic acid showed decreasing concentrations with increasing salinities. Finally, changes in alanine concentration showed little dependence on the external salinity. Aspartic acid and alanine each comprised from 0 to 13 $g.100g^{-1}$ total FAA in all test salinities.

Fig. 5. Concentration of non-essential free amino acids (FAA) from adductor muscle of clams exposed to test salinities for 30 days. (A) Non-essential FAA, (B) Essential FAA. (Vertical bar=s.d.; some s.d. too small to plot)



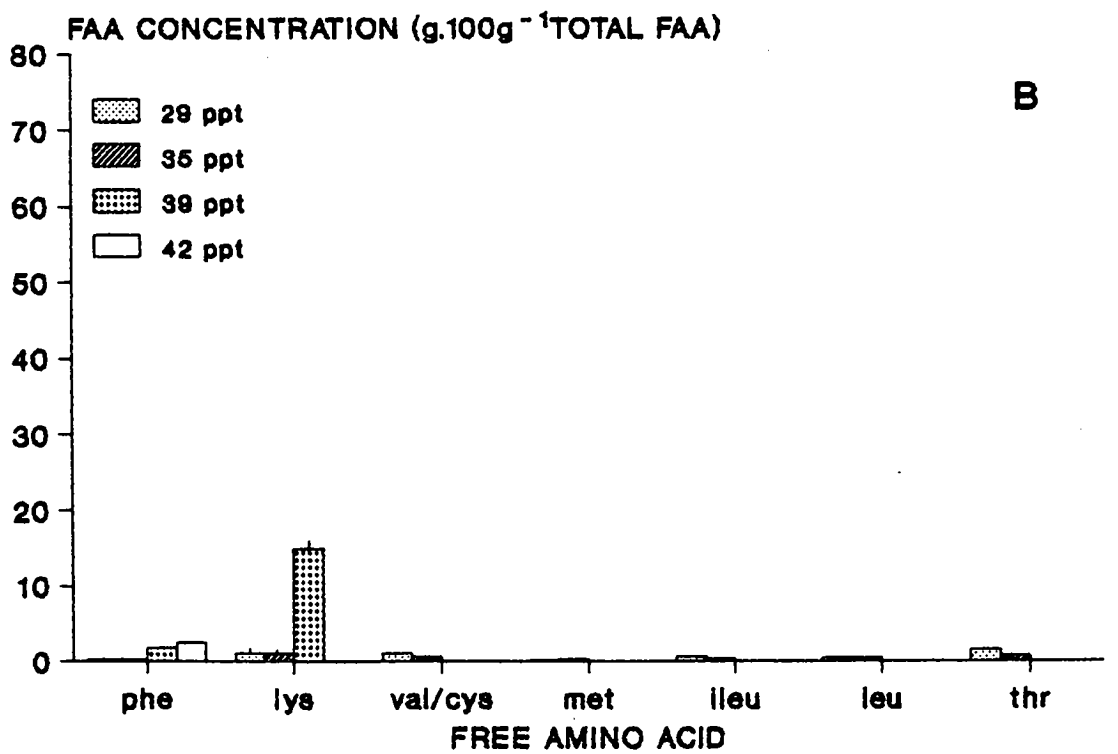
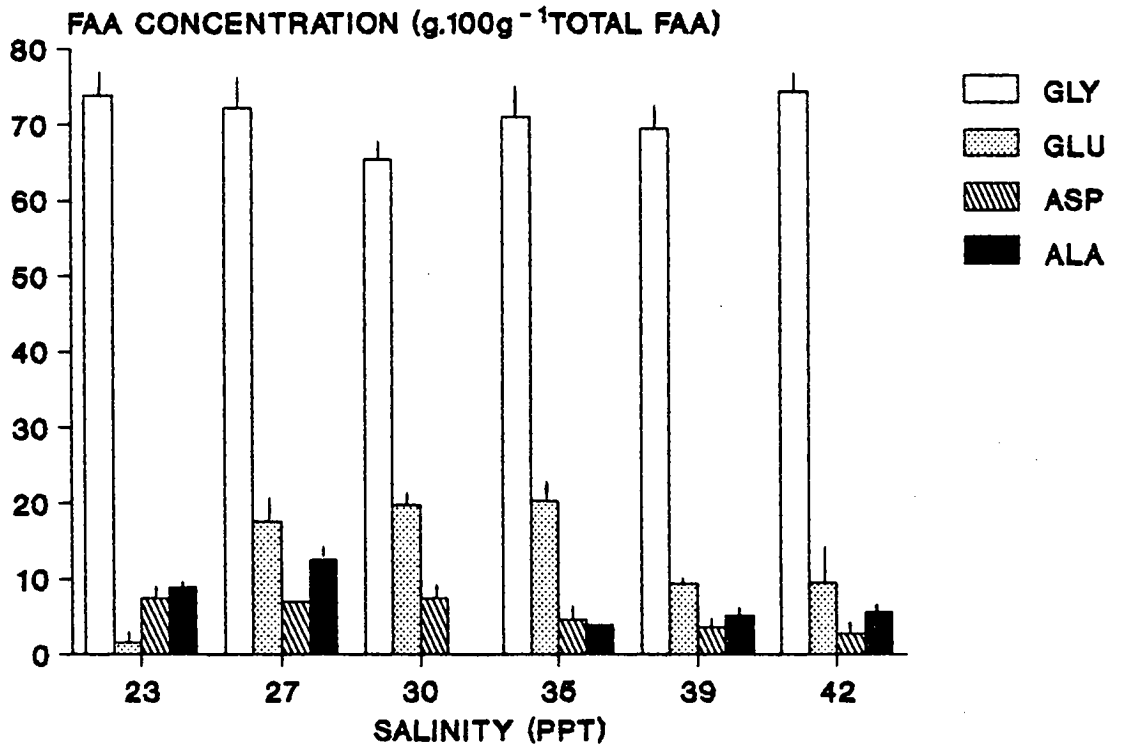


Fig. 6. Concentration of major free amino acids (FAA) from adductor muscle of clams reared in different salinities over 30 days. (Vertibar=s.d.; other s.d. too small to plot)



4.4. Discussion

Tridacnids generally inhabit waters where marine or near marine salinities prevail (e.g. Alcazar and Solis 1986, Heslinga et al. 1984). In this study, juvenile Tridacna gigas about 10 mm SL tolerated salinities from 26 to 36 ppt with little or no mortality over a week. Thus, intolerance of low and high salinities by at least early juveniles is a factor in the near-marine salinity distribution of T. gigas. This is likely to be found for other tridacnid species in view of their similar salinity distribution.

After an acclimatization period of two weeks, the osmotic concentration of the ECF varied with salinity level, indicating that T. gigas is an osmoconformer. Ionic concentrations of the ECF were not determined; however, based on osmotic concentration, the ECF was slightly hyperosmotic to the mantle cavity fluid (by about 18 $mOsm.kg^{-1}$), with the latter also hyperosmotic to the external environment. The average difference between the ECF and test salinity was 37 $mOsm.kg^{-1}$. For Modiolus demissus granosissimus, Pierce (1970, 1971) reported a difference of 20 $mOsm.kg^{-1}$ between the blood and the external salinity. Such hyperosmoticity in Modiolus was attributed by Pierce to a passive Gibbs-Donnan type of equilibrium, primarily because the slope of the curve for blood osmolality is constant and parallels that of medium osmolality, as similarly observed in T. gigas. Active osmoregulation would entail deviation in the slope of the blood osmolality curve at some point relative to the slope of the test salinity. Slight hyperosmoticity has been reported in other molluscs, for instance, Hydrobia ulvae (Todd 1964, cited in Negus 1968), M. demissus granosissimus, M. modiolus and M. squamosus (Pierce 1970), although several workers have reported extracellular fluids as either hypoosmotic or isosmotic with the external medium (Hoyaux et al. 1976, Lange 1963).

In Tridacna gigas, the major FAA were glycine, varying from 65.28 to 74.31 $g.100g^{-1}$ total FAA; glutamic acid, 1.61 to 20.23 $g.100g^{-1}$ total FAA; alanine 3.83 to 12.54 $g.100g^{-1}$ total FAA; and aspartic acid, 2.76 to 7.49 $g.100g^{-1}$ total FAA. These amino acids have also been found in other bivalves (see Table 5). However, whether or not these FAA are osmotically active in T. gigas was not clear from this study. Most studies depict a closely linear relationship between total FAA and external salinity, for example, in Modiolus demissus granosissimus (Pierce and Greenberg 1972) and Crassostrea virginica (Lynch and Wood 1966). With T. gigas, no general pattern can be obtained from the intracellular FAA analysis, although the major amino acids seemed to exhibit specific trends with change in external salinity.

Concentrations of glycine were relatively constant and high, at least 65 $g.100g^{-1}$ total FAA in T. gigas, suggesting that glycine may have some cellular importance in tridacnids. High concentrations have also been reported in M. d. demissus (Baginski and Pierce 1975), C. virginica (Heavers and Hammen 1985) and Mytilus edulis (Hoyaux et al. 1976). The serine cycle, an intermediate cycle in glycolysis, may be a source of glycine, arising from the combination of serine and tetrahydrofolate, catalyzed by serine hydroxymethylase (see Whiteley 1960).

Glutamic acid is a combination of glutamate (glutamate is a source of nitrogen in several transamination reactions in the serine cycle, anaerobic glycolysis and TCA) and free acid. Glutamic acid concentrations were highest at salinities 27, 30 and 35 ppt. A similar pattern was also observed by Pierce (1971) for Modiolus demissus granosissimus, which exhibited peak level at 35 ppt, but reduced levels at 3 and 48 ppt. The applicability of the glutamic dehydrogenase (GDH) mechanism involving glutamate transamination to explain amino acid regulation in molluscs has been criticized (e.g. Pierce and Greenberg 1972, Shumway et al. 1977) for the reason that the enzyme GDH has been detected in

molluscs only in very small amounts (see Matsushima and Kado 1983). So far, the role of GDH in FAA regulation has not been defined. Furthermore, the role of GDH in the tridacnid symbiosis has not been studied. The fact that the nitrogen cycles of the host and zooxanthellae are intricately linked complicates the interpretation of FAA regulation. The present observations do not seem to support the GDH mechanism--the change in glutamic acid concentration was not linear with change in test salinity, contrary to what would be expected had the GDH mechanism been functioning in Tridacna gigas.

Aspartic acid decreased with increasing salinity, contrary to the results obtained from other bivalves, for example, Rangia cuneata (Allen 1961), and Crassostrea virginica (Heavers and Hammen 1985). Whereas, alanine varied independently of external salinity, i.e. increasing as salinity increased from 23 to 27 ppt, disappearing at 30 ppt, and reappearing at 35 ppt with increasing concentrations as salinities were elevated to 42 ppt. In the TCA cycle, transamination of pyruvate (by aspartate and alanine aminotransferases), alpha-ketoglutarate and oxaloacetate gives rise to alanine, glutamate and aspartate, respectively. Additionally, these three amino acids are readily interconverted, i.e., alanine and glutamate are formed from aspartate, and aspartate and alanine from glutamate (Bishop and Barnes 1971, Goddard and Martin 1966, Hammen 1969, Heavers and Hammen 1985, Lowenstein 1972). There was no obvious relationship between the response of aspartic acid or of alanine to external osmotic changes. Other workers have attributed the accumulation of alanine in high salinities to anaerobic metabolism, for instance, in Modiolus demissus demissus (Baginski and Pierce 1975). The influence of anaerobic metabolism on FAA metabolism in T. gigas during osmotic adaptation needs to to be considered in the future.

Since the zooxanthellae translocate certain amino

Table 5. Major amino acids ('+') of the intracellular free amino acid pool of some bivalve species during salinity stress. (nd = not detected; nt = not tested)

SPECIES	FREE AMINO ACID ala arg asp glu gly his pro ser tau thr	SOURCE
Chlamys operculatis	+ + nd + + nd nd +	Shumway et al. 1977
Crassostrea virginica	+ + + +	Heavers and Hammen 1985
	+ + + + +	Lynch and Wood 1966
Mercenaria mercenaria	+ +	Rice and Stephens 1988a
Modiolus demissus demissus	+ + + +	Baginski and Pierce 1975
M. d. granosissimus	+ + + + + +	Pierce 1971
	+ nt + + + nt nt + + nt	Pierce and Greenberg 1972
Mya arenaria	+ + + + nd nd nd + nd	Shumway et al 1977
Mytilus edulis	+ nd + + + + + +	Hoyaux et al. 1976
	nt + nt + + +	Zurburg et al. 1969
Rangia cuneata	+ nt + + + nt nt nt nt nt	Allen 1961
Scrobicularia plana	+ + + + + + + +	Hoyaux et al. 1976
	+ + + + + nd +	Shumway et al. 1977
Tegula funebralis	+ + + + + +	Peterson and Duerr 1969
Tridacna gigas	+ + + + + nt	This Study

acids to their host, it would seem that particular differences in amino acid content would be manifested between symbiotic tridacnids and non-symbiotic bivalves. Comparison of major FAA, however, reveals no obvious differences (Table 5). Despite this, the role of zooxanthellae in tridacnid FAA metabolism needs to be investigated. Griffiths and Streamer (1988) showed that translocated photosynthates from zooxanthellae to the host include alanine, aspartate, glutamate and serine. As mentioned previously, the first three amino acids readily undergo interconversion, therefore, any one of these three amino acids may serve as precursor for another. On the other hand, serine through the serine cycle may serve as source of glycine. Additionally, the effect of osmotic pressure changes in the ECF on extracellularly-occurring zooxanthellae needs to be studied, since any change in cellular integrity of these algae may affect the tridacnid's autotrophic capacity.

Based on growth studies, the optimum salinity for Tridacna gigas is about 35 ppt, i.e. marine conditions. Substantial reductions in growth rate occur when salinity varies from 35 ppt by $\pm$ 4 ppt. T. gigas juveniles, therefore, are relatively stenohaline. Reduced growth at higher salinities is probably due to their limiting effects on metabolic activities such as feeding.

5. SEAWATER FLOWRATE, STOCKING DENSITY, ALGAL FOOD, DISSOLVED INORGANIC NUTRIENTS AND CLEANING

5.1. Introduction

Since resources in land-based mariculture operations are often restricted in comparison to those which are ocean-based, it is important to identify culture conditions that can be manipulated to optimize production during the land-based phase of giant clam culture. The aims of this study were to determine the influence of five environmental factors: seawater flowrate, stocking density, microalgae, dissolved inorganic nutrients and "cleaning", separately or in combination, on juvenile Tridacna gigas. This Introduction focuses on these factors.

5.1.1. Seawater flowrate

Little has been done to critically assess the influence of flowrate on juvenile T. gigas, although a number of mariculture operations have employed specific flowrates with particular nursery tank volumes when rearing juveniles. Flowrate and tank volume affects water turnover time, and hence availability of particulate food and nutrients, and efflux of wastes.

Proper evaluation of flowrate effects involves the consideration of turnover or replacement time (TOT), which is the amount of time required to replace the volume of water in a tank. It is a function of water volume and flowrate, and hence can be adjusted by modifying (within reasonable limits) either of these two factors (Sprague 1969). TOT determines the amount of food present in the volume of water, and also the rate at which wastes discharged by the organism are effectively removed from the seawater medium. A fast TOT results in a large influx of exogenous energy sources. However, the effect of flowrate on feeding depends on the amount of food available in the water (see Spencer 1988).

Maximum growth has been related to flowrate per unit clam. Fast growth rates were found for Mercenaria mercenaria reared at maximum flowrate per unit clam (Hadley and Manzi 1984). In addition, Castagna (1984) stated that the detrimental effects of stocking at high densities can be circumvented by altering the flowrate, such that flowrate per unit clam is enhanced.

5.1.2. Stocking density

While abundance of bivalves in natural populations varies from sparse to dense aggregations (e.g. Braley 1988, Harger 1970, Seed 1969), it is important that commercial species be cultured in unnaturally high densities to yield optimum production. This also suggests that optimum production per unit area may not be fully realized under natural conditions.

Growth rates of juvenile bivalves have been shown to vary with stocking density. Slow growth has been related to a high stocking density (or a level resulting in excessive physical contact between clams) (e.g. Hadley and Manzi 1984). The effect of stocking density on growth of bivalve juveniles varies with age and this has been found from early studies on Tridacna derasa. A stocking density level of $1000.m^{-2}$ has been employed for this species at 5-10 mm shell lengths, with regular thinning to lower densities as juveniles increase in size (Heslinga et al. 1984). Although a maximum density of $2000.m^{-2}$ specifically for 10 mm seed has been recommended for most tridacnids (Heslinga and Fitt 1987), the higher limits of stocking density applicable to T. gigas have not been investigated.

Results on juvenile growth in stocking density experiments have been attributed to crowding, and to lack of food and poor food distribution due to overgrazing (Castagna 1984). There is an apparent critical margin in net production, beyond which the restrictive effects of stocking density are then manifested, as has been

demonstrated in the hard clam Meretrix lusoria (Jeng and Tyan 1982).

Studies on Mercenaria mercenaria have shown that the effect of stocking density on growth is interrelated with flowrate, indicating the predominant influence of food (Manzi et al. 1986). Hence it has been recommended that fast flowrates be employed where densities are high, in order to circumvent the detrimental effects of high stocking density (Castagna 1984). Manipulation of stocking density levels for M. mercenaria in relation to temperature has been suggested by Manzi et al. (1986) Since M. mercenaria exhibits little growth in winter (below 15°C), stocking levels may be increased to minimize maintenance costs on the nursery system for that time of year.

5.1.3. Microalgae

Juvenile Tridacna gigas are effective filter-feeders (D. W. Klumpp, pers. comm.), and this section focuses on their heterotrophic nutrition (for discussion on autotrophy, see Chapter 2, Section 2.3.4). The importance of filter-feeding relative to autotrophy in the nutrition of tridacnids has been debated (see Mansour 1946, Morton 1978, Ricard and Salvat 1977, Yonge 1980). Various modifications of the gills for feeding have been described in other bivalves, demonstrating the close relationship between gill structure and function (Newell 1964). Tridacnids are well-equipped to filter-feed, with large gills, active palps, a digestive system with diverticula and a crystalline style which are all fully functional (Goreau et al. 1973). Various phytoplankton and zooplankton have been found in the stomachs of giant clams (Mansour 1946). Stained thin sections from several specimens of T. crocea dissected at intervals over a 24-hour period showed an apparent diurnal feeding rhythm (Morton 1978).

The relative contribution of plankton in clam nutrition may vary with age, species and habitat. Reid

and King (1988) suggested that, because of the late development of the gills and siphons in post-larvae, suspension-feeding may not contribute to nutrition until some days later after metamorphosis. Thence, filter-feeding rates may vary as the clam increases in size, since the gill surface area changes approximately proportionate to the square power of the length of the clam, while clam mass changes in proportion to the cubic power of clam length. Thus, there is likely to be a changing relationship between filter-feeding rate and mass.

Species differences in gill morphology have been observed. For instance, size of the outer demibranch in Tridacna crocea is reduced and the individual gill filaments are quite broad. Whereas in T. derasa, the demibranchs are symmetrical and the filaments are not as broad as those of T. crocea. These differences probably reflect dissimilarities in filter-feeding capabilities and habitat preferences (Yonge 1936).

D. W. Klumpp and his associates (pers. comm.) have demonstrated substantial particulate filter-feeding in tridacnids. Specifically, for a juvenile T. gigas with a dry tissue weight (DTW) of 50 mg (35 mm shell length, SL), 56% of the total organic carbon requirements (for growth and respiration) is satisfied by filter-feeding. This value decreases with increasing clam mass, i.e. 24% at 15 g DTW (189 mm SL). This supplements phototrophy, which provides from 32% (clam size: 25-30 mm SL) (Griffiths and Streamer 1988) to 95% (clam size: 10-380 mm SL) (Fisher et al. 1985) of the clam's metabolic carbon requirements. In T. maxima, this value is 40% (clam size: about 90 mm SL) (Trench et al. 1981). Trench (in Goreau et al. 1973) pointed out that, "if algal products [are] not available at the necessary rate, other resources [will] have to be drawn on". Small juvenile T. gigas seem to utilize zooxanthellar photosynthates and particulate food simultaneously.

Since tridacnid clams are often found in oligotrophic waters, it seems reasonable that supplementary feeding in culture with microalgae and inorganic nutrients (see Section 5.1.4.) should lead to increased growth rates. A study by Crawford et al. (1986) on juvenile Tridacna gigas supplementing the seawater with microalgae, Isochrysis galbana and Chlorella sp. alternately, was inconclusive.

There has been much interest in identifying the best microalgal food for maricultured bivalves (e.g. Whyte 1987). Studies on Crassostrea virginica and Mercenaria mercenaria have shown that growth of hard and soft tissue is dependent on specific combinations of microalgae, indicating synergism of relative food values of the algal species (Epifanio 1979). From preliminary studies on T. maxima, Estacion et al. (1986) found better growth rates for juveniles fed a combination of two microalgae, I. galbana and Tetraselmis sp., than single species diets. The ultimate value of microalgae as food, however, depends on other factors such as the available nutrients, the alga's relative digestibility and its degree of toxicity (Whyte 1987).

There is also the possibility that heterotrophic feeding in tridacnids, while it directly supports host metabolism, may also support zooxanthellar productivity. This has already been illustrated in the symbiotic sea anemone Aiptasia pallida using the mitotic index (MI) (Cook et al. 1988). MI, an indicator of zooxanthellar productivity, is the ratio of the number of dividing algal cells against a specified number of non-dividing cells (Wilkerson et al. 1983). Cook et al. (1988) observed that algal mitosis is stimulated when the host is fed.

5.1.4. Dissolved Inorganic Nutrients

Munro and Heslinga (1983) suggested that growth of juvenile tridacnids may be enhanced by assisting the phototrophic process, for instance, with the use of inorganic fertilizers. The use of fertilizers or supplementary nutrients in bivalve mariculture is not new.

It has been shown that the addition of fertilizers (nitrogen, phophorus and silicon) to the water enhances phytoplankton growth, and concomitantly, bivalve growth (oysters, Spencer et al. 1986, Spencer 1988). For tridacnids, the effect of fertilizers would be to enhance production of the zooxanthellae within the clams, as the inorganic nutrients are made available to the zooxanthellae through the clam's tissues.

Present knowledge on the effects of inorganic nutrients on symbiotic algae is largely based on studies on symbiotic corals and sea anemone. Gunnersen et al. (1988) demonstrated ammonium uptake by freshly isolated or cultured zooxanthellae of Acropora formosa and Montipora verrucosa. Ammonium-starved zooxanthellae showed higher uptake rates than those cultured in ammonium-rich media or that have been freshly isolated. Enhancement of photosynthesis has been observed in the symbiotic sea anemone Aiptasia pallida after addition of inorganic nitrogen and phosphorus (Cook et al. 1988).

Nitrogen, phosphorus and sulfur are required for protein synthesis in giant clams (Heslinga and Fitt 1987, Yonge 1974). Absorption of nitrogen as amino acids, ammonium or nitrate has been demonstrated in tridacnids (Burris 1983, Fankboner 1971, Goreau et al. 1973). Such capability for absorption has been attributed to their algal endosymbionts (Deane and O'Brien 1981a, Wilkerson and Trench 1986, Yonge 1936). Compared to the several symbiotic soft and hard corals studied, Burris (1983) found that Tridacna crocea displays a higher affinity for ammonium.

Summons et al. (1986) showed that ammonium can stimulate photosynthesis in mantle tissue slices from T. maxima, T. derasa and T. squamosa. They further found that carbon fixation involved zooxanthellae that are in the log phase, and not those in the stationary phase. In T. maxima, the amount of photosynthetic carbon assimilated increased with the level of ambient ammonium.

Tridacna gigas (75 to 100 mm SL) takes up both ammonium and nitrate (Wilkerson and Trench 1986). However, a previous light exposure is required to sustain uptake, while dark incubation results in reduced uptake. Ammonium absorption by T. gigas is not influenced by the ammonium concentration, which is important when living in nutrient-limited environments. In addition, for the concentration range (up to 28 umol NH_4) studied by Wilkerson and Trench (1986), there was no manifestation of a saturation level for ammonium absorption.

Another nutrient that may be considered in nutrient-enrichment studies in tridacnids is phosphate. Fitt (1988) stated that additional phosphates may increase either photosynthetic rates or numbers of zooxanthellae, or both, hence increasing the amount of photosynthate available to the host for growth.

Little is known about phosphate uptake in giant clams. Studies on corals have shown that zooxanthellae can take up phosphorus (Snidvongs and Kinzie 1987, Yonge and Nicholls 1931a, b). Light intensity (Wynne and Rhee 1985) and phosphorus deficiency (Healey 1978) may influence phosphorus uptake as has been found with other algal cultures. Generally, phosphorus is vital to cellular metabolism, for instance, in ATP formation and DNA synthesis. Its effect on growth of zooxanthellae still needs to be established. Phosphate spiking of Stylophora pistillata colonies yielded no difference in the zooxanthellar ratio of carbon to nitrogen (Muscatine et al. 1989).

As studied in Mercenaria campechiensis, phosphates are required in metal detoxification (Evtushenko et al. 1986, Miller et al. 1985). Deposits of calcium phosphate, together with oxalate concretions, have been found in several bivalve kidneys (Hignette 1980). Much speculation has been generated by the size and contents of the tridacnid kidney, relating these to the host's symbiosis with endosymbiotic algae (Yonge 1936, Trench et al. 1981b). There has been some interest in the biogenic

phosphorite found in the kidney of Tridacna gigas (Trench et al. 1981b) and T. maxima (Hignette 1980). Phosphorite is a type of phosphate deposit similar to apatite but lacking a crystal form. It is composed of calcium phosphate and is believed to occur in reducing environments. In T. maxima, significant amounts of metals such as aluminum, cobalt, copper, magnesium, manganese and zinc were also found in these concretions (Hignette 1980). Little is known about the significance of these phosphorite concretions.

While phosphorus may serve in important metabolic processes, it may also inhibit some of these processes. It has been proposed that complex phosphates may act as a crystal poison in calcification. By settling on the surface of a crystal, phosphates may interfere with the continued formation of the crystal lattice (Neuman et al. 1951, Simkiss 1964a). In vitro experiments with Rangia cuneata showed that the crystallization rate of calcium carbonate was significantly inhibited by inorganic phosphates at certain concentrations. However, in vivo inhibition of calcification requires concentrations higher than those required for in vitro inhibition (Bernhardt et al. 1985).

The final focus of this discussion on nutrients is on sulfur. Sulfur is present in most commercial fertilizers in the form of ammonium sulfate. Little is known about the sulfur requirements of tridacnids, although uptake has been demonstrated in T. maxima using sulfates and sulfur-containing amino acids (Deane and O'Brien 1981b). Like phosphates, sulfates are important in metal detoxification (Perna viridis: Baby and Menon 1987, T. maxima: Edmonds et al. 1982).

5.1.5. Cleaning

For the nutrient and microalgae experiments, it was required that clams be cleaned periodically. Cleaning involved handling the juveniles to remove epibionts on their shells, and also removing filamentous algae that

grow over and around these clams. Hence cleaning is discussed here in relation to disturbance.

Studies on other bivalves have indicated that disturbance may be a factor influencing the survival and growth of juveniles. For instance, in Mytilus galloprovincialis, a high loss of mussels following thinning was observed. The loss was attributed to mechanical stress to mussels brought about by handling, possibly leading to discontinued byssus formation (Ceccherelli and Barboni 1983).

Natural mussel populations, particularly M. edulis, show significant differences in growth, size, body weight and check ring formation, depending on the extent of wave impact. A reduction in growth, size and body weight are correlated with less severe wave impact, while a greater number of check rings have been observed for mussels experiencing more severe wave impact (Harger 1970).

Rearing of juvenile tridacnids involves a number of maintenance procedures, such as culling, and periodic cleaning of raceways as a means to control fouling by benthic algae (see Crawford et al. 1987, Heslinga et al 1984). The effect of disturbance, or handling, on juvenile tridacnids needs to be investigated.

5.2. Materials and Methods

The survival and growth of juvenile Tridacna gigas were studied under controlled environmental conditions at the Orpheus Island Research Station (OIRS) of James Cook University (JCU) between April and June 1987. The five environmental factors, seawater flowrate, stocking density, dissolved inorganic nutrients, microalgal food and cleaning, were considered according to the experimental design shown in Table 1.

Four-month old juvenile clams were placed in 0.8 l plastic containers ("pots") with running 1-µm filtered seawater (FSW) at a standard flowrate of 1.5 l.h-1. The initial mean SL of these clams (5.68 mm ± 0.31 mm SL) was based on the initial shell lengths of 8 representative batches of clams (n per batch varied from 10 to 59). A One-Way ANOVA on the SLs of these groups of clams showed no significant difference ($F = 1.46$, $df = 7,266$, $P>0.10$). Each replicate per treatment (except for stocking density) consisted of 50 clams. Gentle aeration was also provided to maintain good water circulation. The pots were held in a large water bath with running water to minimize fluctuations in water temperature. Water temperature was monitored daily with a minimum-maximum thermometer. All treatments were under 50% shade using a shade screen. The actual irradiance under 50% shade screen was measured on several clear days at noon using a Li-Cor light meter with a submersible sensor, and was shown to approximate 50% of the underwater irradiance under full sunlight.

Survival was monitored every week. Shells were collected and their SL measured. Mortalities at 4-week intervals were compared.

Clam growth was based on SL, which was initially measured under a dissecting microscope with a calibrated micrometer eyepiece, and later with a vernier caliper (± 0.02 mm). Measurements were taken every 4 weeks. Unfortunately, all experiments had to be terminated after 10 weeks because of equipment failure. Growth results

Table 1. Experimental design of small-scale experiments.

FACTOR	TREATMENT	REPLICATE PER TREATMENT	CLAMS MEASURED
Seawater flowrate (FR)	Fast FR	3	20
	Moderate FR	3	20
	Slow FR	3	20
Stocking density (SD)	High SD	3	20
	Moderate SD	3	20
	Low SD	3	20
Cleaning and Supplements	Cleaned: AF	2	20
	DIN	2	20
	Control	2	20
	Not Cleaned: AF	2	20
	DIN	2	20
	Control	2	20

(AF = algae; DIN = dissolved inorganic nutrients; Control = no supplement)

were then compared for the 10th week, based on SL measurements.

The flowrate experiment consisted of 3 treatments: 1.5 $l.h^{-1}$ (fast FR), 0.2 $l.h^{-1}$ (moderate FR) and 0.02 $l.h^{-1}$ (slow FR). The stocking density experiment also used 3 treatments: 5 clams per cm^2 (or 250 clams per pot; high SD), 1 clam per cm^2 (or 50 clams per pot; moderate SD) and 0.2 clam per cm^2 (or 10 clams per pot, low SD).

Experiments on inorganic nutrients (DIN), microalgal food (AF) and cleaning employed a two-Factor experimental design (with replication). DIN was provided as a "Dix Mix" fertilizer solution, containing superphosphate (a mixture of monobasic calcium phosphate and hydrated calcium sulfate) and ammonium sulfate (Appendix 1). Clams were gravity-fed with DIN by a continuous drip method at a flowrate of 0.02 $l.h^{-1}$. Final concentrations of these inorganic nutrients were approximately: 50 μM NH_4^+, 8 μM PO_4^{2-} and 15 μM SO_4^{2-}.

The unicellular alga Isochrysis galbana (Tahitian isolate) (AF), cultured in a Dix Mix-enriched medium, was fed to juveniles at a concentration of 10^5-10^6 $cells.ml^{-1}$. I. galbana is high in cellular lipid and protein. Its carbohydrate component is a mixture of glucose, galactose, mannose, xylose, arabinose, ribose, fucose and rhamnose (Whyte 1987). I. galbana has been used to feed various bivalves (e.g. Crassostrea virginica, Mercenaria mercenaria) with favorable results for growth relative to other microalgal feeds (Epifanio 1979). Clams were gravity-fed with AF separately from DIN by a continuous drip method at a flowrate of 0.02 $l.h^{-1}$. The algal supply was checked daily, refilled or replaced with new stock when necessary.

Clams were placed on a petri dish at the bottom of the pot. Every third week, clams were cleaned by carefully cutting their byssal threads with a scalpel and gently scrubbing shells to curtail filamentous algal growth. Pots were replaced with clean ones weekly.

5.3. Results

5.3.1. Seawater Flowrate

Survival of clams reared in fast, moderate and slow flowrates (FR) is shown in Table 2. One-Way ANOVA on clam survival (using arcsine transformation: $p' = \arcsin \sqrt{p}$) in different flowrates over 4 and 8 weeks showed significant differences in survival after 4 weeks ($F = 24.84$, $df = 2,6$, $P<0.005$), with 100% survival in moderate and fast FR, compared to 97% in slow FR. However, after 8 weeks, there was no significant difference between the FR treatments ($F = 0.18$, $df = 2,6$, $P>0.50$); the average percentage survival was 94%.

Shell lengths of clams reared at 3 FR levels were significantly different after 10 weeks (Tables 3, 4A). Fig. 1 shows minimum and maximum water temperature values for the duration of the experiment.

Growth rates were enhanced in fast FR (1.5 $l.h^{-1}$), while reduced in slow FR (0.02 $l.h^{-1}$) (Fig. 2). After 10 weeks, shell lengths had increased by 10%, 20% and 54% in slow, moderate and fast FR, respectively, indicating that shell growth was dependent on the FR level. Based on water temperature trends using intervals of 0 to third (W0-3), 4th to 7th (W4-7), and 8th to 10th week (W8-10), shell growth at different flowrates showed some dependence on temperature (see Fig. 1 and 2), except in fast FR for W0-3 and W8-10.

5.3.2. Stocking Density

Survival of juveniles at high, moderate and low SD is shown in Table 2. One-Way ANOVA on survival of clams at different SD levels after 4 and 8 weeks showed clam survival to be significantly different only after 8 weeks (after 4 weeks: $F = 3.87$, $df = 2,6$, $P>0.10$; after 8 weeks: $F = 20.49$, $df = 2,6$, $P<0.005$). Greater mortalities occurred at high SD (5 $clams.cm^{-2}$) than at moderate SD (1 $clam.cm^{-2}$); no clams died at low SD (0.2 $clam.cm^{-2}$).

Table 2. Percent survival among juveniles reared under controlled conditions of flowrate, stocking density, nutrients (DIN), algal food (AF) and cleaning over an 8-week period.

FACTOR	TREATMENT	PERCENT SURVIVAL PER REPLICATE	
		TIME	
		4th WEEK	8th WEEK
FLOWRATE	Fast	100,100,100	98,88,98
	Moderate	100,100,100	90,92,98
	Slow	94,98,98	94,96,90
STOCKING DENSITY	High	100,98,97	76,77,88
	Moderate	100,100,100	98,88,98
	Low	100,100,100	100,100,100
CLEANING AND SUPPLEMENTS	Cleaned: AF	96,100	84,82
	DIN	98,100	96,98
	Control	100,100	96,90
	Not Cleaned: AF	100,100	92,96
	DIN	100,100	92,92
	Control	100,100	98,88

Table 3. Average shell lengths (± s.d.) of clams reared under controlled conditions of flowrate, stocking density, nutrients (DIN), algae (AF) and cleaning over a 10-week period.

FACTOR	TREATMENT	SHELL LENGTH (mm) MEAN ± S.D.
FLOWRATE	Fast	8.74 ± 0.36
	Moderate	6.84 ± 0.34
	Slow	6.27 ± 0.23
STOCKING DENSITY	High	6.79 ± 0.28
	Moderate	8.74 ± 0.36
	Low	10.02 ± 1.11
CLEANING AND SUPPLEMENTS	Cleaned: AF	8.02 ± 0.17
	DIN	8.54 ± 0.13
	Control	8.72 ± 0.45
	Not Cleaned: AF	8.79 ± 0.71
	DIN	8.63 ± 0.66
	Control	8.74 ± 0.36

Table 4. 1-Way ANOVA (with replication) of shell lengths of clams reared under controlled levels of (A) flowrate and (B) stocking density, for 10 weeks.

A.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P(LEVEL OF SIGNIFICANCE)
Flowrate	2	10.0540	5.0268	50.05***	« 0.001
Within	6	0.0626	0.1004		
TOTAL	8	10.6560			

B.

Stocking density	2	15.9080	7.9542	16.85**	<0.001
Within	6	2.8655	0.4776		
TOTAL	8	18.7740			

Fig. 1. Minimum and maximum mean water temperatures for the duration of experiments on flowrate, stocking density, cleaning and supplements. From the 8th week, heaters were installed in the water bath to raise water temperature to about 27 °C. (Broken line = average of minimum and maximum temperatures; vertical bar = s.d., other s.d. too small to plot)

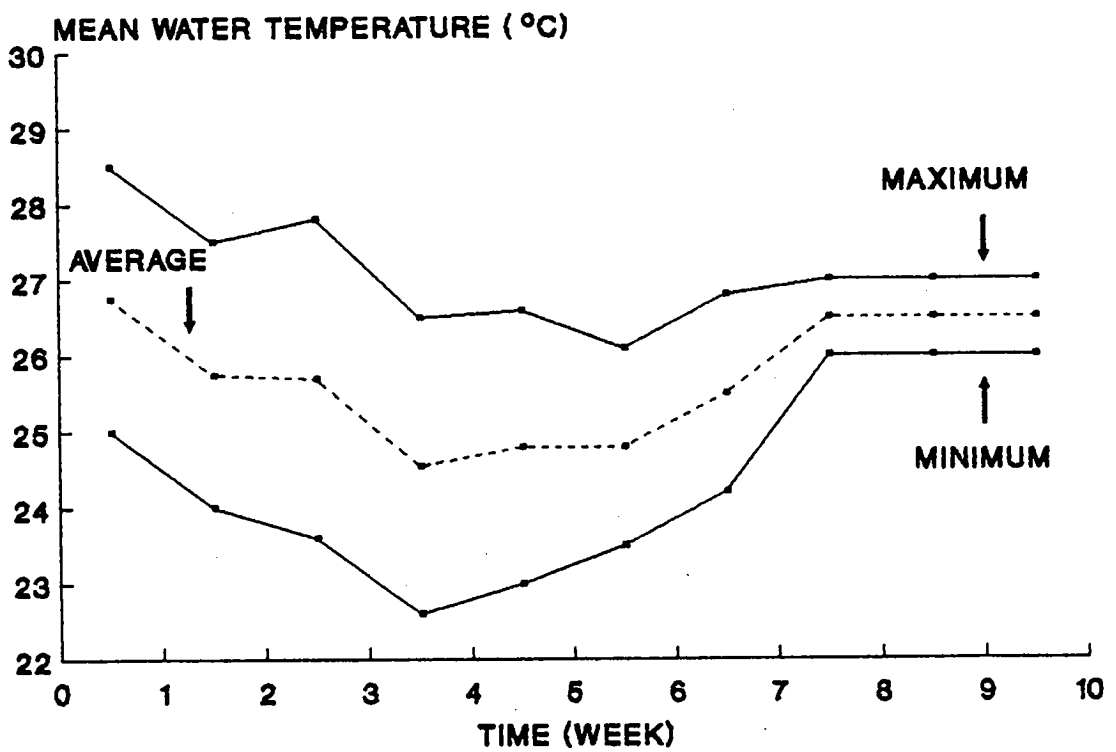
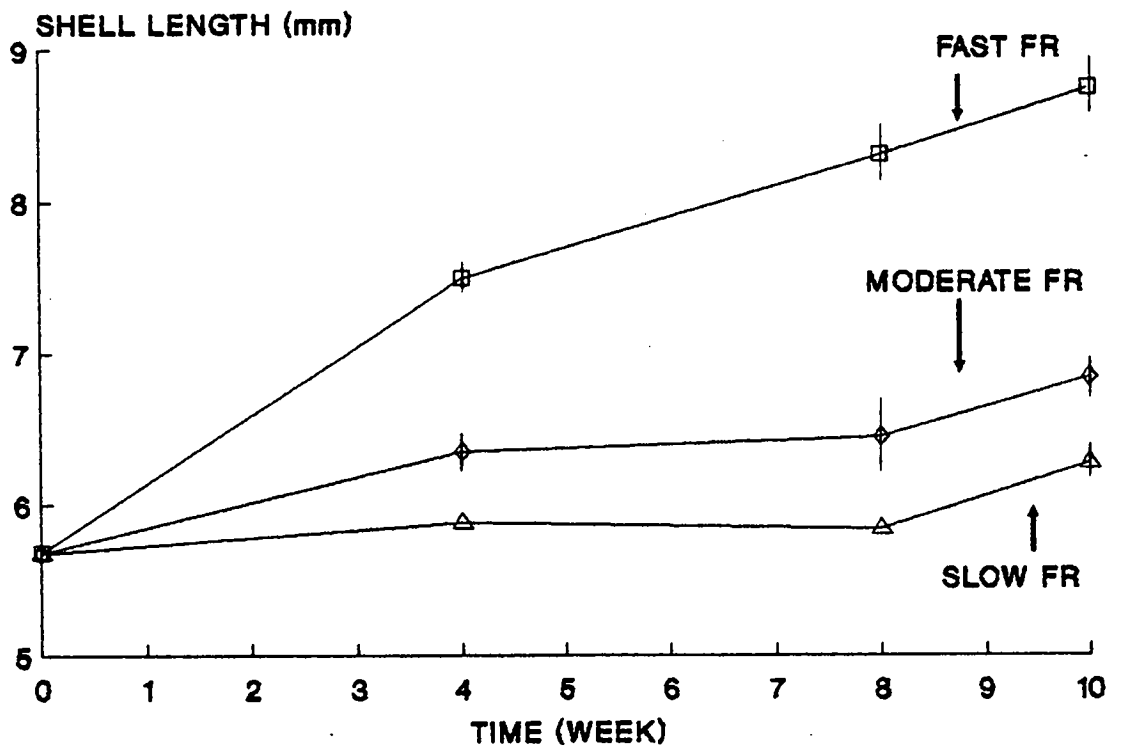


Fig. 2. Growth of juvenile clams at 3 flowrate (FR) levels: 1.5 $l.h^{-1}$ (fast FR), 0.2 $l.h^{-1}$ (moderate FR) and 0.02 $l.h^{-1}$ (slow FR). (vertical bar=s.d.; other s.d. too small to plot)



In order to determine whether there were size-related causes for mortality at high SD, size-class distributions were compared for live and dead clams using 8-week SL measurements of live clams (a total of 60 measurements) and SL measurements of dead clams that were collected from week 5 to week 8 (Table 5). A 2-Way ANOVA of percentage frequencies (arcsine transformed) showed no significant difference between proportions of dead and live clams ($F = 0.50$, $df = 1,7$, $P>0.50$). There was also no significant difference between proportions of live and dead clams per size class ($F = 4.52$, $df = 7,7$, $P>0.05$).

Growth of clams reared at high, moderate and low SD for a period of 10 weeks is shown in Fig. 3. Shell lengths were significantly different between treatments after 10 weeks (Table 4B). Growth was greatest at low SD (76% increase from initial SL), and lowest at high SD (increase was 20%). Clams at moderate SD exhibited 54% growth (Table 3). Temperature effect on clam growth (compare Figs. 1 and 3) was also observed for the SD treatments, except for low and moderate SD at W0-3 and W8-10.

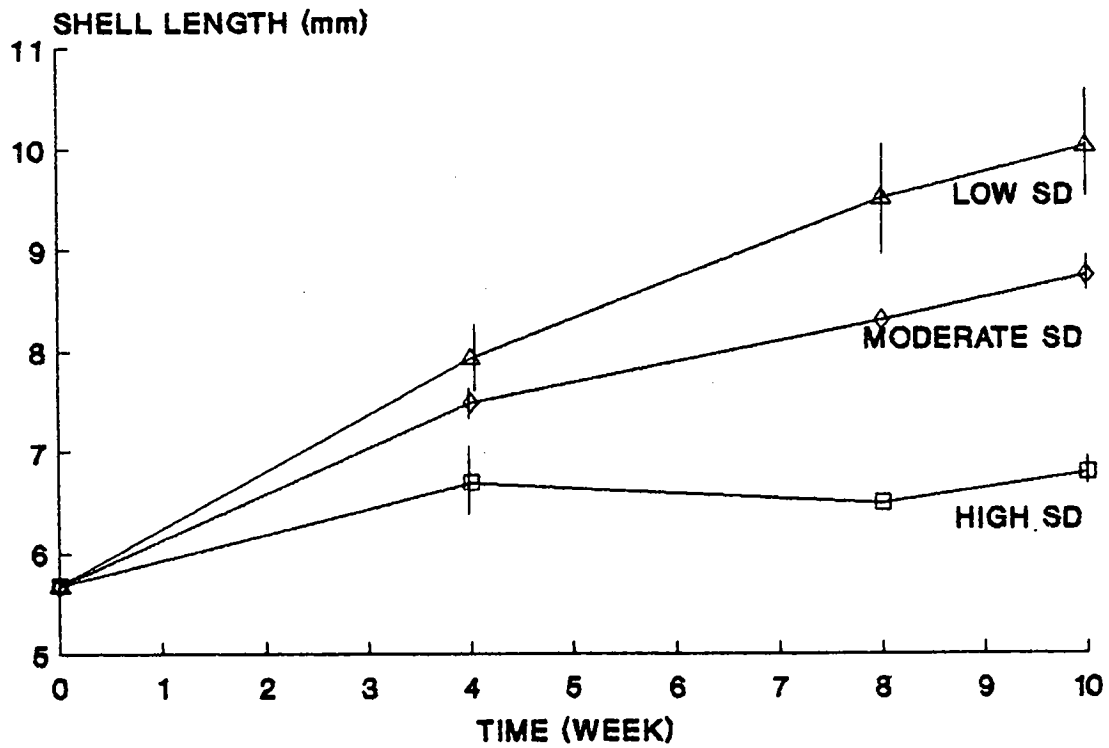
5.3.3. Dissolved Inorganic Nutrients, Algal Food and Cleaning

Survival of clams fed with microalgae (AF) or dissolved inorganic nutrients (DIN), and subjected to cleaning is shown in Table 2. Two-Way ANOVA on clam survival under conditions of: 1) cleaning and AF; 2) cleaning and DIN; 3) cleaning and no supplements (control); 4) no cleaning and AF; 5) no cleaning and DIN; and 6) no cleaning and no supplements (control) showed no significant differences after 4 weeks (Cleaning/No Cleaning: $F = 1.94$, $df = 1,6$, $P>0.20$; Supplements: $F = 0.53$, $df = 2,6$, $P>0.50$; Interaction: $F = 0.53$, $df = 2,6$, $P>0.50$) and after 8 weeks (Cleaning/No Cleaning: $F = 0.35$, $df = 1,6$, $P>0.50$; Supplements: $F = 2.04$, $df = 2,6$, $P>0.20$; Interaction: $F = 3.72$, $df = 2,6$, $P>0.10$).

Table 5. Size-class distribution for clam mortality at high stocking density from week 5 to 8, compared with the distribution for live clams under the same conditions for 8 weeks. Values in parentheses are percentage frequencies.

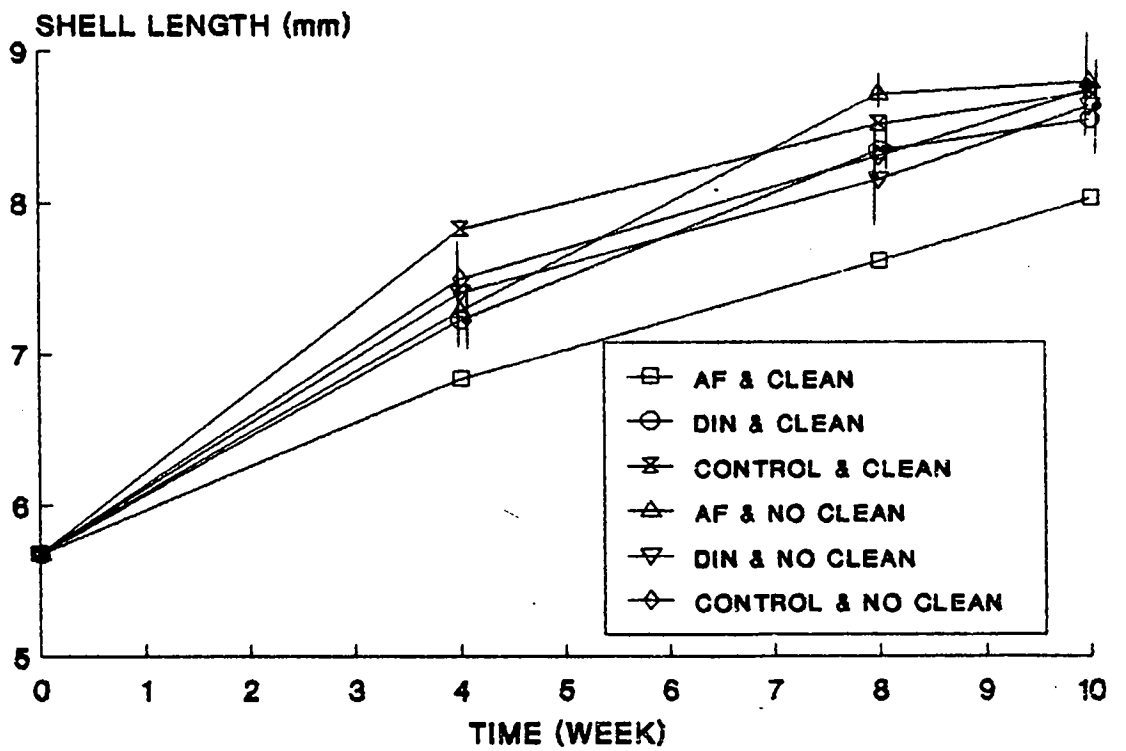
	SIZE-CLASS (mm)							
	4.1-5.0	5.1-6.0	6.1-7.0	7.1-8.0	8.1-9.0	9.1-10.0	10.1-11.0	11.1-12.0
DEAD	12 (9.23)	41 (31.54)	46 (35.38)	19 (14.62)	6 (4.62)	4 (3.08)	1 (0.77)	1 (0.77)
LIVE	4 (6.67)	17 (28.33)	19 (31.67)	14 (23.33)	5 (8.33)	0 (0)	0 (0)	1 (1.67)

Fig. 3. Growth of juvenile clams at 3 stocking density (SD) levels: 5 clams.cm^{-2} (high SD), 1 clam.cm^{-2} (moderate SD) and 0.2 clam.cm^{-2} (low SD). (Vertical bar=s.d.; other s.d. too small to plot)



Growth of clams is shown in Fig. 4. Final SLs are presented in Table 3. A 2-Way ANOVA of shell lengths after 10 weeks revealed no difference between treatments (Cleaning/No Cleaning: $F = 1.44$, $df = 1,6$, $P>0.50$; Supplement: $F = 0.66$, $df = 2,6$, $P>0.50$; Interaction: $F = 0.62$, $df = 2,6$, $P>0.50$).

Fig. 4. Growth of juvenile clams under Cleaning and Supplements treatments. AF=algae added, DIN=dissolved inorganic nutrients added, Clean=with cleaning, No Clean=without cleaning, Control= without AF or DIN. (Vertical bar=s.d.; other s.d. too small to plot)



5.4. Discussion

Stocking density (SD) was the main factor that influenced survival, with overcrowding accounting for some mortalities. This has also been observed for juvenile Tridacna maxima, <5 mm SL (Alcazar and Solis 1986) and T. gigas, 40-50 mm SL (Govan 1990). Some shells of T. gigas grown in high densities were deformed, as has been noted by Heslinga et al. (1984) for T. squamosa. With T. gigas, high stocking densities (5-6 clams per cm^2, or 50,000-60,000.m^{-2}) resulted in 20% mortality over 10 weeks. Braley et al. (1988) noted mortality rates of 21%-73% for 2-month old juveniles kept at similar stocking densities for 4 months. Heslinga et al. (1984) reported very low mortalities of less than 2.0% for tridacnids between 10 mm and 30 mm SL, when reared at a stocking density of 1000.m^{-2}. In the present study, clams reared at 0.2-0.3 per cm^2 (or 2000-3000.m^{-2}) showed no mortality. This agrees with the recommendation of Heslinga and Fitt (1987) that maximum production may be at 2000.m^{-2} per rearing cycle for 10 mm seed.

Significant mortalities at high stocking densities may be attributed to the restriction of resources available per clam. Specifically, water volume per clam was reduced from 80 ml at low SD to 3.2 ml at high SD; while for flowrate per clam (FRC) decreased from 150 ml.h^{-1} to 6 ml.h^{-1} at low and high SD, respectively. These results imply that, at high SD, supply of food (inorganic and organic dissolved nutrients, particulates and phytoplankton)is limited, and that toxic waste products may build-up, together with increasing bacterial populations. In addition, the allocated space per clam was reduced from 5 cm^2 to 0.2 cm^2 from low to high SD, hence confining the clam's activities (e.g. locomotion, mantle expansion) to a smaller area. Clams interfere with each other in close proximity so that some are not properly positioned towards the light.

For the size range studied (4.0-12.0 mm SL), mortality was shown not to be a function of size, although on several occasions clams have been observed to interfere

with each other by clamping their valves on their neighbors. An 8-month study on stocking density using clams belonging to the 40-50 mm size class (initial size) has given indications that small clams suffer higher mortalities than large clams (Govan 1990). In the present study, the size class studied for Tridacna gigas was probably not large enough to show any size-dependence of mortality. Such dependence may be evident with a wider size class that emphasizes the gap between small and large juvenile clams.

Similar to the effect of stocking density on survival, a diminished flowrate results in a restriction of food supply. Moreover, there is a decrease in the rate of water change and, hence, a reduction in water turnover time (TOT). For the volume of water (0.8 l) used in this study, TOT in fast FR was estimated at 1.5 h (95% replacement, based on Sprague 1969), 15 h in moderate FR (0.2 $l.h^{-1}$), and 6 1/4 days in slow FR.

Different researchers on tridacnids have used different values for TOT (Table 6). Braley et al. (1988) working on T. gigas applied a TOT of 24 h using 2000-10,000 l tanks. Compared to the present study, a TOT of 24 h is close to a flowrate of 0.1 $l.h^{-1}$, falling between moderate FR and slow FR (TOT approximation based on Sprague (1969)). Other workers have used a TOT of 1.3 h (Heslinga et al. 1984, Solis et al. 1988). Relative to the present study, this is comparable to a TOT of 1.5 h resulting from fast FR.

The value of a good flowrate in juvenile-rearing is related to the flowrate per clam. This is shown by comparing flowrate per clam (FRC) at SD and FR treatments (Table 7). Moderate FR/moderate SD and high FR/high SD treatments having almost similar FRC of 4 $ml.h^{-1}.clam^{-1}$ and 6 $ml.h^{-1}.clam^{-1}$, respectively, produced similar clam growth rates (compare Figs. 1 and 3, see Table 7). At an FRC of 30 $ml.h^{-1}.clam^{-1}$, the increase was 54%. The major increase in growth with increasing FRC was achieved in the range 6-30 $ml.h^{-1}.clam^{-1}$ with SL increment increasing from

Table 6. Comparison of water turnover time (TOT) employed for tridacnids. (Determination of TOT was based on Sprague's (1969) approximation of times required for partical replacement of water in tanks in constant flow situations).

SPECIES	TANK VOLUME (l)	FLOWRATE ($l.h^{-1}$)	PARTICAL REPLACEMENT	TOT (h)	SOURCE
Hippopus hippopus	5000	1800	95%	1.3	Heslinga et al. 1984
	1440	3360-3600	95%	1.3	Solis et al. 1988
H. porcellanus	1440	3360-3600	95%	1.3	Solis et al. 1988
Tridacna derasa	5000	1800	95%	1.3	Heslinga et al. 1984
	1440	3360-3600	95%	1.3	Solis et al. 1988
T. gigas	5000	1800	95%	1.3	Heslinga et al. 1984
	2000-10,000	---	---	24	Braley et al. 1988
	1440	3360-3600	95%	1.3	Solis et al. 1988
	0.8	1.5	95%	1.5	This Study
	0.8	0.2	95%	15	-ditto-
	0.8	0.02	95%	150	-ditto-
T. maxima	1400	3360-3600	95%	1.3	Solis et al. 1988
T. squamosa	1400	3360-3600	95%	1.3	Solis et al. 1988

Table 7. Percentage shell length (SL) increment of clams over 10 weeks under controlled conditions of flowrate (FR) and stocking density (SD). (Moderate SD and fast FR refer to the same experimental pot.) FRC=flowrate per clam.

TREATMENT	FR ($l.h^{-1}$)	SD ($clam.cm^{-2}$)	FRC ($ml.h^{-1}.clam^{-1}$)	SL INCREMENT (%)
LOW SD	1.5	0.2	150	76.4
MODERATE SD / FAST FR	1.5	1	30	53.9
MODERATE FR	0.2	1	4	20.4
HIGH SD	1.5	5	6	19.5
SLOW FR	0.02	1	0.4	10.4

ca. 20% to 54%. A further 120 $ml.h^{-1}$ increase in FRC resulted in raising SL increment only from 54% to 76%. In view of the cost of pumping seawater through nursery tanks, a balance must be struck between achieving high growth ratio versus the cost of high FRC. These data suggest that the balance will be in the range of FRC approximately 30 $ml.h^{-1}.clam^{-1}$ for this size range of juveniles. These results also suggest that availability of particulate foods, DOM or inorganic nutrients (or two factors or all three) are limiting to growth at low FRC.

After 10 weeks, shell lengths of inorganic nutrient-fed clams (DIN: ammonium, phosphate and sulfate), algal-fed clams (AF: Isochrysis galbana) and those not given any supplements were not significantly different (Table 3), implying that improvement of growth in juvenile Tridacna gigas was not dependent on the supplementary food (algae or nutrients). Similar findings on juvenile T. gigas were obtained by Crawford et al. (1986). However, contrary preliminary findings were found by Estacion et al. (1986), reporting improved growth in T. maxima with supplementary food (combination of I. galbana and Tetraselmis sp). However, their comparison of growth was made between algal-fed clams in unfiltered seawater (UFSW)and unfed clams in filtered seawater (FSW) (control). Better controls (e.g. unfed clams in UFSW) or additional treatments (e.g. algal-fed clams in FSW) would have given more conclusive results. In addition, the lack of effect of supplements on growth found here is contrary to the finding of the stocking density and flowrate studies (see preceding paragraph).

Based on growth results at different FRC, optimum growth was not achieved at 30 $ml.h^{-1}.clam^{-1}$ (Fig. 3), which was also the FRC in the algae and dissolved inorganic nutrients (DIN) experiments. Clearly, some resources were limiting, and it would seem that addition of either algae or nutrients or both would promote growth. It was therefore surprising that neither supplementation

improved clam survival nor shell growth (Fig. 4). This lack of effect may be attributed to a number of factors:

1) Relatively high phosphate concentration in the Dix Mix and algal culture. Phosphates may act as crystal poisons hence inhibiting shell growth (see Section 5.1.4).
2) Requirements for inorganic nutrients by the clam or zooxanthellae that may not be satisfied by either the simple Dix mix solution or microalgae.
3) Competitive absorption of the inorganic nutrients by fouling filamentous algae. Supplementary food presents some technical difficulties. Biofouling as a result of benthic algal overgrowth has been reported (Crawford et al. 1987, Heslinga and Fitt 1987). The presence of an enriched medium, as happens when algae or nutrients are added, encourages the growth of other organisms that confound the effect of supplementary food on clam growth. Fouling leads to poor survival rates in juveniles (Crawford et al. 1987). Crawford et al. (1986) reported that in cultures of juvenile Tridacna gigas provided with Isochrysis galbana and Chlorella sp., filamentous algal overgrowth was observed to such an extent that juvenile clams were outcompeted in terms of space and probably nutrients. This was similarly observed in the algae- and nutrient-enrichment experiments of the present study. It was surprising that the cleaning treatment was not effective in improving growth or survival. This may be attributed to the fast growth rates of the fouling algae and their greater efficiency compared to zooxanthellae in absorbing the nutrients.
4) The growth parameter (shell length) used in the present study. Another ammonium-enrichment study on T. gigas has used the ratio of wet tissue weight against shell length, and yielded significant differences in tissue growth for the same shell length for fed and unfed clams (Mingoa, unpubl. data). Furthermore, DIN-enrichment studies on juvenile T. maxima showed that discernible differences in growth are obtained when based on a condition index using

flesh weight against total live wet weight, instead of other parameters based on shell growth, total live wet weight and the ratio of shell weight to total live wet weight (Trinidad-Roa 1989).

5) The duration of the experiments. Estacion et al. (1986) stated that differences become apparent only after 2 months of observation. Thus, the 10 weeks of this study may not be sufficient time to allow for differences to become evident.

6) The effect of cool weather months on growth rate. As observed by Manzi et al. (1986), Mercenaria mercenaria grows at a slower rate during the winter months. For the Tridacna gigas juveniles used in the present study, differences in shell lengths that can be attributed to the different treatments may not be large enough to be detected.

Other workers have shown for other bivalves that the influence of algae on growth is dependent on interaction with other factors. Rodhouse and O'Kelly (1981) found that slow flowrate increases the rate of filtration in Crassostrea gigas, and hence the efficiency of food collection. Despite this, Spencer (1988) found growth of kC. gigas to be very much reduced. Spencer (1988) demonstrated the dependence of growth of juvenile Ostrea edulis and C. gigas on a combination of factors, specifically filtration, flowrate, temperature, food concentration and oyster size. It is possible that the food concentration and seawater flowrate used in the present study for Tridacna gigas were not compatible. This needs further study.

6. EMERSION

6.1. Introduction

Only recently have emersion effects been considered in studies on juvenile tridacnids, and these mainly for mariculture needs. Juveniles of Tridacna derasa 10-50 mm SL have high survival at 24-28°C for emersion periods of up to 24 hours (Lopez and Heslinga 1985). Furthermore, Solis and Heslinga (1989) showed enhanced survival among juveniles (10-30 mm SL) emersed in oxygen-saturated atmospheres at 27-32°C. These studies aimed at improving survival during "seed" clam transport demonstrate that juveniles are able to withstand substantial periods of emersion under favorable environmental conditions.

Four tridacnid species (T. gigas, T. maxima, T. crocea and Hippopus hippopus) occur in intertidal areas subject to frequent tidal exposure. Hence, their capacity for physiological tolerance of air exposure is not surprising; however, it will probably vary within species by virtue of size differences and between species due to differences in physiology and morphology. Long-term studies by Nash (1988) on emersion effects on T. gigas showed higher survival for larger juveniles (about 100 mm size) than smaller ones (about 30 mm size) over prolonged tidal exposure periods. In addition, growth rates correlate positively with exposure for periods of up to 3-4 hours (Lucas et al. 1989, Nash 1988), apparently due to higher air than water temperatures.

There are three important considerations for intertidal organisms during emersion: water conservation, energy conservation and energy acquisition (Shick et al. 1986, 1988). Emersion inhibits several of the organism's metabolic functions, for instance, feeding, respiration and excretion. Bivalves in general show adaptive strategies in behavior and metabolic regulation to exposure-related stresses, although these strategies often entail a cost in energy for the maintenance of vital functions during exposure or in recovery following

emersion. While a number of commercially important bivalves have been studied, there is a gap in knowledge regarding physiological responses of tridacnid juveniles to air exposure.

6.1.1. Phototrophy

The significance of phototrophy (Chapter 2, Section 2.1) in tridacnids during air exposure needs to be investigated. It is known that Tridacna gigas juveniles keep their valves agape during air exposure and Lucas et al. (1989) hypothesized that "it is highly likely that at least some photosynthesis continues in air due to very high incident light levels on the withdrawn mantle tissue.... It is possible that giant clams continue to gain energy and nutrients during emersion." Aerial photosynthesis has already been demonstrated in the symbiotic sea anemone Anthopleura elegantissima, with the algae still photosynthesizing during air exposure (Shick and Dykens 1984). By estimating that portion of energy input that can be attributed to zooxanthellar photosynthesis, the importance of aerial photosynthesis in T. gigas during air exposure can be assessed.

6.1.2. Respiration

Gas exchange in bivalves may occur in water: 1) by diffusion across general cutaneous surfaces, and 2) by means of the gills (see Ghiretti 1966). The gills of most bivalves serve primarily for filter-feeding, and secondarily for gas exchange (Nicchitta and Ellington 1983). In mussels, Famme and Kofoed (1980) showed that oxygen is transferred to the tissues primarily via the mantle cavity lining, and less importantly via the gills. However, this may not be the case for tridacnids. In T. maxima, photosynthetic oxygen does not diffuse across the mantle surface (J. J. Childress, unpubl., cited in Trench 1987). Trench et al. (1981a) suggested that photosynthetic oxygen may be transported to and unloaded at the gills, resulting in high levels of oxygen in the

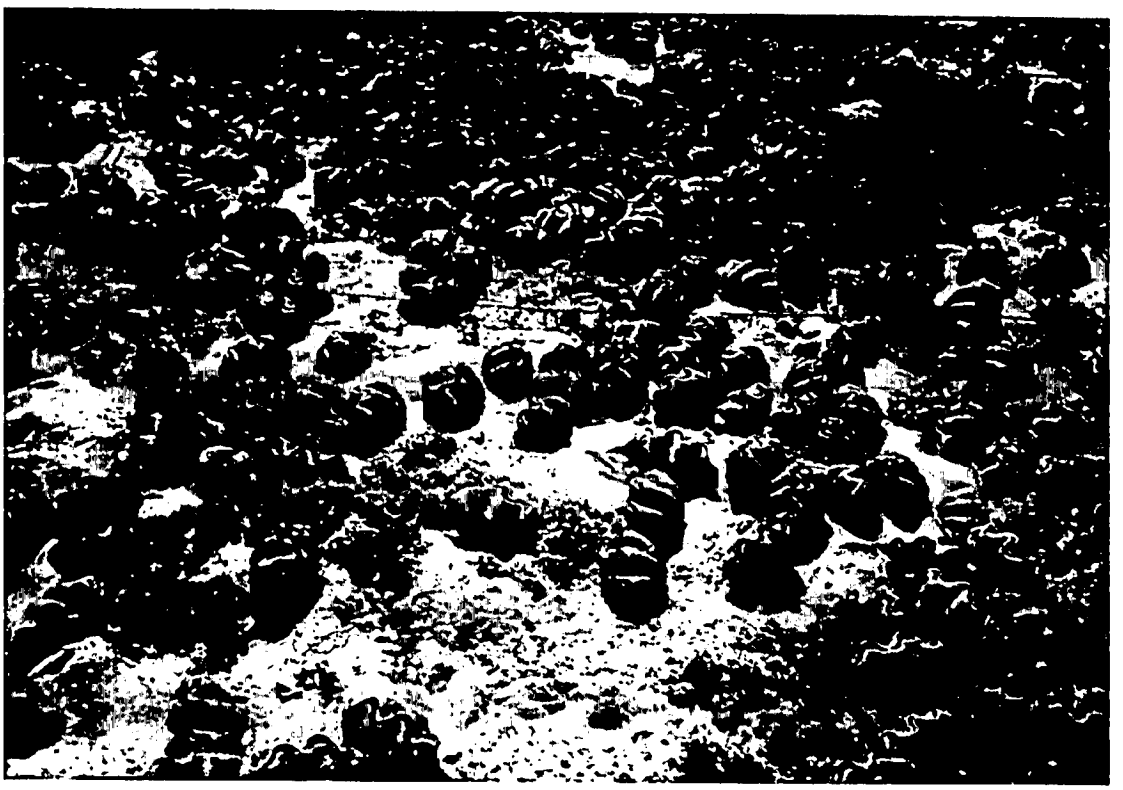
blood. Trench (1987) further suggested that some mechanism must be employed to remove oxygen, thence guarding against photorespiration and oxygen inhibition of photosynthesis.

Most bivalves are capable of aerial respiration, although aerial respiratory rate is generally lower than the aquatic rate (e.g. Widdows and Shick 1985). The gills' efficiency in absorbing atmospheric oxygen diminishes as the large, soft gills collapse out of water, reducing their surface area for gas exchange. Emersed bivalves differ in behavior, such as valve adduction, the degree of shell gape and the retraction of sensitive body parts, in response to exposure stress (see Fig. 1), such that bivalves may be classified as "gapers" and "non-gapers" (Pamatmat 1983, see Shick et al. 1986, Widdows et al. 1979). Controlled air gaping promotes evaporative cooling, which may be more pronounced in small individuals (Gnaiger 1983, Lent 1968). Gapers like Cardium edule and Geukensia demissa (=Modiolus demissus) have a higher rate of oxygen uptake (28-78% of the aquatic rates) than non-gapers like Mytilus edulis and M. galloprovincialis (4-17% of the aquatic rate) (Widdows and Shick 1985, Widdows et al. 1979).

The capacity for aerial respiration differs among bivalves and may be related to their capacity for anaerobic metabolism. A reduction of oxygen uptake during air exposure may lead to the utilization of the anaerobic metabolic pathway and the accumulation of anaerobic end-products (Widdows et al. 1979). It has not been shown that tridacnids employ anaerobic pathways.

Changes in whole organism respiration have been used to measure metabolic adjustments during emersion. By comparing the aerial respiration rate with the "normal" aquatic rate, the level of physiological stress on the individual can be assessed. However, recent studies have shown that respirometry methods may underestimate the total metabolic energy expenditure, particularly of non-gapers that utilize anaerobic metabolic processes under

Fig. 1. Tridacna gigas juveniles displaying gaping behavior during tidal exposure. (The clams shown here are part of the grow-out phase of the JCU Giant Clam Project at Orpheus Island Research Station.)



hypoxic or anoxic conditions. For gapers, energy expenditure based on oxygen uptake during air exposure approximates the predicted rate based on catabolic heat dissipation, indicating a largely aerobic type of metabolism during emersion (Widdows and Shick 1985).

There are apparently different anaerobic pathways in bivalves with various end-products, for instance, in Mytilus edulis, propionate (Shick et al. 1988) and strombine (de Zwaan et al. 1983); in Modiolus squamosus, alanine, succinate, D-lactate (Nicchitta and Ellington 1983), alanopine and strombine (Fields 1976); and in Cardium edule, alanine, succinate and octopine (Gade 1975). Such pathways are characterized by: 1) greater biochemical efficiency than lactogenic glycolysis (de Zwaan 1983, Gnaiger 1983); 2) a reduction in ATP demand; and 3) the suppression of the Pasteur effect (or enhanced anoxic rate of glycolysis). These lead to the conservation of glycogen reserves (Shick et al. 1988).

Direct calorimetry coupled with respirometry measurements determines the anaerobic component of total aerial metabolism. A reduction in metabolic rate (in terms of total heat dissipation) has been observed, and is considered to be an energy-saving mechanism during air exposure. In Mytilus edulis (a non-gaper), the total heat dissipation is 14 to 20% of the aquatic rate. By comparison, C. edule (a gaper) exhibits higher aerial energy expenditure rates of 50 to 75% the aquatic rate (de Zwaan 1977, Pamatmat 1980, Widdows and Shick 1985).

Aerial respiration rate is influenced by temperature, season, the availability of food and acclimatization conditions (whether subtidal or intertidal) (e.g. Shick et al. 1988). For instance, in the case of the filter-feeder M. edulis, it not only faces desiccating conditions, but also a lack of food supply. By closing its valves, M. edulis conserves water and energy; additionally, by controlled air gaping, it permits some aerobic metabolism to meet the costs of digestion and

assimilation which continue during emersion (Shick et al. 1988).

After air exposure, bivalves generally exhibit a metabolic deficit, referred to as "oxygen debt", which is manifested as an overshoot in oxygen uptake upon re-immersion. The size of oxygen debt has been correlated with length of exposure (Shick et al. 1986), temperature (Widdows et al. 1979) and ration (Widdows and Shick 1985), and its payment may occur within two hours of aquatic recovery as indicated by the decrease in rate of oxygen uptake approximating pre-exposure levels (Widdows and Shick 1985).

6.1.3. Excretion

The major nitrogenous excretory product in bivalves is ammonia (Bishop et al. 1983). Other excretion products include uric acid (Hammen 1969), amino acids (Heavers and Hammen 1985, Rice et al. 1980), and purines (Campbell and Bishop 1970).

Ammonia excretion in tridacnids is of particular interest because of their algal endosymbionts. Zooxanthellae *in vivo* and *in vitro* are known to absorb ammonium from their surroundings (Burris 1983, Wilkerson and Trench 1986). The rate of uptake by zooxanthellae is dependent on the clam's previous light exposure, as well as the ambient ammonium concentration levels (Wilkerson and Trench 1986). Internal recycling of nutrients occurs between the tridacnid host and its zooxanthellae (Heslinga and Fitt 1987, Summons et al. 1986). It is surprising, however, that ammonia excretion in tridacnids has received little attention, considering its obvious and significant implications for the algal symbiosis.

Studies on other bivalves have shown that ammonia results from amino acid catabolism (see Bishop et al. 1983), and from chemical oxidation of amino acids and other organic residues by aminifying bacteria (Odum 1971). Ammonia diffuses into the surrounding water. Excretion rates have been shown to be a function of body size (Bayne

et al. 1976a, Emerson 1969), temperature (Bayne and Scullard 1977), salinity (Emerson 1969) and season (Widdows and Shick (1985).

Bayne and Scullard (1977) have shown that small and large individuals of Mytilus edulis are not in phase when utilizing their protein reserves. During winter and spring, smaller individuals rely more on carbohydrate catabolism, as evident from the depressed rate of ammonia excretion; whereas larger individuals have marked increases in ammonia excretion, indicating the utilization of protein reserves during this time.

Salinity fluctuations influence the rate of ammonia excretion. For instance, ammonia excretion rate in Macoma inconspicua increases with transfer from 100% to 50% salinity. This is due to the involvement of free amino acids (FAA) in the maintenance of internal solute equilibrium (see Emerson 1969). Catabolism of FAA during FAA pool reduction produces ammonia as an end product. Changes in the rate of ammonia excretion and in levels of ammonia in the tissues of Crassostrea virginica have been detected with change in salinity (Heavers and Hammen 1985).

Ammonia production in bivalves during air exposure has been studied, e.g. in M. edulis, ammonia accumulates not in the tissues, but in the hemolymph and mantle cavity fluid (Shick et al. 1988). By contrast, ammonia was not detected in hemolymph samples from Tridacna maxima (Deane and O'Brien 1980). Ammonia is known to participate in the acid-base balance of the extracellular fluids during low oxygen conditions, for instance, by restricting the rate of shell decalcification (Shick et al. 1988). It has also been suggested that ammonia may be involved in anaerobiosis (Bayne et al. 1976a).

Widdows and Shick (1985) found different ammonia excretion responses for intertidal and subtidal M. edulis. Whereas no change in excretion rate upon re-immersion was shown by subtidal individuals, intertidal individuals reduced their excretion rate by 50% of the pre-exposure

aquatic rate (see de Vooys and de Zwaan 1978). This reduction in excretion rate was also observed in Mytilus californianus (Bayne et al. 1976b): re-immersion excretion rate of intertidally-acclimatized individuals was only 14% of the normal aquatic rate, while for subtidally-acclimatized individuals it was 65% of the normal rate (Widdows and Shick 1985).

6.1.4. Aims of this Study

The aims of this study were to investigate the following aspects of emersion effects on juvenile Tridacna gigas:

a) survival rates following emersion at different exposure conditions, considering the duration of emersion, availability of atmospheric oxygen, and humidity levels;
b) oxygen debt related to duration of aerial exposure;
c) ammonia excretion rates related to duration of air exposure;
d) significance of phototrophy to clam nutrition during emersion.

6.2. Materials and methods

All laboratory studies were conducted at James Cook University (JCU) unless otherwise stated. Experiments were performed at ambient temperature 27 ± 1°C. Exposure treatments were conducted in the dark.

6.2.1. Survival with exposure to aerobic and anaerobic atmospheres at different humidities

The effects of atmospheric conditions and exposure period on survival of juvenile Tridacna gigas (SL ± s.d. = 76.43 mm ± 3.19 mm) were investigated at high (>90%) and low (<10%) relative humidity (R.H.), in aerobic and anaerobic gaseous environments and for two exposure periods (9 and 27 hours). There were 3 replicates (n = 10 clams) per treatment.

The clams were kept in darkened airtight chambers (2.7 l), provided with an inlet and an outlet, both valve-controlled, for gas-flushing. To achieve aerobic and anaerobic conditions, the chamber were slowly flushed with oxygen and nitrogen gas, respectively, for 20 minutes. R. H., measured using a humidity meter, was maintained within the chamber by the addition of filtered seawater (FSW) for high humidity, or desiccant (silica gel) for low humidity (see Boyden 1972, von Brand et al. 1950).

After exposure, the clams were re-immersed in running seawater. Percentage survival was observed after 24 and 48 hours. The criteria used to assess mortality were: gaping valves, retracted mantle and lack of response to gentle prodding.

6.2.2. Water Loss

The influence of humidity conditions on the rate of water loss in juveniles during air exposure was determined. Clams (SL ± s.d. = 76.76 mm ± 2.39 mm) were exposed to humid and to desiccating environments, both oxygen-saturated, in darkened, tightly sealed chambers (see Section 6.2.1). Ten tagged clams were used per

treatment, with three replicates each. Clams were drained of cavity fluid, then the total wet weight (TWW) was measured prior to exposure and measured again after cumulative exposure periods of 3, 9, 18 and 27 h. Treatment conditions were maintained for the duration of the experiment by re-flushing the chamber with oxygen and checking the desiccant.

Clams were sacrificed after 27 h to obtain corresponding wet tissue weight (WTW) and dry tissue weight (DTW). The relative difference between WTW before and after exposure was used as a measure of the extent of water loss (see Coleman 1973). Percent water loss was calculated from the equation:

$$\text{Percent water loss} = \frac{WTW1 - WTW2}{WTW1 - DTW} \times 100\%$$

where the numerator refers to water loss; the denominator to water content; and 1 and 2 indicate WTW values before and after exposure, respectively.

6.2.3. Oxygen debt

Oxygen debt for two size classes (50-65 and 100-115 mm SL) was studied at three air exposure periods: 3, 9 and 18 h. The size of oxygen debt for each clam was determined by comparing the rate of oxygen consumption before and after emersion (see Boyden 1972, Moon and Pritchard 1970). Twelve clams were used per treatment. All clams were scrubbed of adhering organisms on the valves, and held overnight in running 1-µm filtered seawater (FSW) in the JCU recirculating seawater system.

Each clam was placed in a darkened, tightly-sealed container filled with FSW (salinity = 27 ppt): 0.2 l for small clams and 1 l for large clams. The seawater medium was gently stirred by a magnetic stirrer, and carefully sampled initially and after an hour using a 20-ml syringe. The medium was changed every hour for 3-4 hours before and after the exposure period. Additional samples were taken during the first hour of re-immersion, specifically after 10, 20 and 30 min of re-immersion. A separate control

experiment, following this procedure except for the exposure period, validated the sampling method. Samples were immediately transferred into a thermostatic cell (5 ml volume) for oxygen measurements. Oxygen concentration was measured as in Chapter 2, Section 2.2.4. The clam's respiratory rate was derived from the relative difference between the initial and subsequent samples.

Clams were sacrificed after each experiment to obtain corresponding DTW.

6.2.4. Ammonia excretion

Rates of ammonia excretion of juveniles before and after emersion were compared. Three air exposure periods were considered: 3, 9 and 18 h, with 12 clams (SL $\pm$ s.d. = 89.46 mm $\pm$ 3.27 mm) per treatment. Clams were scrubbed of all epibionts adhering to the valves, then held in running 1 µm FSW overnight (see Section 6.2.3).

Each clam was placed in a darkened, tightly-sealed container filled with 1 l of 1-um FSW. The FSW medium was gently stirred by a magnetic stirrer, and carefully sampled initially and after an hour using a 20-ml syringe (see Lum and Hammen 1964). The medium was changed every hour for 3-4 hours before and after the exposure period. Samples were stored in sterile "Whirlpak (NASCO)" bags and kept in a freezer (-20^{o}C). After each experiment, the clams were sacrificed to obtain DTW.

Three replicates (20 ml) per sampling were analyzed for ammonia using the phenolhypochlorite method (Solorzano 1969), and ammonia concentration was expressed as µg-atom NH_3-N.gDTW^{-1}.h^{-1} (see Parsons et al. 1984, Sharp 1983). The rate of excretion was derived from the relative difference between initial and hourly samples.

The initial excretion experiments were conducted with clams that had been kept in a recirculating seawater system at JCU. This system was characterized by high ammonia levels. To determine whether high ammonia levels in the recirculating seawater had an effect on the ammonia

excretion, a similar experiment was conducted at JCU, Orpheus Island Research Station (OIRS), where fresh seawater was available from a nearshore intake.

6.2.5. Photosynthesis and Respiration

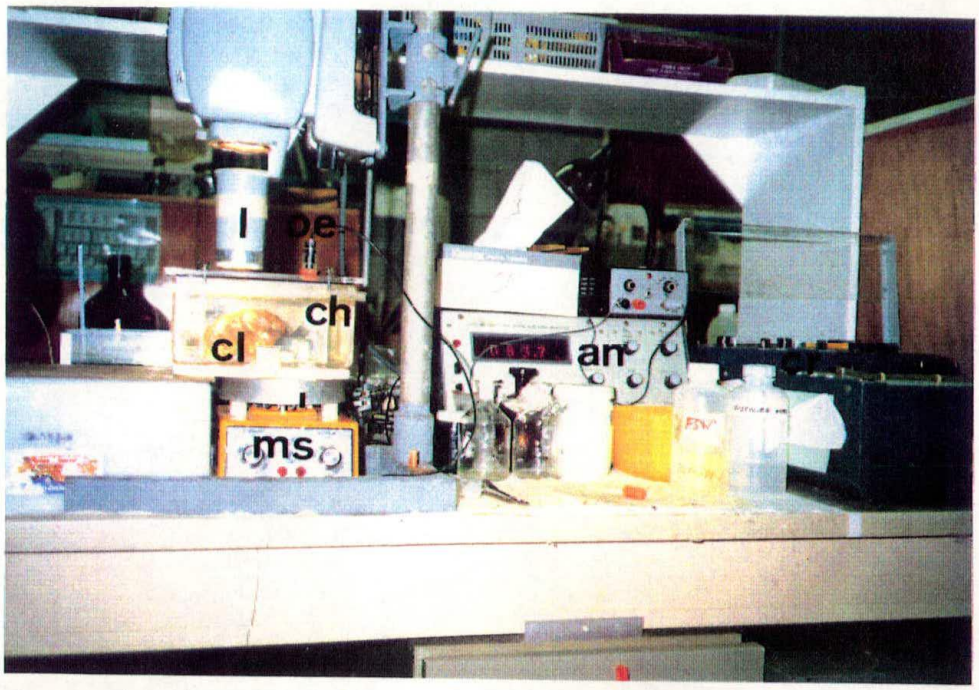
6.2.5.1. Photosynthesis and respiration of the intact clam

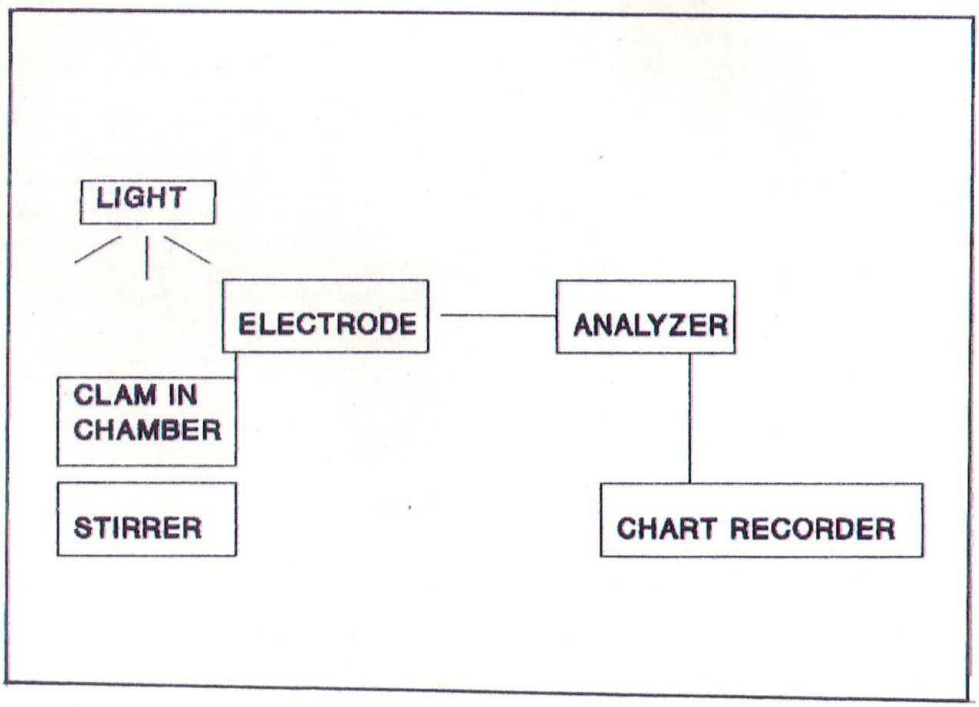
Rates of photosynthesis and respiration of juveniles (110-115 mm SL) during immersion (aquatic) and air exposure (aerial) were compared. The methodology for determining aquatic photosynthesis and respiration was similar to that in Chapter 2, Section 2.2.4. Fig. 2A outlines the experimental set-up (compare with Fig. 2B). A clam was placed in a hermetically sealed chamber filled with 0.45-µm FSW. Chamber volumes were 737.1 and 321.8 ml, depending on the size of the clam. A stirrer bar maintained the water circulation, while the temperature within the chamber was regulated by a water bath at 27°C. Seawater salinity was 33 ppt. Oxygen levels of the FSW medium were maintained above 75% saturation level. Respiration was measured in darkness. Oxygen production was measured at different light intensities (40-1670 $\mu E.m^{-2}.s^{-1}$). Illumination was provided by a slide projector (Prado 250/500, Leitz Wetzlar). All measurements were conducted between 7 a.m. and 7 p.m.

Aerial photosynthesis and respiration rates of juveniles were also determined with an oxygen electrode (Delieu and Walker 1972, 1981, Pamatmat 1978). Each clam was placed in a tightly-sealed chamber (802.8 ml chamber volume) with temperature regulated at 27°C by a water bath. A vial of carbon dioxide (CO_2) buffer (diethanolamine solution) capable of 95% absorption (see Krebs 1951, Pardee 1949, Umbreit 1964) was placed in the chamber. This buffer binds reversibly with atmospheric CO_2, maintaining the CO_2 level at 3%. The buffer ensures that atmospheric CO_2 is not depleted with continued photosynthesis (Umbreit 1964).

Fig. 2. (A) Experimental set-up (at James Cook University) for measuring rates of photosynthesis and respiration for whole Tridacna gigas juveniles. (cl=clam, ch=chamber, l=light, oe=oxygen electrode, ms=magnetic stirrer, an=oxygen analyzer, cr= chart recorder)

Fig. 2. (B) Diagram of experimental set-up shown in Fig. 2A.




LIGHT
ELECTRODE
ANALYZER
CLAM IN CHAMBER
STIRRER
CHART RECORDER

Photosynthesis during air exposure was measured at different light intensities, from 60 to 980 $\mu E.m.^{-2}.s^{-1}$. Aerial photosynthesis was not measured beyond 1000 $\mu E.m.^{-2}.s^{-1}$ for the reason that it required longer measurement times per clam. The gaping (or lack of gaping) behavior of the clam in the chamber largely contributed to longer measurement times. Hence, a sufficiently high irradiance about 1000 $\mu E.m.^{-2}.s^{-1}$ (see Fisher et al. 1985) was set as the maximum exposure irradiance.

Aerial respiration was determined in the dark. Measurements were conducted between 7 a.m. and 7 p.m. All clams were sacrificed after each experiment to obtain WTW.

6.2.5.2. Zooxanthellae isolation

Methods were as those described in Chapter 2, Section 2.2.6.

6.3. Results

6.3.1. Survival

All or most clams survived aerobic atmospheric conditions for 9 and 27 h periods (Table 1). Survival was significantly higher in oxygen-saturated conditions by about 60% at 9 h exposure, and by 100% at 27 h exposure than in anoxic conditions.

Table 2 shows a 3-Way Analysis of Variance (ANOVA) testing for differences between the effects of exposure period, aerobic and anaerobic conditions, and humidity on clam survival. There was significant interaction between exposure period and oxygen availability, indicating that the effect of exposure period on survival of juvenile Tridacna gigas was also dependent on respiratory oxygen being available. The effect of relative humidity on survival was not significant.

Clams kept in anaerobic conditions for 27 h appeared to lose large amounts of body fluids, unlike those kept in aerobic conditions. This was observed but not quantified. It was due to the longer periods of gaping during anaerobic conditions.

6.3.2. Water loss

Clams showed slight shell gaping during air exposure, allowing for surface evaporation. In addition, leaking of the remaining cavity fluid through the byssal orifice contributed to the apparent water loss.

Water loss in juveniles 70-80 mm SL continued for the duration of exposure, up to 27 h, with greater loss in low than in high humidity (Fig. 3). However, the extent of water loss was not substantial: a little over 3% of the clam's total water content after 27 h in humid conditions, and about 5% in desiccating conditions for the same exposure period.

6.3.3. Oxygen debt

Juveniles exposed to air incurred an oxygen debt, reflected by increased rates of oxygen consumption upon

Table 1. Clam survival upon re-immersion (for 48 h) after exposure to air for 9 and 27 h, in aerobic and anaerobic conditions, and low and high relative humidities (R.H.). n=10, 3 replicates per treatment.

9 h AIR EXPOSURE

	AEROBIC		ANAEROBIC	
REPLICATE	LOW R.H.	HIGH R.H.	LOW R.H.	HIGH R.H.
1	10	10	2	6
2	10	10	4	2
3	10	9	5	2

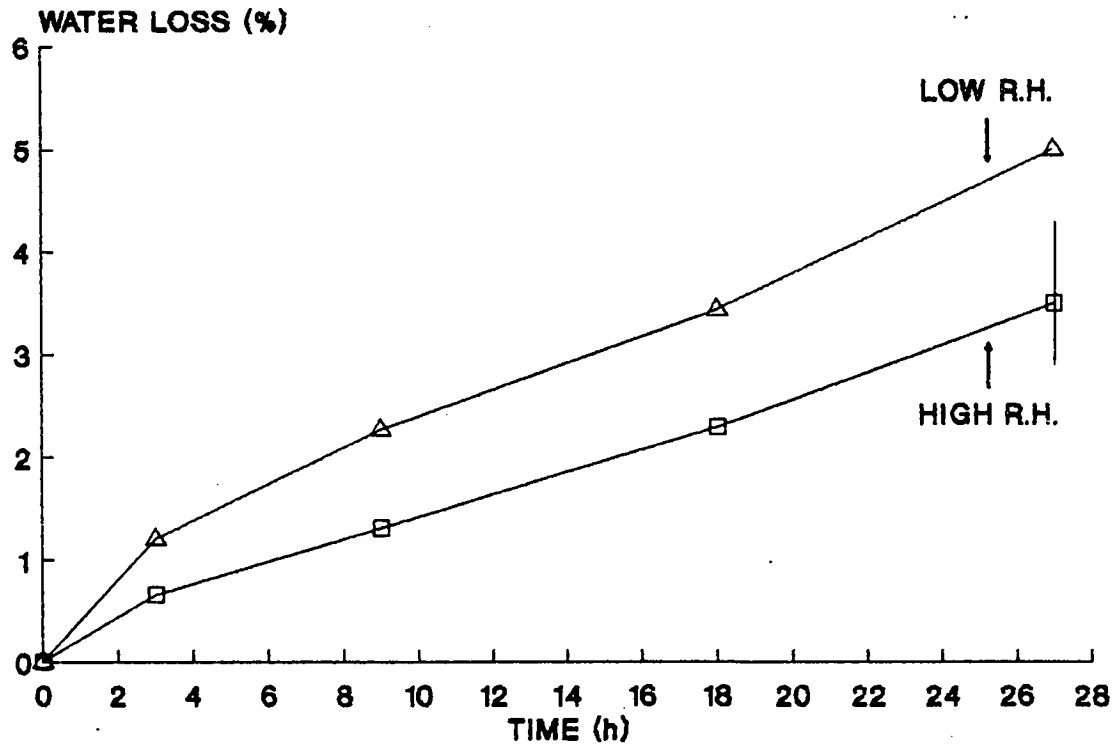
27 h AIR EXPOSURE

	AEROBIC		ANAEROBIC	
REPLICATE	LOW R.H.	HIGH R.H.	LOW R.H.	HIGH R.H.
1	10	10	0	0
2	10	10	0	0
3	10	7	0	0

Table 2. 3-Way ANOVA on the effect of air exposure (9 vs. 27 h), aerobic and anaerobic conditions, and relative humidity (<10 vs. >90%) on clam survival (see Table 1. Data were transformed using log(X+1)). P=level of significance.

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F	P
EXPOSURE PERIOD (A)	1	0.6178	0.6178	64.35***	«0.001
AEROBIC/ ANAEROBIC (B)	1	3.0555	3.0555	318.28***	«0.001
HUMIDITY (C)	1	0.0047	0.0047	0.49	»0.50
A x B	1	0.5571	0.5571	58.03***	«0.001
A x C	1	0.0001	0.0001	0.01	»0.50
B x C	1	0.00003	0.00003	0.003	»0.50
A x B x C	1	0.0026	0.0026	0.27	»0.50
WITHIN	16	0.1529	0.0096		
TOTAL	23	4.3907			

Fig. 3. Percent water loss (see Section 6.2.2. for formula) in juveniles during exposure to low (<10%) and high (>90%) relative humidities (R.H.) in oxygen-saturated atmosphere. n=10, 3 replicates. Vertical bar=s.d., other s.d. too small to plot.



re-immersion. For small clams (SL ± s.d. = 55.90 mm ± 7.20 mm), the rate of oxygen consumption after 3 h air exposure increased from 3.4 (the average pre-exposure rate) to 15.9 $\mu mol.gDTW^{-1}.h^{-1}$ (Fig. 4A); and after 9 h exposure, from 4.5 to 22.8 $\mu mol.gDTW^{-1}.h^{-1}$ (Fig. 4B). The oxygen debt was paid within 2 h of re-immersion, with respiration rates returning to pre-emersion levels.

For large clams (SL ± s.d. = 109.64 mm ± 7.64 mm), respiration rates increased from 3.6 to 14.1 $\mu mol.gDTW^{-1}.h^{-1}$ after 3 h air exposure (Fig. 5A); and after 9 and 18 h exposure, from 3.4 to 18.6 $\mu mol.gDTW^{-1}.h^{-1}$ (Figs. 5B and C). Payment of oxygen debt occurred within an hour of re-immersion for clams emersed for 3 h, while those emersed for 9 and 18 h took 3-4 h.

The size of the oxygen debt varied with clam size. Re-immersion respiration rates of large clams after 3 and 9 h air exposure were 16% and 18% lower, respectively, than those of small clams.

Juveniles (SL ± s.d. = 89.64 mm ± 12.84 mm) exposed to anaerobic atmospheres also manifested high rates of oxygen consumption relative to pre-emersion rates upon re-immersion. After 3 h exposure, re-immersion respiration rates increased from 13.0 to 23.4 $\mu mol.gDTW^{-1}.h^{-1}$ (Fig. 6A); after 9 h exposure, rates increased from 8.4 to 35.4 $\mu mol.gDTW^{-1}.h^{-1}$ (Fig. 6B). Recovery of normal oxygen consumption levels occurred within an hour during re-immersion for clams emersed for 3 h in anaerobic conditions. Those subjected to a 9 h exposure period did not show recovery of normal levels of respiration rates during monitoring of re-immersion rates (within 4 hours). Rates were, however, reduced within an hour, but maintained at levels higher than pre-exposure rates.

It was also observed that pre-emersion respiration rates of this last group of clams (70-100 mm size class) were higher (e.g. Fig. 6A) than those measured from preceding experiments, specifically the 50-65 mm (e.g. Fig. 4A) and 100-115 mm (e.g. Fig. 5A) size classes. The

Fig. 4. Aquatic respiration rates of small clams (50-65 mm shell length) before and after emersion for (A) 3 and (B) 9 h. n=12: vertical bar=s.d., other s.d. too small to plot.

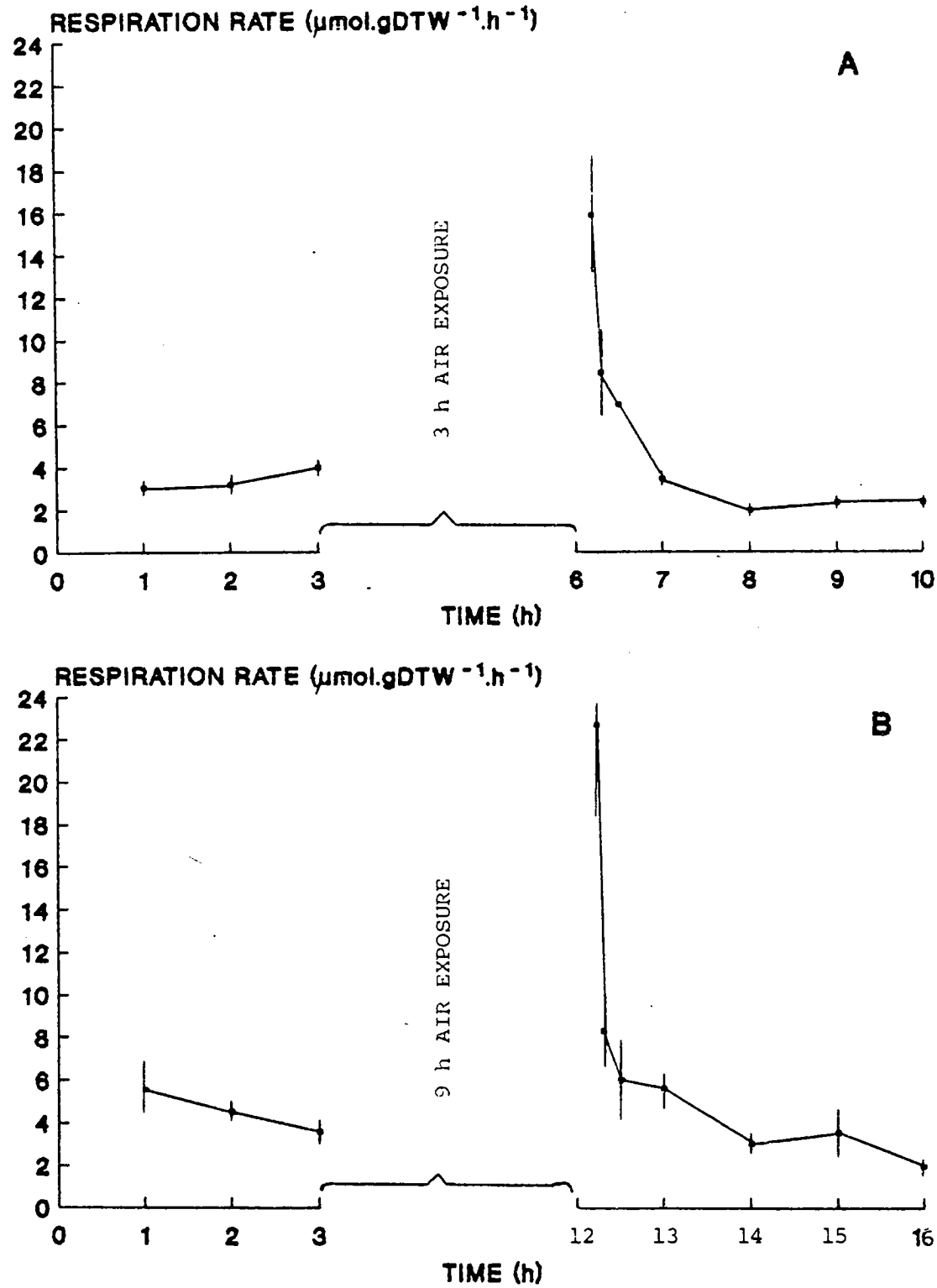


Fig. 5. Aquatic respiration rates of large clams (100-115 mm shell length) before and after air exposure for (A) 3, (B) 9 and (C) 18 h. n=12, vertical bar=s.d., other s.d. too small to plot.

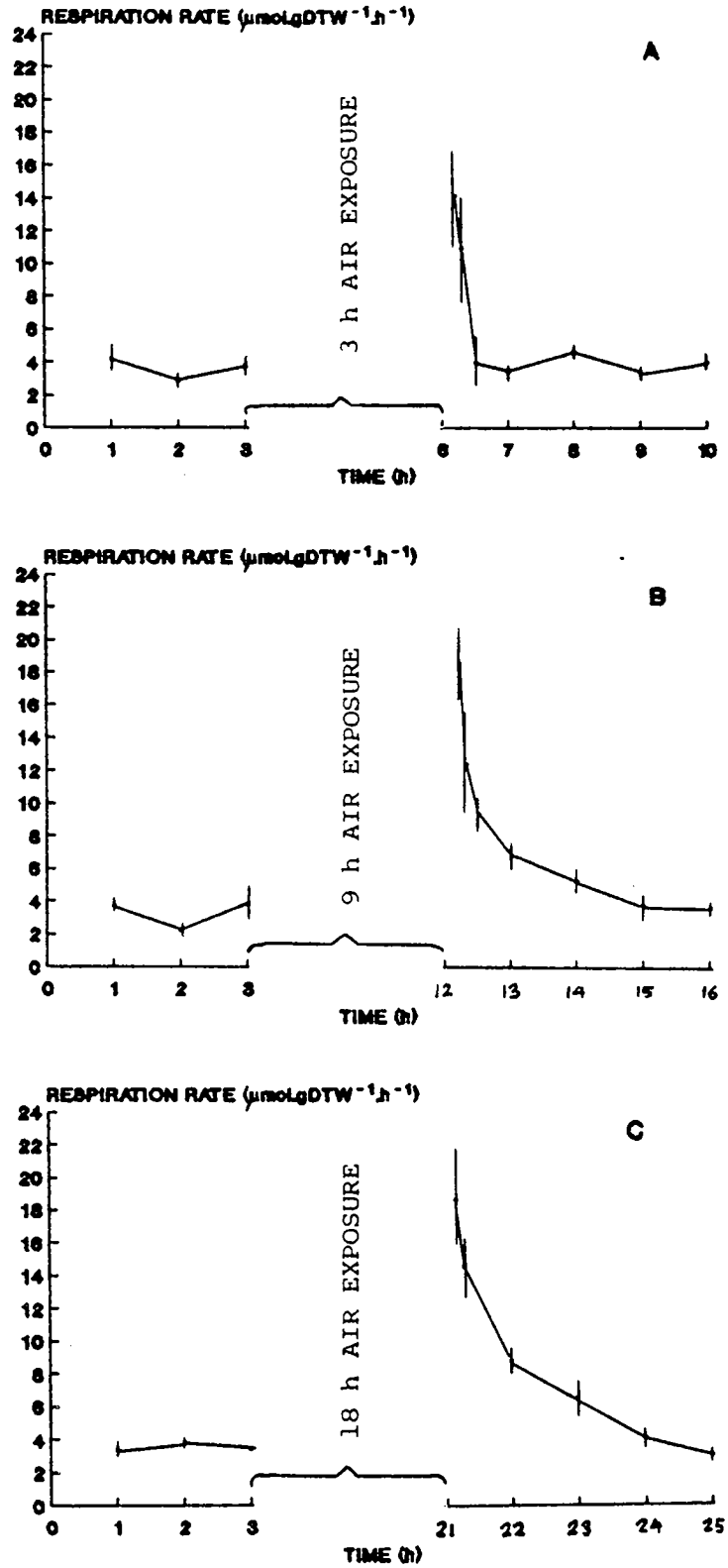
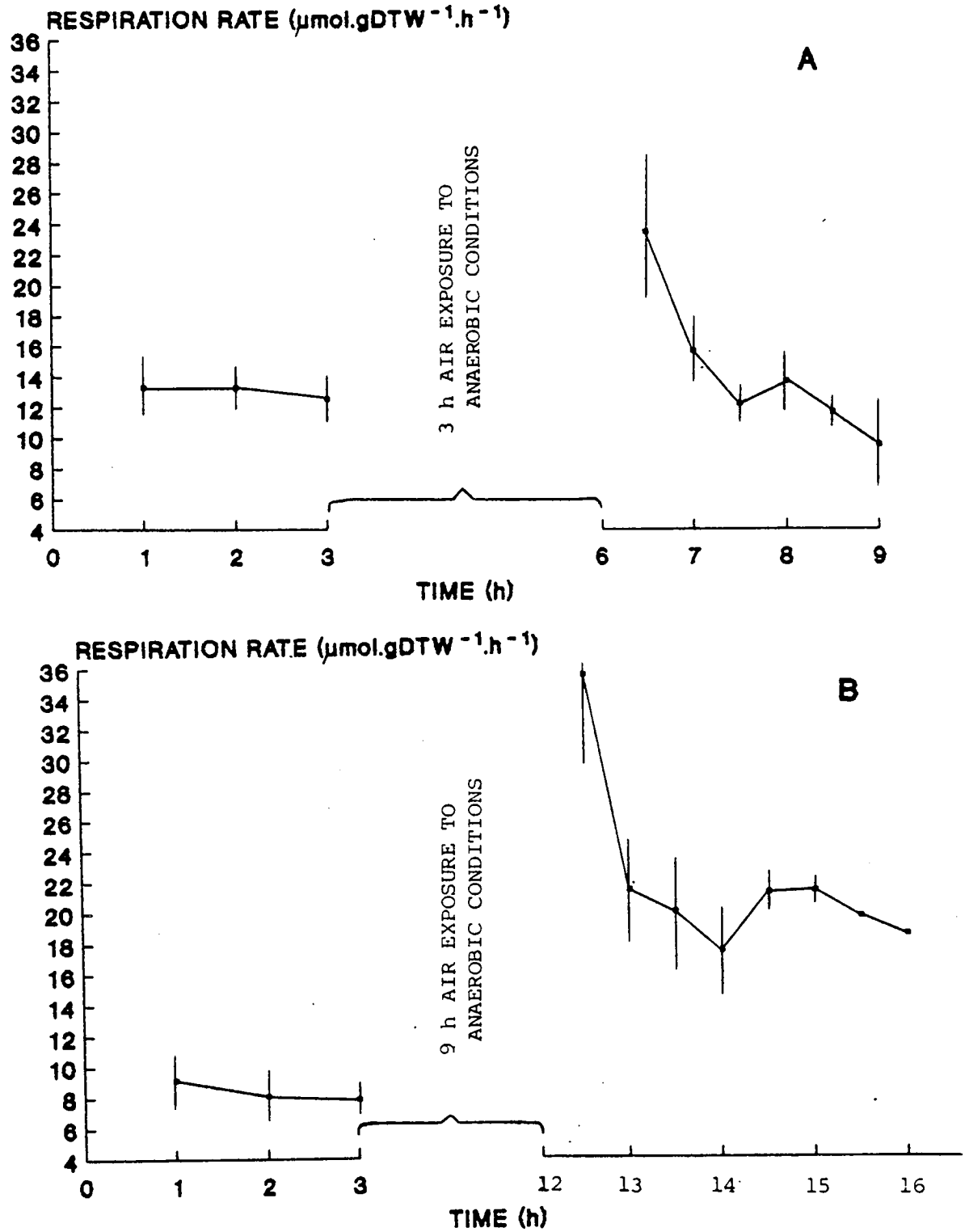


Fig. 6. Aquatic respiration rates of small clams (70-100 mm shell length) before and after emersion in anaerobic conditions for (A) 3 h, n=8, and (B) 9 h, n=9. Vertical bar=s.d., no s.d.= single observation.



difference cannot be attributed to a methodological factor since the same methods were employed in these experiments. However, there may be a seasonal acclimatization factor involved. Measurement of respiration rates of clams exposed to anaerobic conditions were conducted in summer, January to March 1990; whereas, the other experiments were conducted in winter, June to July 1989.

6.3.4. Ammonia Excretion

Ammonia production in Tridacna gigas juveniles continued during emersion. Ammonia excretion rates for clams, 80-100 mm SL, before air exposure were about 1.25 µg-atom NH_3-N.gDTW^{-1}.h^{-1} (e.g. Fig. 7A). Post-exposure rates upon re-immersion were higher than pre-exposure rates, indicating that ammonia accumulated during emersion. After a further hour, excretion rates declined to lower levels, although slightly higher than pre-emersion rates.

Post-exposure excretion rates varied with duration of air exposure, increasing from 4.57 µg-atom NH_3-N.gDTW^{-1}.h^{-1} for 3 h emersion (Fig. 7A), to 7.14 µg-atom NH_3-N.gDTW^{-1}.h^{-1} for 9 h (Fig. 7B), and 14.11 µg-atom NH_3-N.gDTW^{-1}.h^{-1} for 18 h (Fig. 7C). The increase in ammonia levels was not proportional to the duration of air exposure. Clams emersed for 3 h excreted (on the first hour of re-immersion) additional ammonia at a rate of 1.52 µg-atom NH_3-N.gDTW^{-1}.h^{-1} per hour exposure. On the other hand, clams exposed for 9 and 18 h excreted additional ammonia at a rate (on the first hour of re-immersion) of 0.79 µg-atom NH_3-N.gDTW^{-1}.h^{-1} per hour exposure.

In relation to Fig. 7, Fig. 8 compares rates of ammonia excretion and uptake by clams ("JCU clams") acclimatized to JCU recirculating seawater and clams ("OIRS clams") acclimated to OIRS seawater, respectively. There was a decrease in external ammonia concentration (indicating ammonia uptake by endosymbionts) using OIRS clams, rather than an increase (indicating ammonia

Fig. 7. Ammonia excretion rates of clams before and after emersion for (A) 3, (B) 9 and (C) 18 h. These clams were held in high ambient ammonia concentration (about 3 μg-atom NH_3-N.l^{-1}). (n=12; vertical bar=s.d., other s.d. too small to plot)

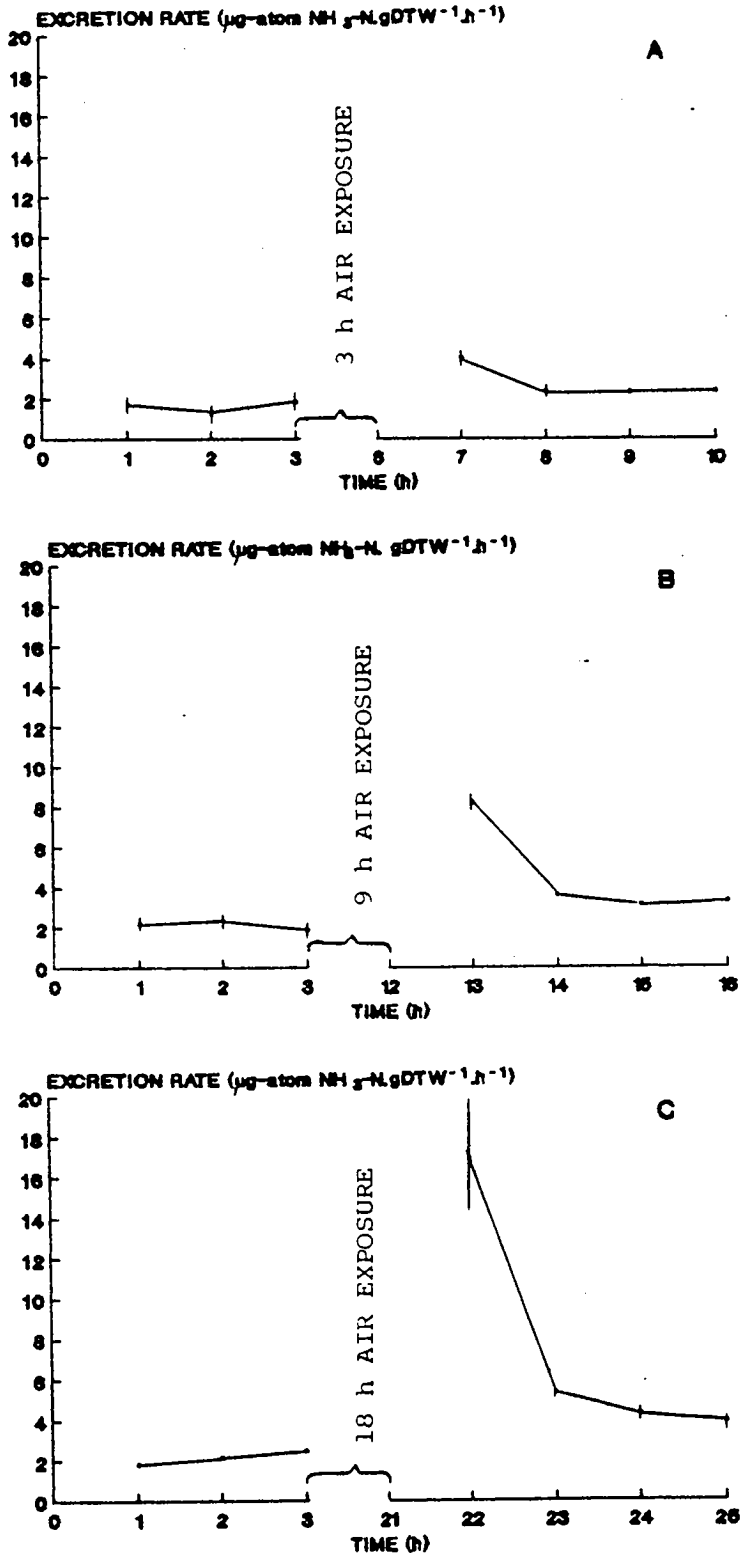
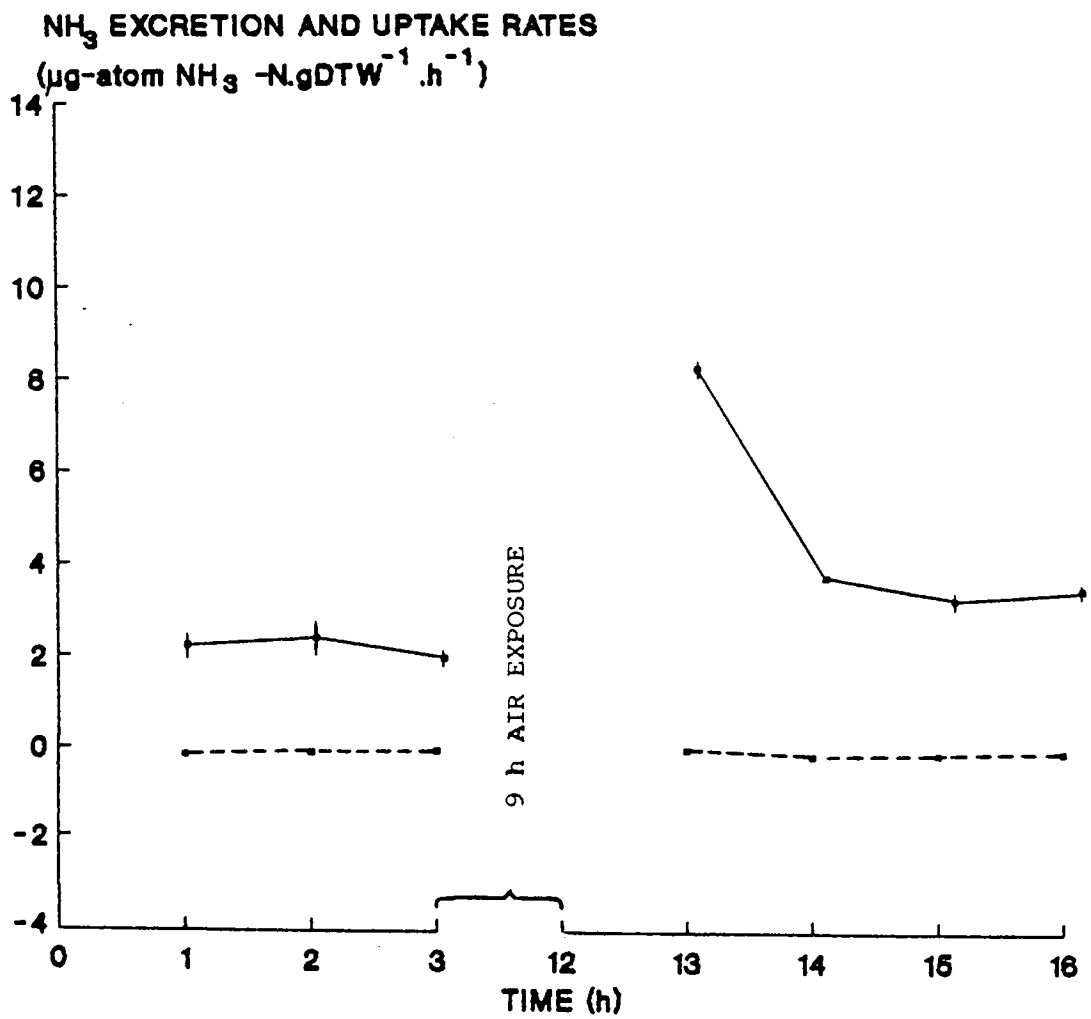


Fig. 8. Rates of ammonia excretion and uptake by juveniles acclimated to 3 (unbroken line) and 0.2 (broken line) µg-atom NH_3-N.l^{-1} ambient ammonia, respectively, before and after 9 h of emersion. n=6, vertical bar=s.d.; other s.d. too small to plot.



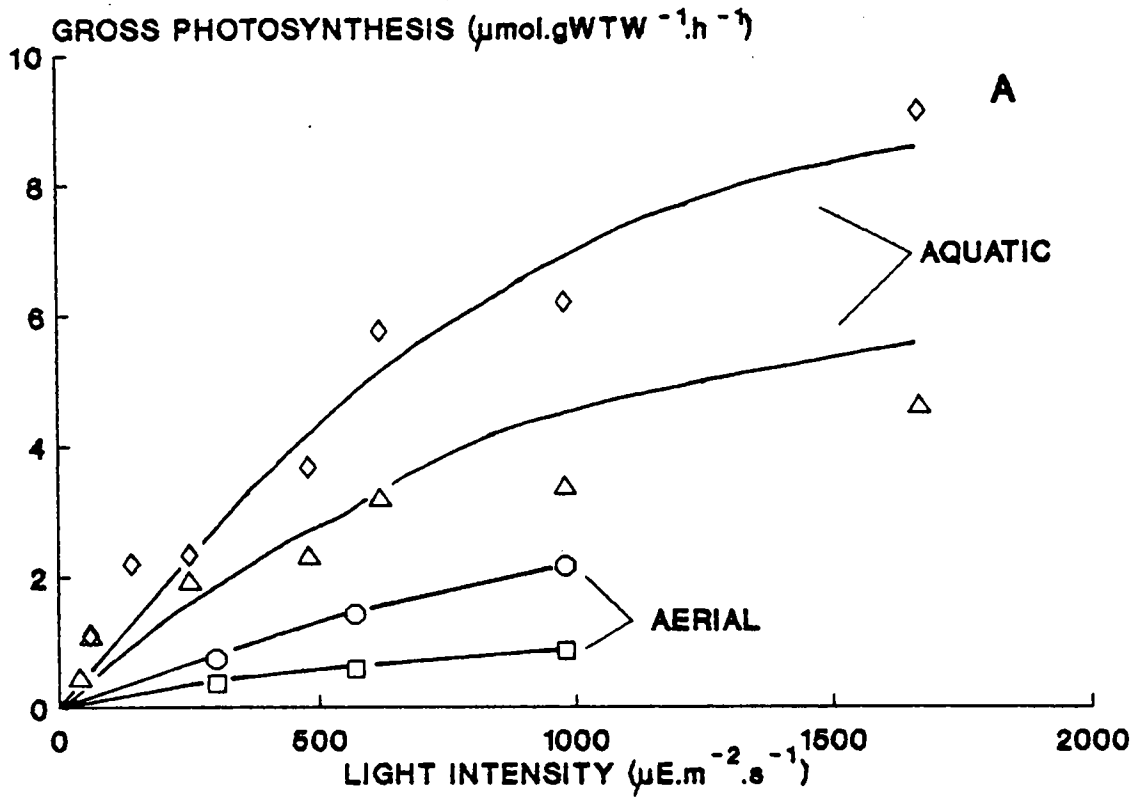
excretion by the host) using JCU clams. This means that the ammonia excretion rates depicted in Fig. 7 were obtained from clams acclimatized to high concentrations of ammonia.

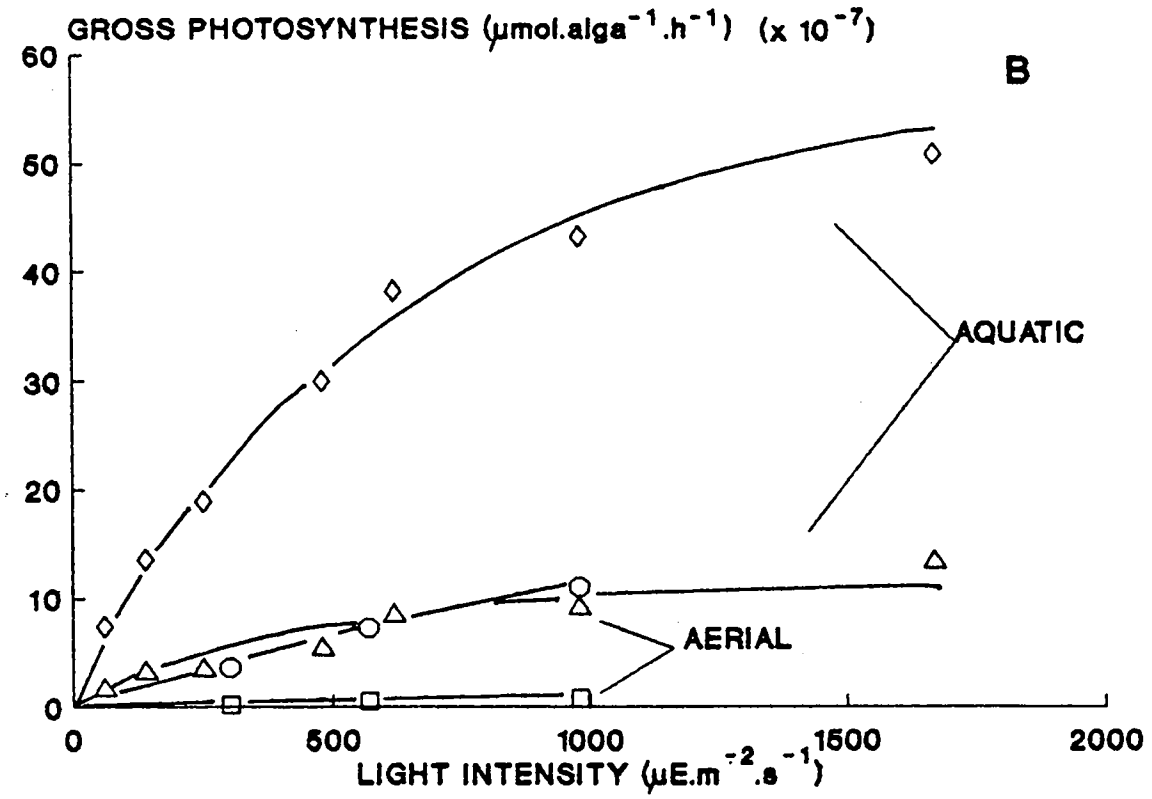
6.3.5. Photosynthesis and respiration

Rates of photosynthesis and respiration during immersion and air exposure were compared. Aquatic and aerial rates of photosynthesis (based on wet tissue weight, WTW) at different light intensities were plotted to obtain photosynthesis-irradiance (P-I) curves. The curves showed the characteristic shape of light-saturation curves for clams about 100 mm SL, i.e., a gradual increase in oxygen production with increasing irradiance within subsaturating levels, then decreasing in slope beyond a certain irradiance level, but not forming an asymptote with further increase in irradiance (see Fisher et al. 1985).

Aquatic P-I curves for intact clams based on clam WTW, number of zooxanthellae per clam (expressed as per alga) and total chlorophyll a per clam (expressed as per chl. a) are shown on Figs. 9A, B and C, respectively. Table 3A and B show aquatic and aerial photosynthetic parameters (P_m, Ik and alpha), respectively, derived from these sets of P-I curves obtained from intact clams. On a WTW basis (Fig. 9A, Table 3), immersed clams had higher values for P_m (or rate of photosynthesis at light saturation, Ik) and alpha (or P_m/Ik), and lower Ik (or minimum irradiance required to attain maximum rate of photosynthesis, P_m) values than emersed clams. The range of aquatic photosynthesis rates obtained here (using clams of 110-115 mm SL) were lower than those presented in Chapter 2 (see Fig. 8) for clams not more than 20 mm SL, in agreement with previous studies showing clam photosynthesis rates as dependent on clam mass (e.g. Fisher et al. 1985). Aerial values for alpha and P_m based on WTW were 9-16% and 8-25% respectively of the aquatic values (Tables 3A and B). Emersed clams required very

Fig. 9. Aquatic and aerial photosynthetic rates of intact clams, based on (A) clam wet tissue weight (=per WTW), (B) number of zooxanthellae per clam (=per alga), and (C) total chlorophyll a per clam (=per chl. a). Minimum and maximum P-I curves are shown. (Fig. 9C continued on page 134)





(Continuation of Fig. 9)

Fig. 9C. Aquatic and aerial photosynthetic rates of intact clams, based on total chlorophyll a per clam (= per chl. a). Minimum and maximum P-I curves are shown.

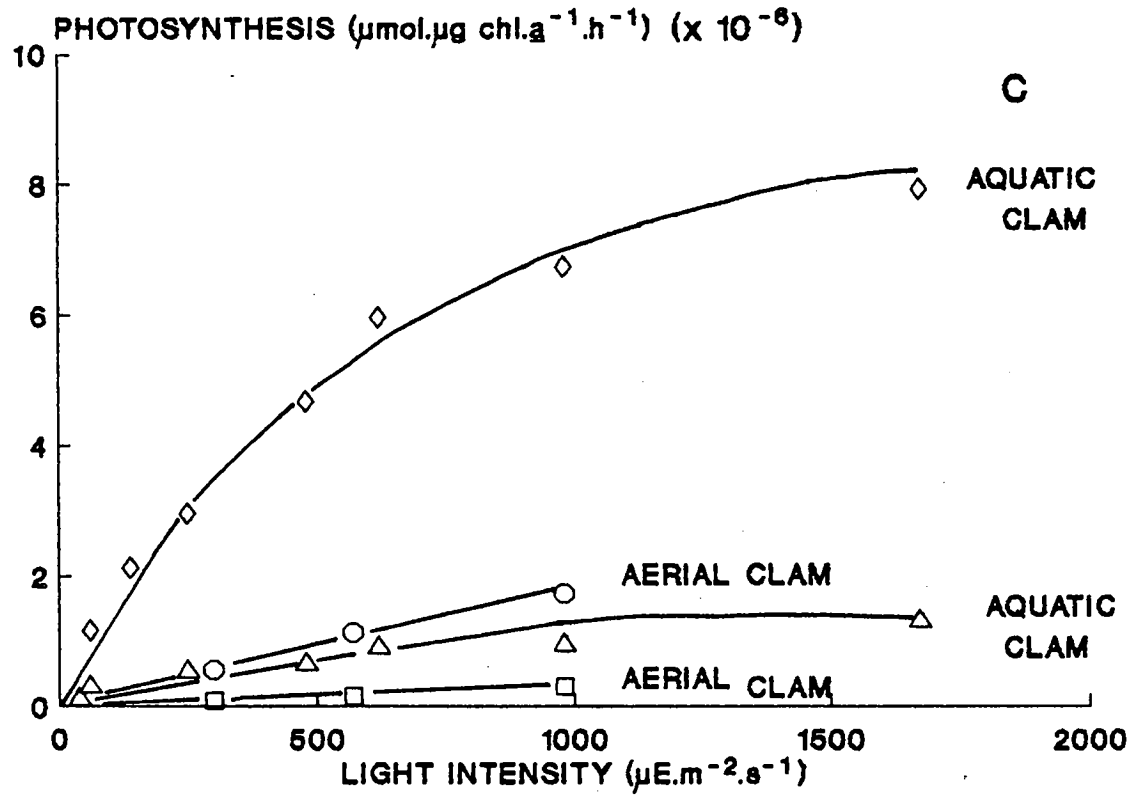


Table 3. Aquatic (A) and Aerial (B) photosynthetic parameters from P-I curves of intact clams, based on wet tissue weight (=per WTW), number of zooxanthellae per clam (=per alga) and total chlorophyll *a* per clam (=per chl. *a*). Alpha (α) = P_m /Ik.

A. DURING IMMERSION

CLAM NO.	PER WTW			PER ALGA			PER CHL. a		
	P_m [a]	Ik [b]	α ($\times 10^{-3}$)	P_m [c] ($\times 10^{-7}$)	Ik [b]	α ($\times 10^{-3}$)	P_m [d] ($\times 10^{-6}$)	Ik [b]	α ($\times 10^{-9}$)
1	5.94	755	7.87	17	790	21.52	2.83	890	3.18
2	4.37	706	6.19	16	740	21.62	1.28	1242	1.03
3	6.74	634	10.63	54	520	103.85	8.40	628	0.13
4	5.75	643	8.94	35	700	50.0	3.25	775	4.19
5	9.62	1053	9.15	13	850	15.29	5.08	1128	4.50
MEAN	6.64	778	8.54	27	720	42.46	4.17	933	5.26
S.D.	1.79	161	1.47	17	125	36.87	2.73	252	4.74

B. DURING EMERSION

CLAM NO.	PER WTW			PER ALGA			PER CHL. a		
	P_m [a]	Ik [b]	α ($\times 10^{-3}$)	P_m [c] ($\times 10^{-7}$)	Ik [b]	α ($\times 10^{-10}$)	P_m [d] ($\times 10^{-6}$)	Ik [b]	α ($\times 10^{-10}$)
1	1.28	>1000	1.28	3.4	432	7.87	0.80	978	8.18
2	0.85	>1000	0.85	0.74	840	0.88	0.44	1212	3.63
3	1.48	>1000	1.48	11.40	890	12.81	2.0	1040	0.19
4	1.45	>1000	1.45	7.90	430	18.37	1.12	661	0.17
5	0.80	>1000	0.80	1.30	735	1.77	0.50	990	5.05
MEAN	1.17	>1000	1.17	4.95	665	8.34	0.97	976	10.61
S.D.	0.33		0.33	4.58	221	7.41	0.64	200	7.07

[a] In units of $\mu mol.gWTW^{-1}.h^{-1}$

[b] In units of $\mu E.m^{-2}.s^{-1}$

[c] In units of $\mu mol.alga^{-1}.h^{-1}$

[d] In units of $\mu mol.\mu g\ chl.a^{-1}.h^{-1}$

high light intensities (Ik) to reach P_m, possibly as a result of mantle retraction during air exposure.

On an algal basis (Fig. 9B, Table 3), P_m and alpha were higher for clams in water than in air. Ik values for this set of P-I curves were similar (2-Sample t Test, t = 0.453, v = 8, P>0.50).

On a chl. a basis (Fig. 9C, Table 3), P_m and alpha were again higher for clams in water than in air, but Ik values were similar (2-Sample t Test, t = -0.286, v = 8, P>0.50).

Ik values from aquatic P-I curves (Table 3A) per WTW, per alga and per chl. a for intact clams were similar (One-Way ANOVA, F = 1.77, df = 2,12, P>0.10). On the other hand, Ik values from aerial P-I curves (Table 3B) showed higher values for P-I curves based on WTW than on an algal or chl. a basis. Ik values from P-I curves per alga and per chl. a were not significantly different (2-Sample t Test, t = -2.20, v = 8, P> 0.50).

Respiration rates of seven clams during immersion and air exposure were also compared (Table 4). The range of aquatic respiration rate values (for clams 100-115 mm SL) obtained here were lower than those (for clams not more than 20 mm SL) presented in Chapter 2, Table 1. This may be attributed to differences in size, hence clam mass, also observed by Fisher et al. (1985). Aquatic and aerial respiration rates were significantly different (2-Sample t Test, t = -5.806, v = 12, P<0.001). Aerial respiration rates were 22-52% of aquatic rates (mean = 35%).

CZAR, or the contribution of zooxanthellae to total clam respiration, was determined as discussed in Chapter 2 (see page 30), following the same assumptions, except for tday, which was assumed here to be 12 h. Table 5 shows calculated P/R ratios and CZAR values of whole clams during immersion and emersion. CZAR values were significantly different (2-Sample t Test, t = -4.129, v = 8, P<0.005). For juvenile Tridacna gigas about 100 mm size, the mean CZAR value during immersion was 88% at a Translocation Factor of 32% (Table 3A). During air

Table 4. Aquatic and aerial respiration rates of intact clams.
(In units of $\mu mol.gWTW^{-1}.h^{-1}$)

CLAM NO.	RESPIRATION RATE		AERIAL/AQUATIC RATIO
	AQUATIC	AERIAL	
1	1.74 ± 0.20	0.38 ± 0.09	0.22
2	2.06 ± 0.33	0.63 ± 0.02	0.31
3	2.37 ± 0.11	0.62 ± 0.18	0.26
4	1.17 ± 0.13	0.44 ± 0.18	0.38
5	1.16 ± 0.08	0.57 ± 0.16	0.49
6	1.14 ± 0.05	0.59 ± 0.09	0.52
7	1.66 ± 0.08	0.43 ± 0.08	0.26
MEAN ± S.D.	1.61 ± 0.49	0.52 ± 0.10	0.35 ± 0.12

Table 5. P/R ratios and CZAR values for intact clams in water and in air.

	IN WATER			IN AIR		
	P/R	CZAR *		P/R	CZAR *	
		T=32%	T=95%		T=32%	T=95%
	1.67	54	159	1.16	37	110
	2.18	70	207	1.10	35	105
	3.42	110	325	1.50	48	143
	2.96	95	282	1.42	45	135
	3.41	109	324	1.06	34	101
MEAN	2.73	88	259	1.25	40	119
S.D.	0.78	25	74	0.20	6	19

* T=32%, based on Griffiths and Streamer (1988); T=95%, based on Muscatine et al. (1984) and Fisher et al. (1985).

exposure, the mean CZAR value was 40%, which is 45% of the mean CZAR value during immersion.

Using 95% as Translocation factor (Fisher et al. 1985, Muscatine et al. 1984; also see Chapter 2, Section 2.3.4), average aquatic CZAR increased to 259%, while average aerial CZAR decreased to 119%.

6.4. Discussion

Desiccation stress was not a major factor responsible for juvenile mortality during emersion in oxygen-saturated conditions (mortality being 3-10%, observed in juveniles 70-80 mm shell length, SL). Water loss was minimal, being only 5% or less of the clam's total tissue water content (without the cavity fluid) even after 27 h in about 10% R.H.. Limited shell gaping minimized the degree of water loss. However, the importance of shell gaping during emersion for oxygen uptake was shown by exposing juveniles to anoxic atmospheric conditions.

Gaping valve movements have been correlated with decreasing oxygen concentrations in Cerastoderma edule (Boyden 1972). With Tridacna gigas, extensive shell gaping, together with high mortalities (87-100%), was observed after 9 and 27 h exposure to anoxic conditions. These were not observed for clams in aerobic atmospheres. Furthermore, statistical analyses showed that humidity conditions did not affect clam survival. Thus, the pattern of shell gaping and shell closure during emersion in juvenile T. gigas served primarily for gas exchange.

T. gigas is able to respire oxygen during emersion. This supports the results of Solis and Heslinga (1989), showing improved survival rates among juveniles exposed to oxygen-saturated atmospheres. In the present study, rates of oxygen consumption in air were about 35% of the aquatic rates, i.e. within the range of values obtained for other bivalves classified as "gapers" (see Table 6). This reduction may reflect the following: 1) a lowered aerobic metabolic rate, which in most intertidal bivalves serves to alleviate the cost of maintenance during air exposure, when external energy sources are not available and internal energy reserves have to be relied on; 2) the onset of anaerobic metabolism; and 3) the collapse of the mantle and gills into the shell for lack of support, reducing the respiratory surface area.

Table 6. Aerial and aquatic respiration rates by some littoral bivalves (modified from Table 1 of Widdows et al. 1979).

SPECIES	RESPIRATION RATE [a] IN AIR	IN WATER	AIR/WATER RATIO	TEMP (°C)	SOURCE
GAPERS--					
Geukensia demissa	0.24	0.38	0.63	20	Kuenzler 1961
	0.23	0.41	0.56	20	Widdows et al. 1979 Widdows and Shick 1985
Cardium edule	0.13	0.20	0.65	15	Boyden 1972
	0.14	0.50	0.28	10	Widdows et al. 1979
	0.35	0.45	0.78	20	-ditto-
Mytilus californianus	0.17	0.23	0.74	13	Bayne et al. 1976
Tridacna gigas	0.52 [b]	1.61 [b]	0.32	27	This study
NON-GAPERS--					
Cardium glaucum	0.012	0.15	0.08	15	Boyden 1972
Mytilus galloprovincialis	0.05	0.45	0.11	25	Widdows et al. 1979
	0.037	0.21	0.17	25	-ditto-
M. edulis	0.017	0.42	0.04	10	-ditto-

[a] in ml O_2.gDTW^{-1}.h^{-1}

[b] in µmol.gWTW^{-1}.h^{-1}

Respiration measurements in clams during aquatic recovery after a dark emersion revealed the incursion by juveniles of an oxygen debt (Figs. 4, 5). This indicates the occurrence of some anaerobic metabolism during emersion. Emersed clams have a P:R ratio (ratio of net photosynthesis to total respiration over 24 h) value of 1.26 (Table 5), implying that photosynthetically-fixed carbon is made available to the host during emersion in light conditions. This also suggests that utilization of an anaerobic pathway by clams during emersion in light conditions would be less, than if clams were exposed to air in the dark.

The oxygen debt increased with longer duration of exposure, and decreased for larger-sized clams. For large clams, there was no further increase in the size of oxygen debt after air exposure for 18 h, suggesting that a steady state of aerobic metabolism had been achieved after 9 h.

Juvenile Tridacna gigas acquired a larger oxygen debt when exposed for protracted periods to anoxic atmosphere. Oxygen debt was greater by 61% after 9 h exposure, compared 3 h exposure. This illustrates the level of stress experienced by clams when exposed to anaerobic conditions for long periods.

Investigations of other bivalves have shown that there is no direct relationship between the size of oxygen debt and the degree of aerobic or anaerobic respiration during emersion, since a distinct overshoot in oxygen uptake has been observed both from bivalves that maintain a fully aerobic metabolism (e.g. Cardium edule) and those that rely on anaerobic metabolism (e.g. Mytilus edulis) (Widdows and Shick 1985, Widdows et al. 1979). In addition, payment of an oxygen debt may coincide with increased rates of metabolic activities, e.g., pumping, ctenidial ciliary beating and re-oxygenation of the hemolymph, after re-immersion (de Vooys and de Zwaan 1978, Shick et al. 1988). The respiration rate of T. gigas during aquatic recovery after exposure to an anoxic atmosphere was maintained at a higher level than the pre-

emersion rate for the duration of the observation period. These results imply that Tridacna gigas is capable of anaerobic metabolism. Calorimetric studies measuring metabolic heat dissipation are required to verify this.

The results on ammonia excretion should be considered in the light of what is known from previous research regarding ammonium and the alga-invertebrate symbiosis. Such symbiotic associations are considered to involve conservative nutrient recycling whereby zooxanthellae transfer fixed carbon and amino compounds to the host, while the host provides catabolic nitrogenous compounds to the zooxanthellae (Taylor 1983). As a result, these symbiotic organisms have been found to release little or no ammonium; however, production of ammonium occurs when the organism is incubated in darkness (Cates and McLaughlin 1976, Muscatine and D'Elia 1978, Szmant et al. 1990, Wilkerson and Muscatine 1984). It has been shown for several symbiotic corals that ammonium uptake occurs when ammonium is limiting (Muscatine et al. 1989), but is inhibited when ambient concentrations are at saturation levels (see Wilkerson and Muscatine 1984). Tridacnid zooxanthellae in vivo and in vitro exhibit a high affinity with ammonium (Burris 1983, Wilkerson and Trench 1986). The ammonia excretion observed here in T. gigas has not been observed in other algal symbioses under natural conditions. In addition, it is not known if tidally-emersed clams would excrete ammonia under natural conditions.

The ammonia saturation level for T. gigas was not determined here. This needs to be addressed in future studies. In the present study, the ambient ammonia level of the recirculating seawater at JCU (about 3 ug-atom NH_3-N.l^{-1}) was very much higher than that of the seawater at OIRS (0.2 ug-atom NH_3-N.l^{-1}) from where clams used for ammonia determination experiments originated. The ammonia level of the recirculating seawater may have been at saturation levels such that ammonia uptake by zooxanthellae was inhibited. This would account for the

presence of excreted ammonia in the incubation trials based on recirculating seawater.

Increased ammonia excretion following air exposure shows that there was continued catabolism of amino-bearing compounds in Tridacna gigas juveniles (80-100 mm SL) despite emersion. There was a transient increase in ammonia excretion rate following air exposure. This has also been observed in other bivalves such as Mytilus edulis (de Vooys and de Zwaan 1978; Widdows, unpubl., cited in Shick et al. 1988), M. californianus (Bayne et al. 1976a) and Cardium edule (Widdows and Shick 1985).

Bivalves in general exhibit a reduction in rate of ammonia excretion during air exposure (Table 7). However, whereas ammonia excretion during emersion by other bivalves occurs at a steady rate (de Zwaan et al. 1983), this may not be so in T. gigas. The ammonia excretion rate value to cover 3 h air exposure was equivalent to 1.52 ug-atom NH_3-N.$gDTW^{-1}.h^{-1}$, while for 9 and 18 h exposure, this value was about 0.79 ug-atom NH_3-N.$gDTW^{-1}.h^{-1}$. The equivalent rate of excretion for the 3 h exposure treatment was higher than the pre-emersion ammonia excretion rate (1.25 ug-atom NH_3-N.$gDTW^{-1}.h^{-1}$), whereas for 9 and 18 h exposure treatments, rates were lower than pre-emersion rates. These results imply that the rate of ammonia excretion following air exposure tends to stabilize at a lower rate after a relatively long exposure period, as in this study, 9 to 18 hours. No measurements were made for other exposure times between 3 and 9 h, hence the exposure time at which stability was reached was not determined.

Clearly, air exposure interferes with important metabolic functions in T. gigas juveniles. Reduced rates of respiration and excretion during emersion indicate decreased metabolic rate, reflecting energy conservation during exposure. For filter-feeding bivalves, food supply is cut off during emersion. Such loss or reduction in energy gain necessitates an adjustment of the energy expended for maintenance during exposure. However, the

Table 7. The effect of emersion on ammonia excretion rate by some bivalves (modified from Shick et al. 1988).

SPECIES	NH_3 EXCRETION RATE IN AIR	IN WATER	AIR/WATER RATIO	SOURCE
Mytilus californianus	0.07[1]	2.14	3-6%	Bayne et al. 1976
M. edulis	0.11[1]	1.04	11%	Widdows unpubl.[4]
Cardium edule	0.213[2]	0.425	50%	Widdows and Shick 1985
Tridacna gigas	0.79[3]	1.25	63%	This study

[1] Ammonia excretion rate determined from samples of the hemolymph and mantle cavity water during air exposure, in μmoles $NH_4 . gDTW^{-1} . h^{-1}$

[2] NH_3 released upon re-immersion, in μmoles $NH_4 . gDTW^{-1} . h^{-1}$

[3] NH_3 released upon re-immersion, in μg-atom NH_3-N.$gDTW^{-1} . h^{-1}$

[4] Cited in Shick et al. 1988.

capability of tridacnids for both heterotrophy and phototrophy sets them apart from most bivalves. The potential for phototrophic nutrition becomes significant during exposure, as has been suggested by Lucas et al. (1989).

Photosynthesis was demonstrated for intact clams during air exposure. P_m of the intact clam also decreased during air exposure. This is attributed to reduced net photosynthetic and respiratory rates of the intact clam at air exposure. Observations on exposed clams showed that, although the valves were held agape, the mantle slightly collapsed into the shell for lack of support. Therefore, the area of mantle and hence the number of zooxanthellae that were exposed to light were reduced.

CZAR is derived from the ratio of net photosynthesis to animal respiration (Muscatine et al. 1981), a percentage (based on the Translocation Factor, T) of which represents the contribution of algae to total clam respiration (Griffiths and Streamer 1988, see Trench et al. 1981b, and Muscatine et al. 1981). The Translocation Factor used in this study was 32% (Chapter 2, Section 2.3.4), with comparative calculations based on 95% Translocation Factor (Fisher et al. 1985, Muscatine et al. 1984).

CZAR will vary depending on the T value used (see discussion on page 34, Section 2.4). For juvenile Tridacna gigas 110 to 115 mm SL (about 33 g wet flesh weight), algae contribute about 40% of clam respiratory carbon requirements during air exposure, nearly half of what the clam would receive (CZAR = 88% with T=32%) during submergence. Notwithstanding this reduction in CZAR, algal photosynthesis provides an important source of energy for the emersed clam which otherwise would cease feeding. These results support the hypothesis of Lucas et al. (1989) that because short periods of emersion during the day promote growth, the clams must be photosynthesizing out of water.

7. CONCLUSIONS AND RECOMMENDATIONS

Juvenile Tridacna gigas was affected by the factors considered in this study: light, temperature, salinity, seawater flowrate, stocking density and air exposure, except supplements and cleaning.

Light influences the photosynthetic capability of the algal endosymbionts of T. gigas, and thence the contribution of zooxanthellae to clam respiration (CZAR). Photoadaptation in small juveniles to ambient light intensities allows them to utilize suboptimal light regimes. This study showed that juvenile clams reared under suboptimal light still continued to exhibit some growth, although clam growth in shell and in tissue was low compared to that under high light intensities. The occurrence of negative tissue growth after a week's exposure to darkness, and of high mortalities with further dark exposure emphasizes the dependence of tridacnids on light, for their algal endosymbionts and for other reasons still unknown. Based on photosynthesis-irradiance curve estimates and CZAR calculations (Translocation Factor = 32%, Griffiths and Streamer 1988), a minimum irradiance level of 200 $uE.m^{-2}.s^{-1}$ is required to attain about 90% CZAR. This CZAR, plus a substantial contribution by filter-feeding (D. W. Klumpp, pers. comm.), and by nutrient absorption to total carbon requirements of the clam for respiration and growth constitute the relatively large energy inputs required for rapid growth of juvenile tridacnids.

The effect of light on growth in juvenile tridacnids may not be the same for shell and tissue. It is clear from this study that light, through phototrophy induces tissue growth in clams. On the other hand, the effect of light on shell growth is less clear for the reason that light's effects may have various manifestations: changes in shell length, weight and thickness, and/or alterations in shell structural deposits. In this study, clams exposed to low light regimes exhibited a decrease in shell

thickness and weight (but not in shell length), together with a decrease in tissue mass. The complexity of shell growth response makes the use of shell growth parameters inferior to those based on tissue. Future studies on light effects must therefore consider these aspects of shell growth simultaneously with tissue growth.

Temperature strongly affects clam metabolic rate. Based on temperature quotient values (Q_{10} = 1.37 to 3.07 for temperature intervals between 19° and 33°C) and upper temperature tolerance, a temperature range of 26° to 32°C is suitable for juvenile rearing. Temperatures below 19°C and above 33°C depress respiration rates, suggesting destabilization of metabolic processes and accounting for mortalities at temperatures 33°C and above. For mariculture, a transplantation schedule for clam seed can be organized based on temperature requirements. For example, when land-based nurseries are experiencing the low (or high) temperature limits, juveniles may then be transferred to shallow (or subtidal) ocean-based nurseries where temperatures are more suitable for juvenile rearing.

Salinity tolerance studies show that salinities in the range of 26 to 36 ppt are favorable to juveniles, with best results on growth at about 35 ppt. Comparison of osmolal concentrations of extracellular (ECF) and cavity fluids against external salinities indicate that Tridacna gigas juveniles are osmoconformers exhibiting slight hyperosmoticity of the ECF. High Performance Liquid Chromatography (HPLC) analysis of free amino acids (FAA, clam adductor muscle extracts) indicate that non-essential FAA are more important in contributing to the intracellular osmotic pressure, and that certain non-essential FAA respond more readily to changes in external osmotic pressure over essential FAA.

The observed FAA changes in T. gigas subjected to hypo- and hyperosmotic stress are difficult to explain in terms of osmotic adaptation alone. The effects of salinity variation on zooxanthellae in situ merits

consideration in the future. Since zooxanthellae are extracellular in tridacnids, a salinity change exposes the zooxanthellae to osmotic changes that are occurring in the host's extracellular fluids, thence leading to corresponding changes in the osmotic pressure of zooxanthellar cells. Furthermore, since zooxanthellae transfer to the host certain organic molecules, including alanine, aspartate, serine and succinate (Griffiths and Streamer 1988), the role of zooxanthellae in tridacnid FAA metabolism during osmotic adaptation needs to be examined.

In order to understand the significance of intracellular FAA metabolism during osmotic stress, several aspects of FAA metabolism should be investigated:
1) Source and fate of glycine. The effects of salts on the activity of serine hydroxymethylase catalyzing the formation of glycine from serine still have to be demonstrated (Whitely 1960).
2) The influence of anaerobiosis on alanine concentrations. A metabolic shift from the TCA cycle at the level of the phosphoenolpyruvate intermediate occurs such that alanine, along with succinate, is formed. This pathway is utilized by other bivalves: Crassostrea virginica (Hammen 1969), Rangia cuneata (Chen and Awapara 1969), Mytilus edulis (de Zwaan and van Marrewijk 1973). However, some controversy still exists regarding the applicability of the anaerobiosis hypothesis and the utilization of one scheme for molluscs in general (Baginski and Pierce 1978, Heavers and Hammen 1985, Shumway et al. 1977, de Zwaan et al. 1976). Although anaerobiosis in tridacnids has been given some consideration in Chapter 6 of this study, this aspect of clam metabolism needs to be investigated further.

Survival and growth studies under different levels of seawater flowrate, stocking density, and supplements (microalgae, dissolved inorganic nutrients) and cleaning confirm the importance of flowrate, particularly water turnover time (TOT) and flowrate per clam (FRC), for

growth; and the dependence of survival on clam stocking density. The largest increase in shell length, over stocking densities equivalent to 2000 - 50,000.m^{-2}, was obtained at the lowest stocking density.

Flowrate levels must be considered together with water TOT and/or FRC. In this study, short TOT (1.5 h) and/or high FRC (150 $ml.h^{-1}.clam^{-1}$) induced best clam growth, by a more generous allocation of natural food resources per clam. A high FRC is deemed essential. However, in commercial mariculture, one is dealing with compromises between the ideal and the economic. Decreasing clam density means more tanks are needed. Increasing FRC means increased water pumping. For instance, with 10^6 juveniles and tanks having 10 m^2 bottom area, stocking at 10,000.m^{-2}, using 10 tanks and yielding somewhat reduced clam growth may be better economics than stocking at 2000.m^{-2} and using 50 tanks, but yielding high clam growth. Under the same hypothetical situation, using a flowrate of 0.72 x 10^9 $l.day^{-1}$ to give a FRC value of 30 $ml.h^{-1}.clam^{-1}$ and yielding reduced clam growth may again be favored for economic reasons, rather than using a flowrate of 2.4 x 10^9 $l.day^{-1}$ to give 100 $ml.h^{-1}.clam^{-1}$, but yielding optimum clam growth.

Based on FRC and stocking density results on growth, tank flowrates may be calculated, for instance, 20,000 juveniles in a 3000 l tank with about 10 m^2 bottom area, as shown in Table 1. An FRC of 30 $ml.h^{-1}.clam^{-1}$ requires 14,400 $l.day^{-1}$ and is equivalent to about 5 turnovers per day (at 90% particle replacement, based on Sprague (1969)). This situation would yield good clam growth and still be economic. By comparison, the Tridacna gigas cultivation study conducted by Braley et al. (1988) using a turnover time of 24 h (or 1 turnover per day) (see Chapter 5, Table 6) is therefore inadequate for good clam growth.

Supplements and cleaning had no effect on survival and growth, contrary to expectations based on improved

Table 1. Required tank flowrates (FR) for corresponding flowrate per clam (FRC) for 20,000 juveniles (<10 mm shell length) in a 3000 l tank (bottom area = 10 m^2). Percent increase in growth was approximated from Table 7 of Chapter 5.

FRC ($ml.h^{-1}.clam^{-1}$)	FR ($l.day^{-1}$)	INCREASE IN GROWTH
0.4	192	<10% (poor)
4-6	1920-2880	10% (weak)
30	14,400	50% (good)
150	72,000	75% (best)

growth from increased FRC. The effects of supplementary food and nutrients on clam growth need to be reconsidered together with specific nutrient requirements and the occurrence of interfering factors, like fouling filamentous algae that compete with juveniles for nutrients and space.

Emersion-related stresses such as desiccation, restriction of respiratory gas exchange and loss of food supply are alleviated by gaping for respiratory gas exchange, and physiological adaptations, such as minimal water loss, reduced aerial respiration (35% of aquatic rate) and ammonia excretion rates, and aerial photosynthesis. Emersion tolerance is dependent on availability of oxygen. Aerial respiration and oxygen debt measurements show that aerobic and anaerobic metabolism occur during emersion, although neither is sufficient on its own to meet the demands of clam metabolism during air exposure.

Ammonia excretion rate, measured under abnormally high ambient ammonia conditions, was 63% of aquatic rate, suggesting a reduction in nitrogen catabolism. This is important for a bivalve that relies to some extent on filter-feeding. For the duration of emersion, food supply is cut off. By reducing the rate of nitrogen catabolism, the clam uses less of its nitrogen reserves during emersion.

Aerial photosynthesis has been demonstrated here for juvenile Tridacna gigas, further distinguishing tridacnids from other commercially-important bivalves (see Crawford et al. 1986, Heslinga and Fitt 1987, Munro and Gwyther 1981). Despite a reduced zooxanthellar contribution to clam respiration (CZAR in air = 40%, whereas CZAR in water = 88%, at T = 32%), tridacnids are assured of continued energy input through zooxanthellar photosynthesis, whereas other bivalves must draw on energy reserves to support their metabolism during air exposure. By virtue of aerial photosynthesis, the metabolic demand

of clams emersed in light conditions (as occurs in an intertidal nursery situation during daytime tidal exposure) can be met largely by aerobic metabolism (which is more efficient in energy production than anaerobic metabolism), with some input from anaerobic metabolism. Aerial photosynthesis, similarly with reduced ammonia excretion, therefore places less demand on internal energy reserves.

The results of emersion studies here are relevant to several aspects of tridacnid mariculture, for instance, in clam transport which may involve long periods of emersion, especially with overseas transport. Better handling procedures before and after transport reducing the physiological stresses of emersion, hence improving survival, may be developed. Moreover, these studies show that Tridacna gigas juveniles are physiologically suited for cultivation in intertidal areas, and hence there is support here for this type of nearshore cultivation (see Lucas et al. 1989).

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APPENDICES

Appendix 1. Components of the Dix Mix solution (originating from Dr. Trevor Dix) used as inorganic nutrient supplement in Chapter 5.

200 g finely ground "Garden King" superphosphate
(granulate phosphate)
520 g "Garden King" sulfate of ammonia
(granulated nitrogen)
3.72 g thiamine Vitamin B_1

Make up to 10 liters with warm tap water.

Appendix 2

Appendix 3. Gain in total wet weight (shell and tissue) of Tridacna gigas after rearing at different salinities for 30 days. (S.D. = standard deviation)

Salinity (ppt)	Weight Gain (g)	Mean (g)	S.D.	Salinity (ppt)	Weight Gain (g)	Mean (g)	S.D.
23	3.94 2.62 6.18 7.31 6.38 7.59 4.50 3.94	5.31	1.80	35	7.94 2.91 8.12 2.74 12.62 1.24 8.91 13.32	7.23	4.55
27	7.20 6.93 1.29 8.23 1.97 5.31 8.83	5.68	2.99	39	3.83 5.50 4.55 5.92 7.17 6.25	5.54	1.20
30	6.89 2.76 2.59 1.62 4.94 5.43 5.03	4.18	1.88	42	6.64 5.31 3.09 2.14 6.09 4.11 3.77 15.77	5.87	4.28
32	7.91 5.27 4.18 12.27 1.82 2.64 4.73 13.46	6.54	4.32	44	2.13 6.58 1.16 4.06 4.06 4.94	3.82	1.95

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Photoadaptation in Juvenile *Tridacna gigas*

S. Suzanne M. Mingoa*

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