CULTURE OF THE BRINE SHRIMP, ARTEMIA SALINA L.

UTILIZING DUNALIELLA TERTIOLECTA GROWN IN

SWINE WASTE-SEAWATER MIXTURES AND IN

DEFINED INORGANIC MEDIUM

by

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ABSTRACT

A comparative study was conducted on the growth of Dunaliella tertiolecta using defined inorganic medium and swine waste-seawater medium as sources of nutrients. The nitrogen concentration was maintained at ca 1200 $\pm$ 50 µg-at N l^{-1} for both media. There were no significant differences in the daily measurements of cell densities and dry weights of D. tertiolecta grown in defined inorganic medium and swine waste-seawater medium in either batch or continuous culture systems.

The algal biomass produced from continuous culture system was used as food for the brine shrimp, Artemia salina L. The brine shrimp fed with D. tertiolecta grown in swine waste-seawater medium and those fed with D. tertiolecta cultured in defined inorganic medium showed no significant differences in their daily total length and percentage survival measurements. A 57-58% Artemia biomass conversion efficiency was calculated. Statistical analysis for biomass conversion efficiency showed no significant differences for Artemia fed with D. tertiolecta grown in swine waste-seawater medium and that fed with D. tertiolecta cultured in defined inorganic medium.

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INTRODUCTION

Artemia salina L. are filter feeding herbivores and
consume only small particulate matter such as unicellular
algae, yeast and bacteria. The suitability of Artemia for
aquaculture is enhanced by the fact that the physical cultural
conditions: temperature, salinity, pH and O_2 tension are the
same for both the larval stages and adults. There is no special
nursery environment.

Most of the phytoplankters used as food for A. salina have
been cultured on defined seawater media (Croghan, 1958;
Provasoli et al., 1959; Mason, 1963; Reeve, 1963a; and Sick,
1976) or media enriched with commercial fertilizer (Helfrich,
1973). Agricultural wastes have been used successfully for
the culture of microorganisms such as bacteria and phytoplankton
(Hephner, 1962; Schroeder, 1974) and have increased the
production of cultured aquatic animals (Hephner and Schroeder,
1974). The utilization of livestock waste as a nutrient source
for phytoplankton culture and subsequently, as food for A. salina
has not been investigated.

The general objective of the present research work was
to evaluate the culture potential of the brine shrimp, Artemia
salina L. fed with Dunaliella tertiolecta grown in swine waste-
seawater as compared to defined inorganic media in a laboratory
scale continuous culture system.

The experiments were conducted in two phases: Phase I was the culture study between D. tertiolecta grown in swine waste-seawater mixture compared to that grown in defined inorganic medium; and Phase II was the comparative study on the survival and growth of Artemia on two feeding regimes:

    i.   D. tertiolecta grown in swine waste-seawater mixture.

   ii.   D. tertiolecta grown in defined inorganic medium.

LITERATURE REVIEW

Algal Production

Early publications by Allen and Nelson (1910) and
Emerson and Lewis (1939) proved that algal culture was a subject
of much interest in the early 1900's. However, much of the
early work dealt primarily with the study of photosynthetic
pathways in green algal cells (Sargent, 1940; Pratt, 1943).

Milner (1961) analysed the chemical composition of a
number of marine and freshwater species. He found that
freshwater species generally ranked much higher in energy content
than the marine species. This difference resulted from the
relatively greater fat content of the freshwater species.
Parsons et al. (1961) observed that among marine algal species
carbohydrate, lipid, protein and ash content varied widely.
Dunaliella salina was found to contain 57% protein, the highest
value among the species analyzed, however, the lipid content
was quite low, 6.4%.

The genus Dunaliella comprises a group of algae withethin
cell envelope whose ability to withstand certain extreme
environmental changes is well known (Ben-Amotz and Avron, 1978).
However, a cell coat of Dunaliella tertiolecta has been recently
observed which appears to be largely composed of glycoprotein
containing neuraminic acid residues in its molecular structure
(Oliveira et al., 1980). For sometime, it has been recognized
that heavy metal resistance can develop in populations of
microorganisms such as fungi, yeasts and bacteria which have

been subjected to prolonged exposure to sub-lethal metal concentrations (c.f. reviews by Ashida, 1965 and Antonovics et al., 1971) but there appear to be very few examples of organisms which possess an innate resistance to metals. This is the case with D. tertiolecta. The specific growth rate of D. tertiolecta was unaffected by mercury II concentrations of at least 2.03 μg-at l^{-1}. At 10 μg-at l^{-1}, the specific growth rate was eventually reduced by 84% but growth continued, giving a final level of cell material only 13% below that in a mercury-free control (Davies, 1976). The alga has been shown to be unaffected by DDT at 1 ppm as measured by cell division (Bowes, 1971). PCB gave an inhibition of 43% at 10^3 ppm (Luard, 1973). In addition, this species could tolerate fluoride concentration from 0 to 100 ppm (Oliveira et al., 1978).

By the mid-1960's, the production of algae on a semi-commercial basis created much interest. Gaucher et al. (1960), Davis et al. (1961), Casey et al. (1963), Loosanoff and Davis (1963) and Ukeles (1965) investigated the possibility of culturing algae on a mass scale. Ukeles and Loosanoff and Davis were primarily interested in obtaining a reliable feed source for clam and oyster larvae. The efforts of Loosanoff and Davis (1963) were directed toward culturing algae in large outdoor vats resembling small ponds.

Another type of mass culture unit was devised by Wisely and Purday (1961), the batch culture system. This unit consisted of large drums containing 200 liters of Isochrysis galbana cultures. Illumination showed light intensity to be reduced

greatly at the periphery of the culture. This reduction in light intensity may have influenced the density of the culture.

A primary drawback to batch culture lies in the handling and maintenance of such units. Also, steady state condition in batch culture cannot occur without additional nutrient supplementation. Furthermore, such units must be periodically dismantled, scrubbed down, and reinoculated with fresh cultures in order to keep a healthy supply of algae.

Because of the inherent difficulties associated with batch culture, many experimenters turned to the continuous culture of algae. Much of the recent work done with continuous algal cultures has been based on bacteriological models pioneered by Monod (1950), Novick and Szilard (1950) and Herbert et al. (1956) and Herbert (1958; 1961) all of whom contributed to the use of mathematical models for continuous cultures.

The essential feature of the continuous culture lies in the fact that it is self-regulating. Once the inflowing medium has been set at a constant value, the system will automatically adjust itself to the steady state (Herbert, 1958). There are essentially two types of continuous culture units, the 'chemostat' (Novick and Szilard, 1950) and the 'turbidostat' (Herbert, 1958). Although these units are similar in operation, the basic principle by which growth rate (μ) is controlled differs. The turbidostat regulates growth through control of cell density while the chemostat regulates the growth rate of the cells by varying the amount of 'limiting growth substrate' (Monod, 1950).

Algal Waste-Recycling System In Aquaculture

Culture of marine algae at high biomass levels in both laboratory and large-scale outdoor cultures using municipal waste and seawater mixtures has been conducted (Dunstan and Menzel, 1971; Goldman and Stanley, 1974). Interest in this culture technique has been generated by the demonstration that waste water nutrients may be recycled in marine aquaculture systems designed for simultaneous tertiary treatment of waste and food production (Ryther et al., 1972; 1975).

A series of papers in the literature indicate the suitability of liquid organic wastes such as sewage and slurry as media for the controlled cultivation of freshwater micro-algae (Garret and Allen, 1976; Dugan et al., 1971; Garret et al., 1976). The composition and nutritional potential of algae have also attracted interest for many years (Burlew (ed), 1953). Of particular interest are reports on the nutritive values of sewage grown algae for rats, chick, pigs, cattle and sheep (Cook et al, 1963; Hintz et al., 1966).

Most of the investigations in the 1960's and mid-1970's were concerned with the culture of algae on municipal sewage e. g., in California, U.S.A. (Oswald et al, 1959), in Thailand (McGarry et al., 1972), Australia (Bureau of Environmental Studies, 1975) and Israel (Shelef, et al., 1972). Initial work with animal wastes during this period included pig wastewater studies in the U. S. A. and in the Philippines. The latter made use of roofs of pig houses as shallow ponds for algal culture (Eusebio, 1976).

One of the areas of uncertainty in the use of waste-grown algae for animal food (either aquatic or terrestrial) is the question of toxicants such as heavy metals which may be present in the substrate and may have possible concentrating effects in pond organisms and carryover as toxic residues in the foods derived from the animals. Although animal wastes may contain toxicants from feed residues and growth stimulants such as copper added to the ration, these are generally more predictable and controllable than with sewage (Dodd, 1979). An important consideration in utilizing livestock waste or sewage is the use of certain species of algae that could tolerate concentration of toxic compounds or could utilize certaine organic nitrogen sources. D. tertiolecta have been known to utilize certain organic nitrogen sources such as hypoxanthine, urea, D-glucosamine (Antia et al., 1975; 1980).

The planktonic microalgae which predominate in high-rate ponds are too small to be satisfactorily filtered or screened from the pond effluent by conventional equipment which employ fabric material such as microstrainers. Consequently, over many years the search for an effective, low cost method of harvesting which does not decrease the quality of algal product has paralleled the research in pond technology. Algal harvesting and removal were studied extensively by Golueke and Oswald (1965) and Oswald (1963). Dodd (1979) gave a review on different technological and managerial advancement of algal harvesting. But still to date no economical method of harvesting has been successful.

One highly efficient method of utilizing the cultured biomass produced is to feed it to aquatic filter feeders, such as some species of carp, zooplankton and _Artemia_.

Use and Potential of _Artemia_ In Aquaculture
In Relation To Its Life Cycle

The brine shrimp, _Artemia_ _salina_ L. is highly adaptable to an extremely wide range of salinities and temperatures. The _Artemia_ is a non-selective particle filter-feeder. Contrary to many other crustaceans, its food requirements do not change during growth (Reeve, 1963a; 1963b) and it has a unique reproductive cycle which is very well-suited to a cultural system even in high densities (Helfrich, 1973; Sorgeloos and Persoone, 1975; Tobias et al., 1979).

Artemia have a rapid generation time (maximum of about 3-4 weeks), a high fecundity (over 100 offspring day^{-1}) and reproduce continuously throughout their six-month to one year life span (Gilchrist, 1960; Bowen, 1962; Nimura, 1967).

The suitability of _Artemia_ for aquaculture is enhanced by the fact that the physical cultural conditions such as: temperature, salinity, pH, O_2 tension are the same for both the larval stages and adults. That is, there is no special nursery environment for all stages.

Differences in growth rate and time to attain maturity cited in the literature are probably a function of: (1) species of algal food available, (2) the presence or absence of bacteria and (3) physiological state of the algal food (Gibor, 1956a;

Mason, 1963; Reeve, 1963a; Sick, 1976). In addition to the above environmental conditions, geographical strains have been included (Gilchrist, 1956; Baid, 1963; D'Agostino, 1965; Sorgeloos et al., 1975). However, recent studies showed that Artemia can be reared and reproduced successfully using inanimate food (Person-le Ruyet, 1975; Jacob, 1978; Sorgeloos et al., 1980).

Technically, the advantagesof Artemia for aquaculture is t that it starts as dry cysts. These cysts are, in fact, inactive embryos and are commercially available, can be stored for years and only have to be incubated for 24-48 hours in seawater to produce free-swimming nauplii.

Nutritional Value of Artemia Nauplii As Food
In Aquaculture Hatcheries

The earliest significant realization of Artemia as an excellent source of food for early stages of larval fish has been documented (Seale, 1933; Rollefsen, 1939). Moreover, recent studies showed that brine shrimp are very well accepted by larval fishes and crustaceans (May, 1970; 1971; Houde, 1972). It is not exactly known whether this can be attributed in their biochemical composition (Benijts et al., 1975; Watanabe et al., 1978b), or their thin carapace (1 μm) or the fact that they are a moving prey (Houde, 1972) or a combination of all these factors. Furthermore, studies showed that a diet of live Artemia gives better results than any preparation of dead Artemia (Serfling et al., 1974; Beck, 1979; Schauer and

and Simpson, 1979). Recent findings showed that whitefish larvae metamorphosed equally well if fed Artemia previously frozen in liquid nitrogen or live Artemia, but not when fed slow-frozen nauplii. Recently, emphasis on larval rearing of finfishes and crustaceans has been placed on the use of geographical strains of the brine shrimp (Reed, 1969; Provenzano and Goy, 1976; Oleynikova and Pleskacheuskaya, 1979; Ucal, 1979; Watanabe, 1979). From these studies, it was concluded that the Artemia from Great Salt Lake (USA) showed poor performance compared with other strains such as those from Buenos Aires (Argentina), Shark Bay (Australia), Macau area (Brazil), Chaplin Lake (Canada), Lavalduc (France), Gujarat area (India), Tientsin (People's Republic of China), Barotac Nuevo (Philippines), Cadiz area (Spain), San Francisco Bay (USA).

Various theories have been suggested to explain the poor performance of the Great Salt Lake (GSL) strain of Artemia. One of these places the blame on accumulation of residual pesticides in the GSL Artemia (Slodbokin, 1968). It is also possible that GSL Artemia might have developed immunity against toxic alkaloid secreted by algal blooms in the lake and con concentrated in the Artemia cysts (Provasoli, 1969). Another suggestion blames mineral dificiency.

Since the use of Artemia is presently limited in most cases to freshly hatched nauplii, the use of adult Artemia has been suggested for further studies. Adult Artemia are twenty times larger and weigh 500 times more than freshly hatched nauplii (Reeve, 1963c). Their nutritional value changes considerably

during growth. Their fat decreases from 20% to less than 10% of the dry weight and the protein content increases from 42% to over 60% (Helfrich, 1973; Benijts et al., 1975). Whereas nauplii are deficient in histidine, methionine, phenylalanine and threonine, adult Artemia are rich in all essential amino acids (Gallagher and Brown, 1975; Watanabe et al., 1978a; Claus et al., 1979).

Present Cultural Practices of Artemia

Exponentially increasing demand for brine shrimp cysts world-wide by aquarium hobbyists and aquaculture hatcheries exceeded the yearly harvest of approximately 30 to 50 metric tons (Sorgeloos, 1976). As a result, commercial aquaculture has been impeded seriously. Macrobrachium and Penaeids are entirely dependent on Artemia diet during their long larval development (Bledsoe et al., 1978; Glude, 1978; Smith et al., 1978). In addition, Third World countries could hardly afford to import the very expensive cysts.

Natural brine shrimp population are still the most important source of commercially available Artemia. However, they are only exploited in a few areas in Canada, France and the USA with the total yearly output from these countries of ca. 1000 metric tons. The potential future harvest from nature where Artemia has, to date, been recorded from more than 150 habitats could considerably increase (Persoone and Sorgeloos, 1980).

A major innovation in the technology of Artemia batch culturing is the air-water-lift raceway, originally developed

for the intensive culture of post-larval Penaeid shrimp (Mock et
al., 1973) but modified for brine shrimp (Sorgeloos et al., 1977;
Bossuyt and Sorgeloos, 1980). Monodiet using whey-powder or
rice bran proved sufficient. However, in view of the differences
in fatty acid composition, it is suggested that studies are
needed to evaluate the nutritional value of brine shrimp raised
on waste products formvarioussacultured organisms (Dobbeleir et
al., 1980). Aside from the air-water-lift raceway technology,
a much more intensified mass production can be achieved in flow-
through systems (Tobias et al., 1979). The flow through test
carried out in St. Croix (US Virgin Islands) were runs with the
effluent of the 2 algal ponds of the local Artificial Upwelling
Project (Roels et al., 1976). Tobias et al. (1979) calculated
that in a 1 m^3 tank 25 kg adult biomass (or 12000 animals l^{-1})
can be produced within 2 weeks from an initial 30 g cysts using
the above system. However, the maximum productivity potential
has not been achieved due to lowwater temperature (22-25°C).

Recently, another interesting source of Artemia production
has come into perspective. Tertiary treatment plants for
industrial effluents of high salinity are capable of producing
substantial amount of adult Artemia (Milligan et al., 1979).
Other similar study was conducted in Bombay, India. The initial
study proved successful in producing gravid females reared in
manured ponds (pig dung) with superphosphate as additional
fertilizer. Phytoplankton population consisting of Navicula sp.,
and Amphiphora sp. bloomed under this condition and served as
feed for the shrimp. However, ground nut oil cake and yeast

were added as supplement (Dwivedi et al., 1979).

Aside from an improved perspective for the use of <u>Artemia</u> in the aquaculture hatcheries, it has become obvious that other applications show very high potential even including direct use in human nutrition. In a taste panelltest in Hawaii of an experimental shrimp tempura prepared from frozen brine shrimp the response was quite favorable.(Davidson, 1974). If not used directly as human food, <u>Artemia</u> meal can be used as a rich source of animal protein in livestock diets (Anonymous, 1978). In this context, brine shrimp may be used as a valuable alternative to fish meal, especially in countries that are entirely dependent on fish meal imports.

MATERIALS AND METHODS

Algae

Dunaliella tertiolecta, a euryhaline algal species obtained
from Dr. N. J. Antia's[1] collections was used in the experiments.
The alga was maintained under axenic conditions in twenty five ml
Erlenmeyer flasks in alternating light and darkness at $18^{o}C$.
The alga was then exposed to a 12 L.: 12 D regime for a couple
of days before transferring to fifty ml Erlenmeyer flasks and
where they were maintained under axenic conditions as a
"starter" culture. When needed, the Erlenmeyer flask cultures
were inoculated into one liter of seawater and allowed to reach
a density suitable for inoculating the larger experimental
culture vessels. This alga was used for the comparative growth
study using two different media, namely, swine waste-seawater
and defined inorganic media. Likewise, algal biomass produced
from each culture media during continuous culture was used for
the feeding experiment of the brine shrimp.

Brine Shrimp

The brine shrimp, Artemia salina L. eggs were obtained
from Dr. J. Marlieve[2]. This strain of Artemia came from San
Francisco Bay, California (USA).

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The brine shrimp eggs were stored at $4^{\circ}C$ prior to use. The eggs were hatched in a funnel shaped bottom plexiglass container at a density of 10 g l^{-1}. The temperature and salinity were maintained at $28 \pm 1^{\circ}C$ and 31 ± 1 ppt respectively in the hatching funnels. The temperature was maintained in a water bath heated by a glass thermo-heater (Supreme Heatmaster Thermostatic 100 watts). The newly hatched nauplii were separated by a separator designed after Sorgeloos and Persoone (1975)

Media and Saltwater Preparation

Swine Waste-Seawater Mixture

Swine waste samples were obtained from the finishing hog in-housed barn facility at the UBC Farm. Wastes were taken from the supernatant portion of the manure pit by using a Bilge pump (Whale gusher 8 pump MK III with 3.81 cm inlet and outlet) manually operated and the waste filtered with a nylon screen mesh (0.3 - 0.5 cm) at the outlet. The filtered swine wastes were then stored in a plastic container (20 l) and transported to the laboratory (Bio-Resource Engineering Department). The swine wastes were stored in a $4^{\circ}C$ walk-in refrigerator. New samples of swine waste were obtained from the UBC barn every four days.

The samples were analysed for total Kjeldahl nitrogen, NH_3-N, NO_3-N $\pm$ NO_2-N and total phosphate within 24 hrs of collection. The ratio between swine waste and seawater volume was varied depending on the nitrogen concentration, but on the

average ca 5-10% wastes and 90-95% seawater. The nitrogen
concentration was maintained at ca 1200 $\pm$ 50 μg-at N l^{-1}.

Defined Inorganic Medium

D. tertiolecta has a conspicuous requirement for sodium
(Mclachlan, 1960). The medium, 'f/2' developed by Guillard
(1975) contains sufficient sodium, other major nutrients such
as nitrogen, phosphorus with trace metal ions and vitamins
served as a nutrient source. The effect of f/2 medium and
swine waste-seawater mixture on algal growth was compared
(see Appendix 1 for f/2 composition).

The sterile nutrients were added to non-sterilized carboys
(20 l) and filled with 15 l seawater before algal inoculation.
There was no sterilization attempt in any of the culture
experiments.

Seawater Preparation

Since the Zoology Department has seawater facilities, the
experimental work was initially conducted at Dr. W. S. Hoar's[3]
laboratory. However, at the onset of spring 1980, the seawater
had a high concentration of mixed algal population due to high
temperature. A preliminary experiment showed that the synthetic
seawater, Forty Fathoms (Bio-crystal Marine Mix; Appendix 2),
when supplemented with nutrients could be used to culture

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$\underline{D}$. $\underline{tertiolecta}$. This artificial seawater was used in further
experiments.

The seawater used for $\underline{Artemia}$ culture was transported
from the Zoology Department to the Bio-Resource Engineering
Department. The seawater was aged for a week before it was
used

Description of Culture Units

Algal Batch Culture Unit

Algal culture units were constructed at Dr. W. S. Hoar's
laboratory at the Zoology Department and at the aquaculture
laboratory of the Bio-Resource Engineering Department.

Batch culture experiments were conducted both in Unit I and
Unit II. Culture containers for Unit II were 20 l borosilicate
carboys. For Unit I (Experiment I),,$\underline{D}$. $\underline{tertiolecta}$ were grown
in one-liter wide mouth Erlenmeyer flasks. Plastic tubing
(Tygon R-3603 0.476 x 0.635 x 0.14 cm) fitted to glass tubing
(0.5 cm o. d.) connected the culture flasks with the aeration
system. Air was filtered before it was introduced into the
culture flasks with absorbent cotton inside a Nalgene filter
unit. Glass tubing outlets were also provided for air pressure
build-up and as sampling ports. Rubber stoppers (no. 8) supported
the glass tubing which also prevented leakage and evaporation.

The temperature in the culture vessels was maintained at
24 ± 1°C by placing the vessels in the wooden water bath (115 cm
x 49 cm x 9 cm) with water flowing through at 1 l hr^{-1}.

Four 40-Watt "Cool White" Fluorescent Tubes (F 24712; H. O. Lamps)
provided continuous illumination. Two tubes were installed
horizontally on both sides approximately 15-20 cm away from the
culture flasks. Fluoresecent fixtures (BL-248, 98 Watts, 0.81
amps, 120 volts) provided power for the lights. These fixtures
were attached on Handy-angle support frames. The frame was
covered with peg board (122 x 45 cm).

Batch cultures using 20 l borosilicate carboys was
conducted in Unit II with a set-up similar to continuous culture
experiments.

Algal Continuous Culture Unit II

Unit II was used for continuous culture experiments as well
as for batch culture experiment using 20 l borosilicate (Pyrex)
culture container. This Unit was housed in a peg board-Handy
angle frame (230 x 131 x 220 cm). A bank of four fluorescent
tubes (4 ft, 40 watts "cool white") were attached on both sides
of the culture containers. Likewise, two tubes were attached
overhead.

The continuous culture apparatus consisted of a nutrient
reservoir connected to three carboys and a common yield 4-liter
aspirator bottles. The algal effluent was collected and stored
in 20 l carboys (Figure 1).

The nutrient mixtures (defined inorganic and swine waste-
seawater media) were stored separately in plastic containers
(20 l) on an elevated platform. A multi-channel peristaltic
casette pump (Manostat, New York, N.Y.) was used to control the
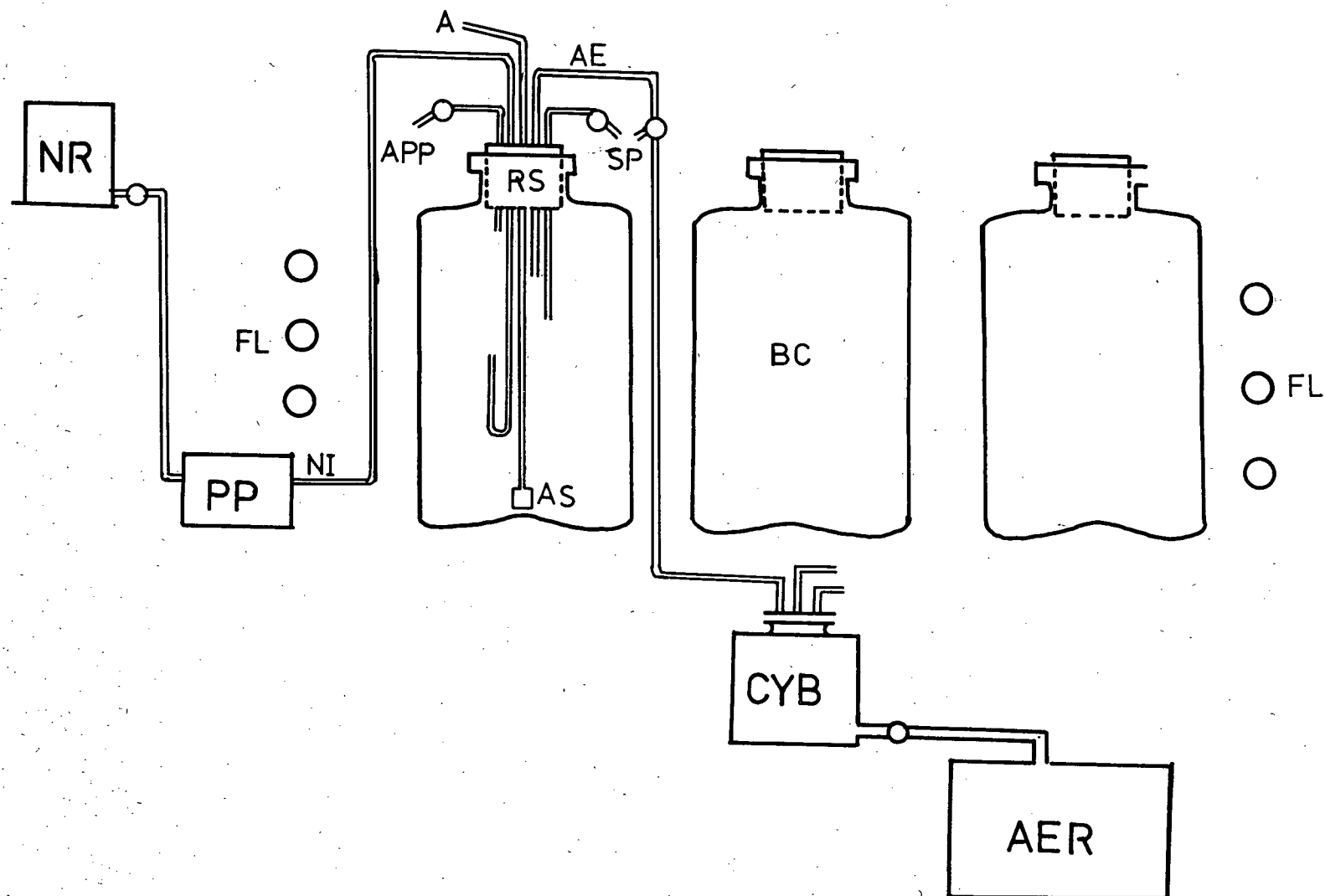
Figure 1. Generalized view of the algal continuous culture system.
A, air; AE, algal effluent; APP, air pressure port; AS, air stone;
AER, algal effluent reservoir; BC, borosilicate carboys; CYB, common
yield bottle; FL, fluorescent lights; NI, nutrient inflow;
NR, nutrient reservoir; PP, peristaltic pump; RS, rubber stopper;
SP, sampling port.

inflow of nutrients to all culture containers. The flow rate was maintained at an average of 2.4 l day^{-1}. Tygon tubing (I. D. 0.476 cm) was used to convey the nutrients. Air was filtered before it was introduced into the culture container through a Nalgene filter unit with absorbent cotton. Glass tubing outlets were provided for air-pressure build-up and as a sampling port. A rubber stopper (no. 11) supported the glass tubing, prevented leakage and evaporation. There was no attempt to sterilize the culture carboys, stoppers, tygon and glass tubings. However, culture vessels were acid-washed and rinsed with distilled water and oven dried.

The temperature in the culture container was maintained at 24 $\pm$ 1°C by placing in wooden water bath (244 cm x 122 cm x 15.2 cm) with water flowing through at 1 l hr^{-1}.

Algal Continuous Culture Unit III

The seawater at the Zoology Department had a high concentration of contaminants which basically consisted of mixtures of algal flagellates during the summer time. Also, the laboratory at the Zoology Department was to be renovated before the anticipated completion of the work. Experimental work was moved to the aquaculture laboratory of the Bio-Resource Engineer Engineering Department.

Unit III set-up was similar to that of Unit II. However, the whole set-up was conducted in a controlled environmental chamber (Conviron Model E8M, Controlled Environmental Ltd., Winnipeg, Manitoba, Canada. Temperature was controlled and

maintained at $24^{\circ}C$. Continuous illumination was provided with 6 fluorescent tubes (Powertubes 48" VHO 'Cool White' F48T1Z-CW, Sylvania, Canada) placed overhead. Illumination was measured by Quantum/Radiometer/Photometer sensors (Lambda LI-185). The average light intensity was ca 0.04 cal min^{-1} cm^{-2} (0.04-0.05 ly min^{-1}) measured inside the carboys without the liquid being present.

Brine Shrimp Culture Unit

Brine shrimp were cultured in cylindrical fiberglass tanks of 40 liter capacity. A PVC (127 mm diamter) stand pipe fitted with nylon screen mesh (0.1-0.3 mm) at the outlet maintained the volume at 20 liters. A handy angle frame held the fiberglass tanks upright and a wooden drainage system was provided. The water column was constantly aerated (600-700 ml^{-1} min^{-1}) in order to resuspend the algae throughout the water column and to provide oxygen for the brine shrimps. Air flow was measured by Gilmont Flowmeter (F-1200). The temperature in the tanks was maintained at $27 \pm 1^{\circ}C$ by glass thermo-heaters (Supreme Heatmaster Thermostatic 100 watts).

<center>Culture Methods</center>

Algal Culture Methods

Although none of the culture units remained bacteria-free, contamination by other algae was avoided by inoculating with an axenic culture and filtering the seawater with a high pressure filter unit (AMF/CUNO Filter Model 1A1) attached to a self-priming pump (Jabsco Model No. 12310-0001).

The density of the inoculant was determined before it was introduced to the culture units. The algal cells were counted daily using a hemacytometer (New Improved Neubauer Chamber, Hausser Scientific). To immobilize the motile D. tertiolecta, 2 or 3 drops of 5% formalin were added to the algal samples before counting. The same four squares on the grid of each chamber were counted each time and the average count was multiplied by 10^4. Thus, given the average Q, the density of (cells ml^{-1}) of the suspension in the hemacytometer was calculated from the expression,

$$d = 10^4 \times Q$$

Three replicate counts were made and the mean value, calculated.

Salinity was maintained at 32 ± 1 ppt and was measured using a hydrometer (Specific gravity for heavy liquids TP $60/60^oF$ range 1000-1220). Temperature readings were taken daily in all culture units. Periodic checks on pH change in the cultures helped to monitor acidic and/or basic conditions within the culture. The pH was measured using a pH meter (Fisher Accumet Model 420 Digital pH/ion meter).

Once the algal growth phase reached the decreasing or declining logarithmic phase (Myers, 1962) at a density of approximately 4 x 10^6 cell ml^{-1}, the nutrient inflow to the culture vessel was initiated. Flow rate was maintained at approximately 2.33 - 2.50 liter day^{-1} $carboy^{-1}$. At this flow rate, the cell density reached steady state, as determined by identical cell counts over a period of 2-3 days. The cell densities would drop to a lower concentration at a higher flow

rate.

Brine Shrimp Culture Method

The incubation period was about 48 hours at 26 ± 1°C. Density determination was conducted after the newly-hatched nauplii were separated from unhatched eggs and empty cysts.

Newly-hatched Artemia were placed in a 4-liter plastic container, moderately aerated to keep them evenly suspended. Five separate samples of 5 ml each were taken from the suspension. An 8 mm diameter, 250 mm long glass tubing was used as sampling tube. Both ends of the tube were opened and when sampling, one end was closed by a thumb. Thus, the entire water column was sampled. Each of the 5 ml samples were placed in separate cylinders (50 ml cap). Then the volume in the cylinders was made up to 25 ml of the same salinity. This volume was aerated slowly to evenly suspend the nauplii. Again, 5 ml were taken from each cylinder and were placed in small glass petri dishes. Several drops of Lugol's solution were added to each petri dish. Then, the petri dishes were placed under a dissecting microscope and the nauplii counted. The number of nauplii in the original container was obtained by the following equation:

$$\text{Total no. of nauplii} = \frac{\text{Total no. of nauplii counted}}{5} \times \text{volume of container}$$

Initial concentration in each tank was 2-3 nauplii liter^{-1}. Algal cell concentration was maintained daily at approximately 5.0×10^5 ml^{-1} in each tank.

Survival counts were conducted daily in each tank.
A 50 ml beaker was used to sample the water column and the
nauplii were counted. The culture was agitated by a relatively
strong aeration for a minute before sampling. Counting was done
3 times for each tank.

To measure growth, ten animals were sampled daily and
preseved from each tank. Preserved animals (in a 5% formalin
solution)wwere measured two weeks after the experiment.
The total body length was measured from the anterior margin of
the head to the base of the caudal furca (Gilchrist, 1956).
During measurement, the animals were placed ventral side down on
a microscope slide.

The temperature in the culture tanks was maintained at
$27 \pm 1^{\circ}C$ by glass thermostatic heaters. Oxygen concentration
was at saturation levels.

<center>Chemical Analysis</center>

Kjeldahl Nitrogen

The method followed was an adaptation of Wall et al. (1974)
and Technicon AutoAnalyser II (1971b). Five ml of the sample
(swine waste or algal suspension)wwere introduced into a 50 ml
digestion tube. To enhance the oxidation reduction, 0.5 g of a
digestion catalyst (composed of 9960 g K_2SO_4, 35 g $CuSO_4$, and 5 g
SeO_2) were added to each tube. The tubes were then placed in a
digestor rack at $360^{\circ}C$ for 6-12 hours. The addition of boiling
chips prevented excessive bumping during digestion and a small
glass funnel on the open end of the tubes prevented spillage

and excessive evaporation. After digestion, the tubes were
removed from the digestor and cooled for about an hour. Distilled
water was then added to each of the flasks to a volume of 50 ml.
Further dilution of the sample with distilled water was necessary
when the auto-analysis graphical recorder went off-scale.
Approximately 10 ml of each diluted sample were placed on a
rotating sampler of the auto-analyser.

Ammonia / Nitrate + Nitrite

The automated procedure for the determination of the above
nitrogenous compounds was adapted to that of Technicon AutoAnalyser
II, 1969; 1971a.

Total Phosphate

Samples were digested before determining total phosphate
through the auto-analyser. The digestion procedure was similar
to that used for the determination of total Kjeldahl nitrogen.
The automated procedure for the determination of total phosphate
was adapted to that of Technicon AutoAnalyser, 1971c.

Dry Weight Analysis

An increase in dry weight of cells is a widely used method
of growth measurement.

Fifty ml aliquot samples from each culture container were
filtered through glass fiber filter (Reeve Angel Grade 934 AH,
size 4.25 cm diameter) using vacuum suction. The filter paper
was placed on the crucible and oven dried at 100^0C for about an

hour and weighed before using. After filtration, crucible and filter paper were oven dried to a constant weight. Three replicates were run for each of the culture vessel. The filter paper and crucible were weighed on a Mettler balance (Type H6 dig cap 160 g, Mettler Instrument, Highstown N. S. Canada). The weight was recorded to four decimal places. Dry weight biomass of <u>Artemia</u> was measured as described above.

Total Suspended Solids

To determine the variability of the solid concentration in the swine waste samples, total suspended solids were analysed. Using suction, 100 ml samples were filterd and washed with 2-5 ml distilled water. The crucible and filter paper (Whatman 7.0 cm no. 541 hardened, ashless) were oven dried at 103-105°C for an hour before using. The oven dried filter paper was cooled in a desiccator and the weight was recorded. After filtration, the filter paper and crucible were then placed in the oven for an hour, cooled in a desiccator and weighed (gross weight). Drying and weighing were carried out untillconstant weight was achieved (normally three times). Five replicates were run for each sample.

Analysis for total suspended solids was conducted for 'fresh' samples taken from the pit-manure. Total suspended solids was calculated using the following equation:

$$\text{TSS ppm (mg l}^{-1}) = \frac{\text{Gross wt - Tare wt (mg)}}{\text{vol of sample (ml)}} \times 10^6$$

RESULTS

Swine Waste Analysis

During the course of the experiments, the total Kjeldahl nitrogen concentration (TKN = organic nitrogen and ammonia nitrogen) and other inorganic nitrogen (NO_2-N + NO_3-N) and PO_4-P of the supernatant part of the swine waste varied only slightly and also there was not much considerable changes in total suspended solids (TSS) as shown in Table 1. Measurement of total Kjeldahl nitrogen of the swine waste samples and three different ratios of swine waste-seawater mixtures (1:1, 2:1 and 1:2) stored at 4°C refrigerator were carried out to determine if changes in N distribution might occur during storage. The results showed no considerable change of the total Kjeldahl nitrogen concentration between day 1 and day 5 (Figure 2).

Algal Batch Culture System

The first experiment on the comparative growth study of D. tertiolecta grown in defined inorganic medium and swine waste-seawater medium was conducted in one-liter wide mouth Erlenmeyer flasks in batch culture system.

Figure 3 shows daily cell density during the seven day culture period. Growth constant ($K_{(10)}$ hr^{-1}) were 0.023 and 0.024 and the mean generation time (t_g hrs) were 13.0 and 12.5 for defined inorganic and swine waste-seawater media, respectively.

Another batch culture study (Expt 2) was conducted in 20 liter capacity Pyrex carboys. However, the culture volume was

Table 1. Total Kjeldahl nitrogen (TKN), NH_3-N, NO_3-N $+ NO_2$-N, PO_4-P and Total Suspended Solids (TSS) of swine waste during the course of the experimental work.

Sampling Date	Experiment No.	Total Kjeldahl Nitrogen (TKN) (μg-at N l^{-1})	NH_4-N (μg-at N l^{-1})	NO_3-N+NO_2-N (μg-at N l^{-1})	PO_4-P (μg-at P l^{-1})	Total Suspended Solids (mg l^{-1})
25 Sept 1979*	1	2.5×10^4	1.0×10^4	3.57	3.2×10^2	3.0×10^3
28 Oct	2	4.9	4.0	5.00	3.87	3.9
29 Nov	3	5.0	3.5	6.43	5.16	3.31
07 Dec	3	4.6	3.0	3.57	3.20	
10 Dec	3	5.4	3.6	3.57	6.12	3.4
13 Dec	3	5.1	3.6	2.14	5.80	4.05
16	3	4.8	3.4	5.0		
04 Fed (Expt 4 was discontinued)		3.9	2.8	5.71	3.20	3.09
10 March		4.2	2.5		3.87	3.50
18 June	5	3.8	2.3	3.57	3.20	3.7
22	5	3.7	2.1	5.0	2.9	3.05
26	5	3.8	2.2	3.57	3.54	3.12
30	5	4.1	2.5	5.0	4.83	
05 July	5	3.6	2.2		3.20	3.8
10	5	4.3	2.5	3.57	4.19	
15	5	3.7	2.2		2.58	3.5

*This particular swine waste sample was obtained from R and H Farm Langley, B. C. The rest of the samples were from U. B. C. No. 1 Research Farm.
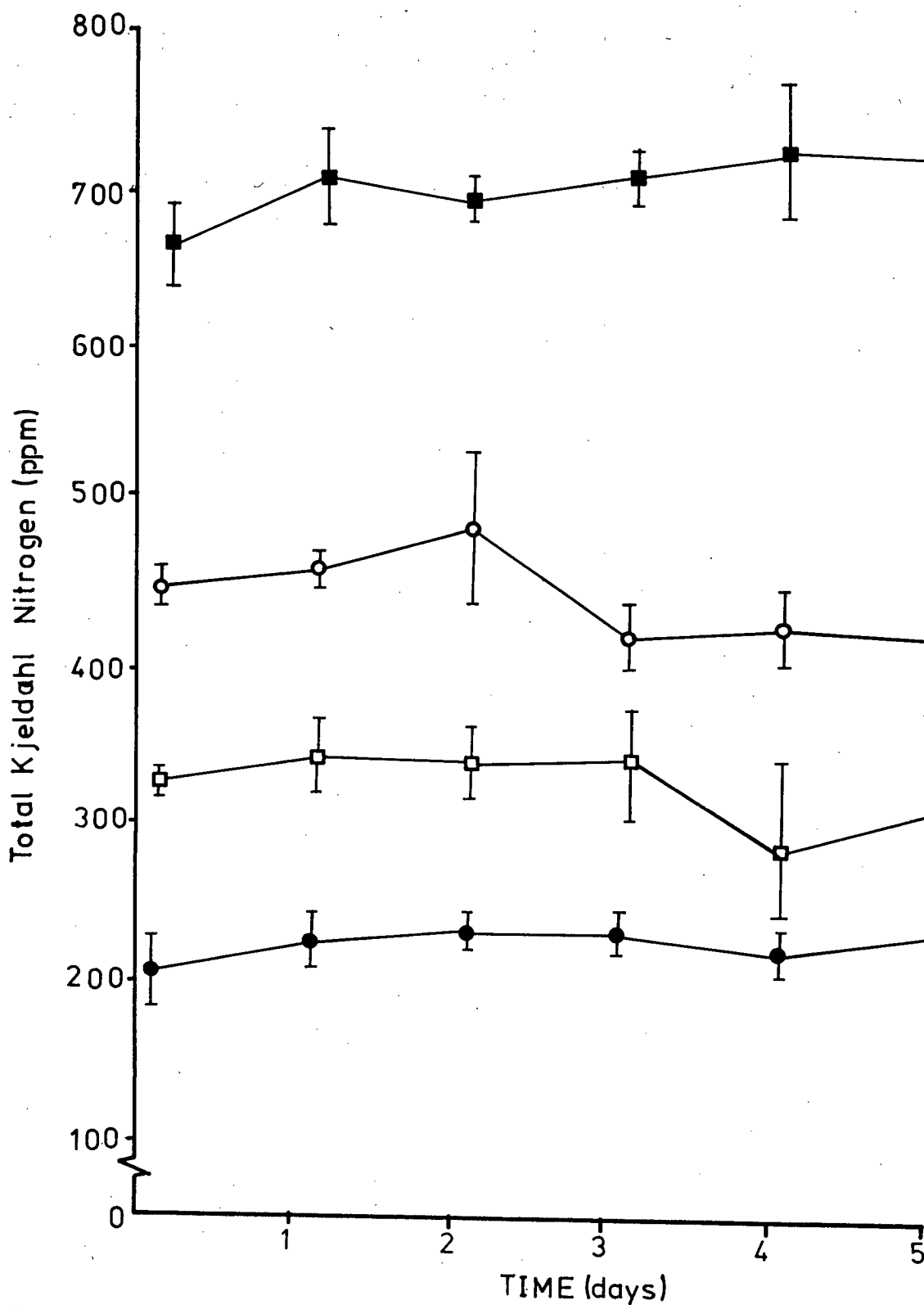
Figure 2. Five-day measurement of total Kjeldahl nitrogen (TKN) of swine waste slurry (■) and three different ratios of swine waste-seawater mixtures (1 : 1 ◻ ; 2 : 1 ○ ; 1 : 1 ●) stored at 4°C. The above values are means and standard deviations.
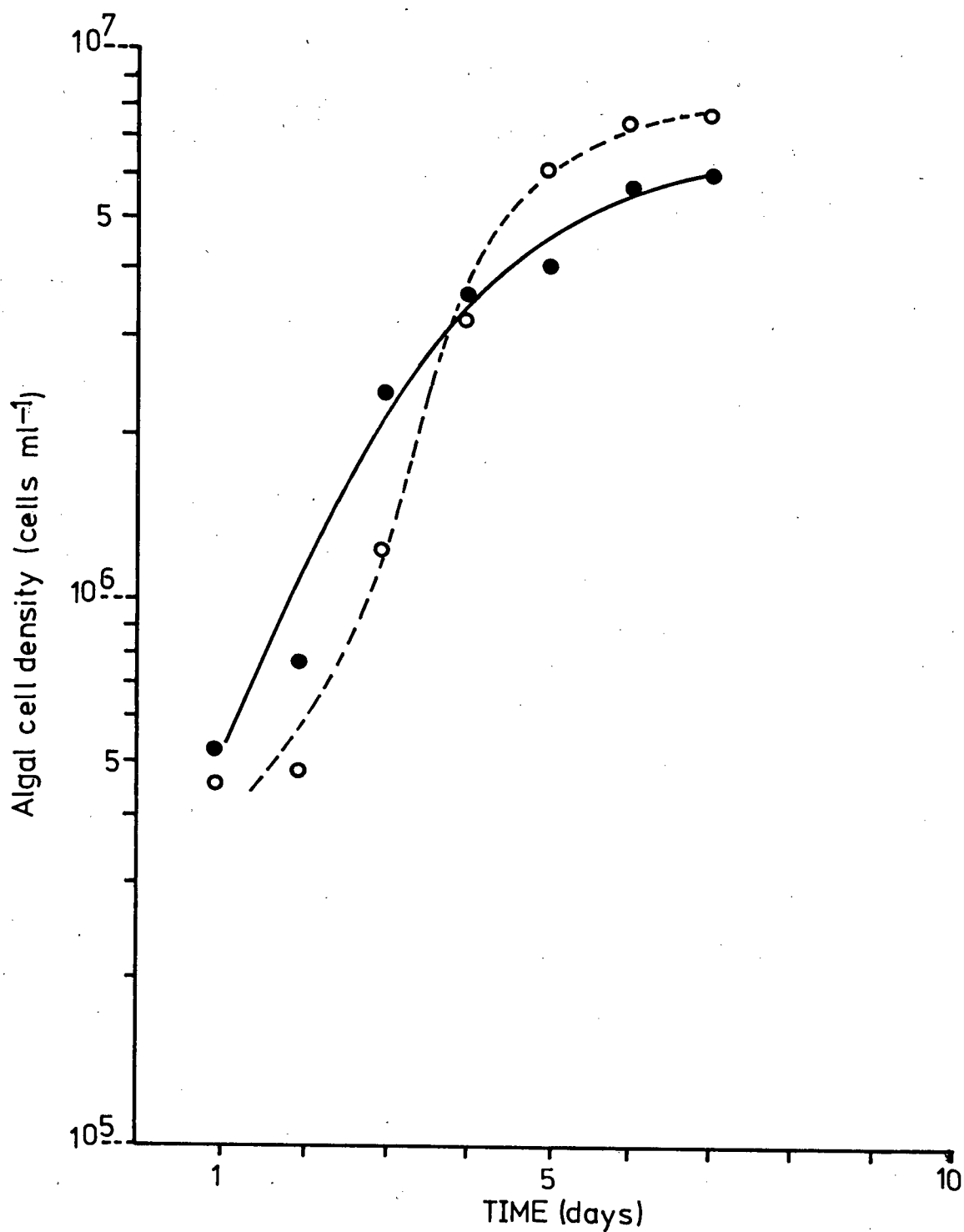
Figure 3. Daily cell density of D. tertiolecta grown
in swine waste-seawater (O) and defined
inorganic media (●) in batch system (Expt 1).

maintained only up to 15 liters. In this experiment and also
with the latter ones, three replicates were used for each
treatment. Figures 4 and 5 show daily cell density and algal
dry weight, respectively. Again, the growth constants were
calculated. Statistical analysis using analysis of variance
(ANOVA) showed no significant differences between treatments and
replicates for growth constant (Appendix 3). The statistical
analysis was done through the UBC Computer System using a
General Least Squares Analysis of Variance Programme known as
GENLIN (Greig and Bjerring, 1977).

Algal Continuous Culture System

Continuous culture studies were conducted in 20 liter
capacity Pyrex carboys. The first continuous culture experiment
was terminated on day 16 because there were some problems with
seawater supply in the laboratory (Dr. W. S. Hoar's laboratory,
Zoology Department). Figures 6 and 7 show daily cell density
and algal dry weight, respectively. Statistical analysis for the
growth constant, cell density and dry weight showed no significant
differences ($P < 0.05$) between treatments and replicates (Appendices
4a, 4b, 4c).

The second continuous culture experiment was terminated on
day 27. The algae produced daily from this experiment were used
in the feeding studies of <u>Artemia</u>. Figures 8 and 9 show cell
density and dry weight, respectively, measured daily during the
culture period. This experiment was conducted in a controlled
environmental chamber at the Department of Bio-Resource
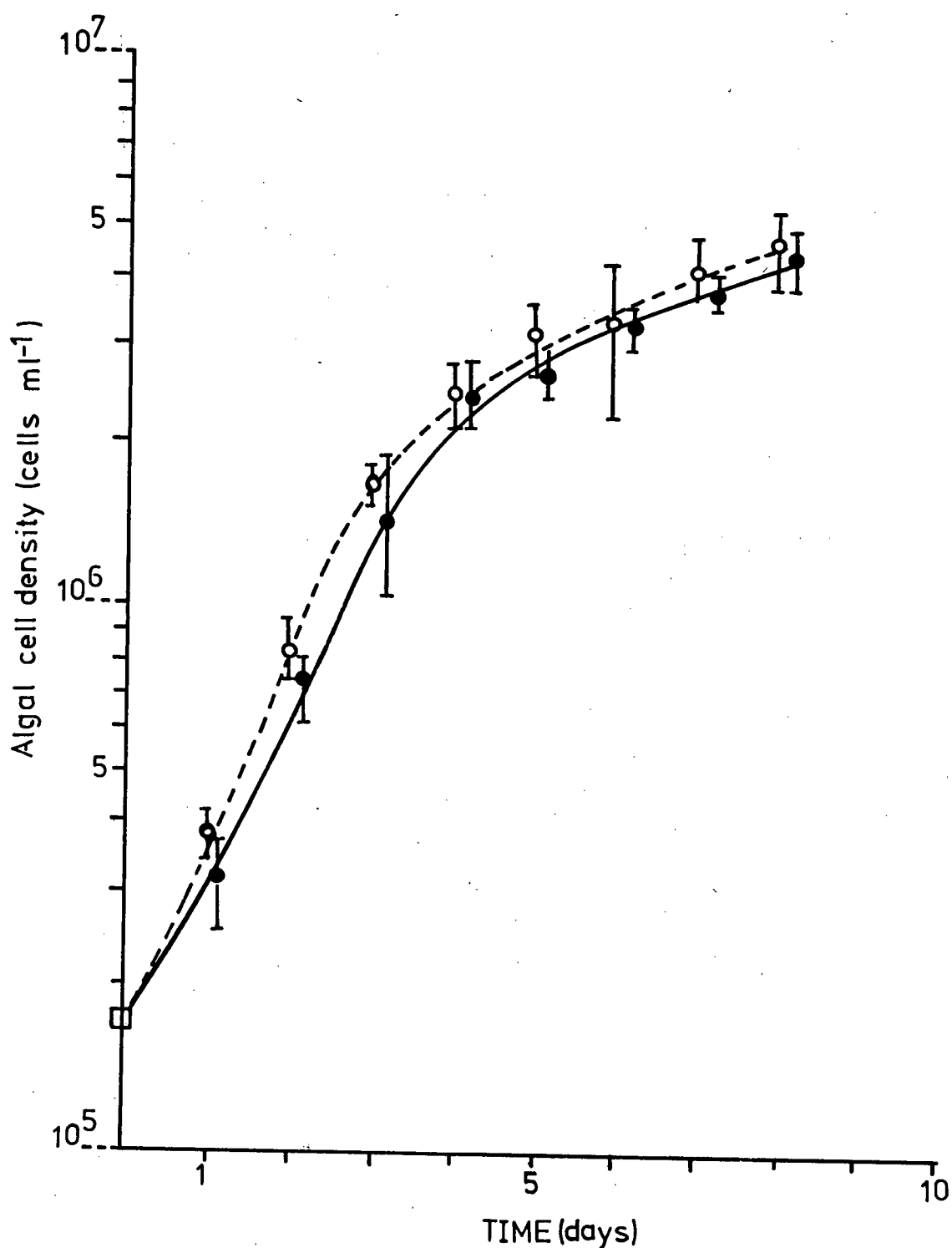
Figure 4. Daily cell density of D. tertiolecta grown in swine waste-seawater (○) and defined inorganic media (●) in batch culture system using 20 l borosilicate carboys (Expt 2). The above values are ranges and means of replicates per treatment.
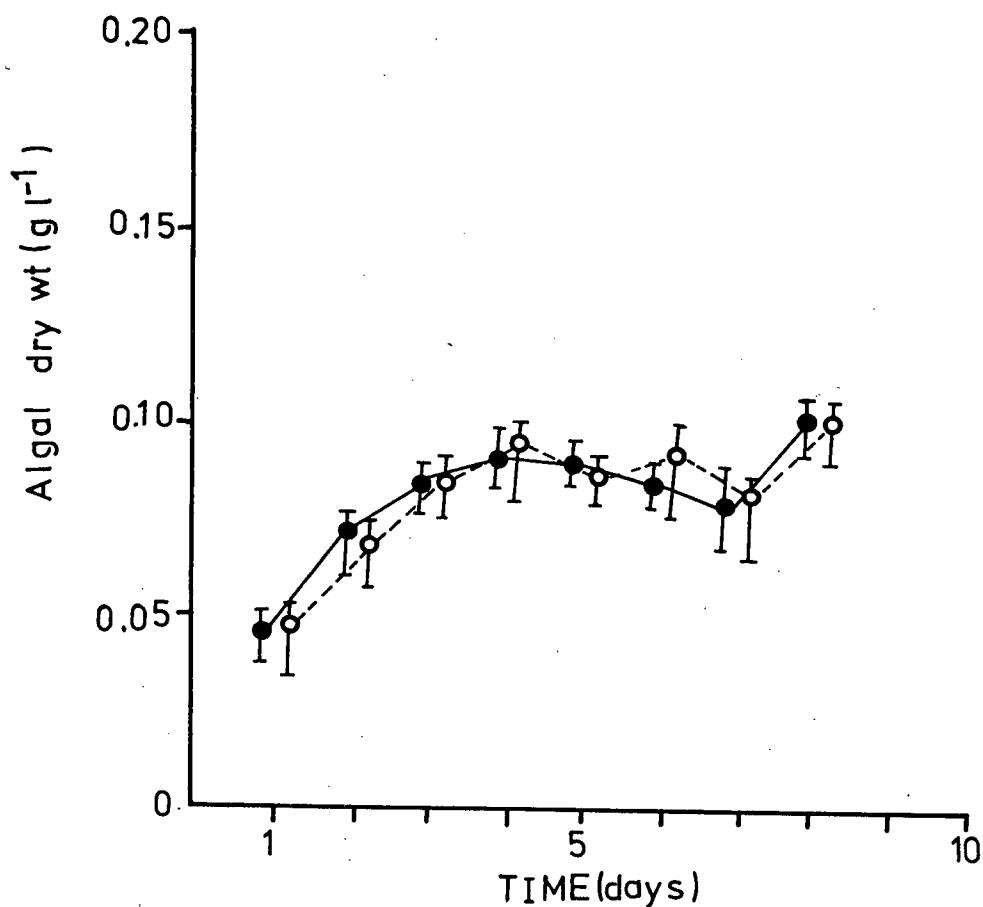
Figure 5. Daily measurement of dry wt of D. tertiolecta
grown in swine waste-seawater (o) and defined
inorganic media (●) in a batch culture system
using 20 l borosilicate carboys (Expt 2).
The above values are ranges and means of
replicates per treatment.
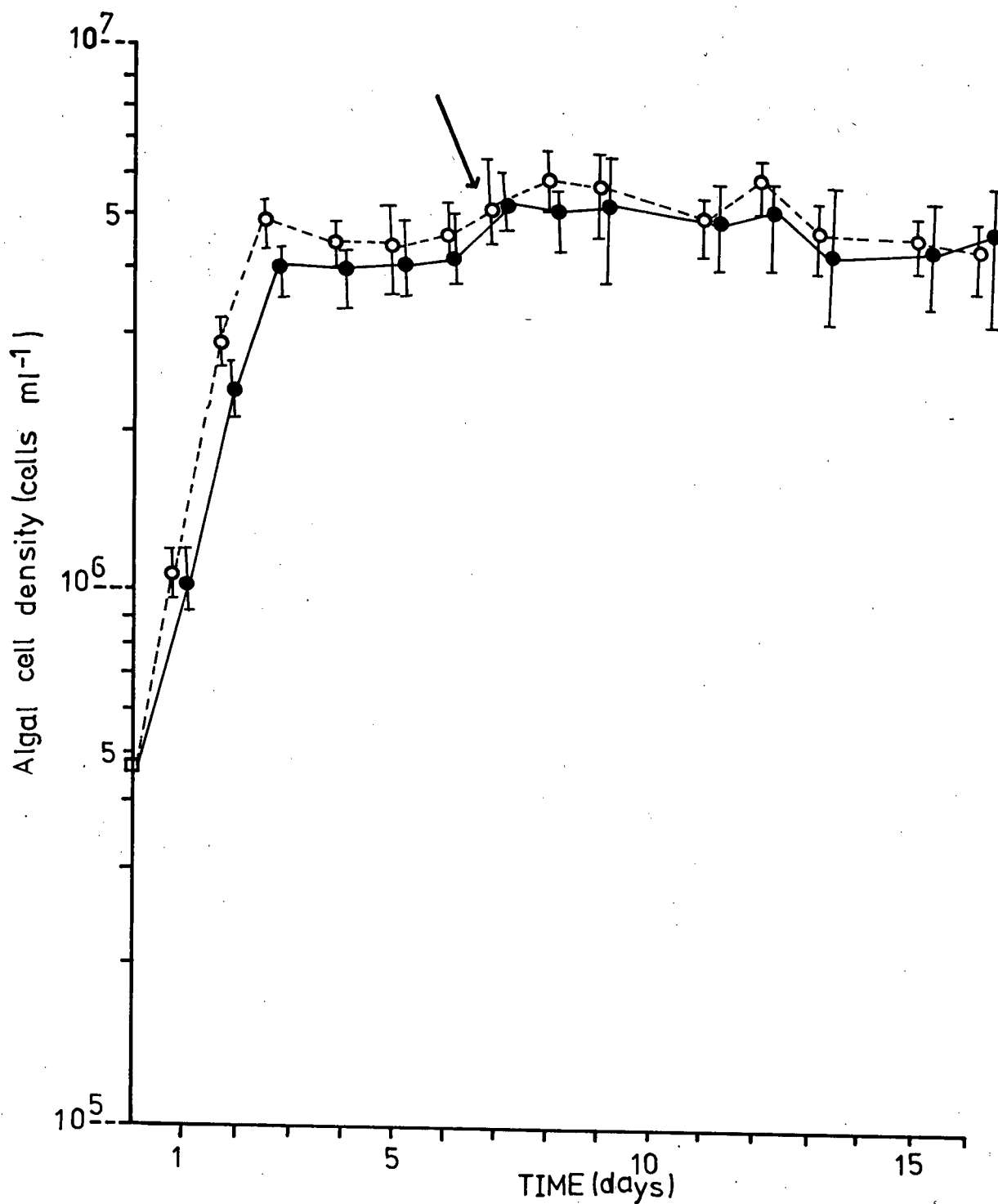
Figure 6. Daily cell density of D. tertiolecta grown in swine
waste-seawater (O) and defined inorganic media (●)
in continuous culture system using 20 l borosilicate
carboys (Expt 3). The above values are ranges
and means of replicates per treatment.
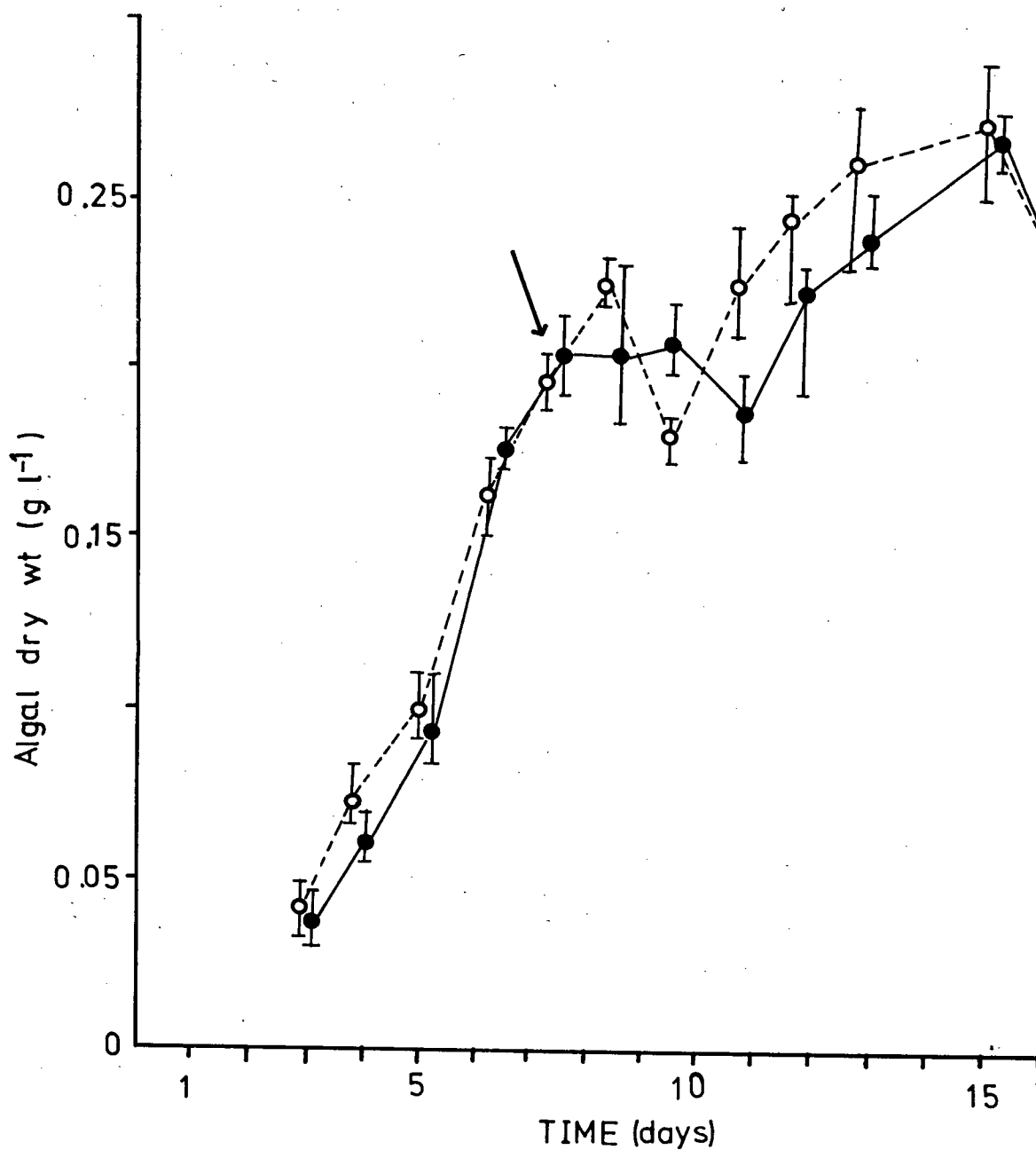
Figure 7. Daily measurement of dry wt of <u>D. tertiolecta</u> grown in swine waste-seawater (○) and defined inorganic media (●) in continuous culture system using 20 l borosilicate carboys (Expt 3). Arrow indicates start of continuous culture. The above values are ranges and means of replicates per treatment.
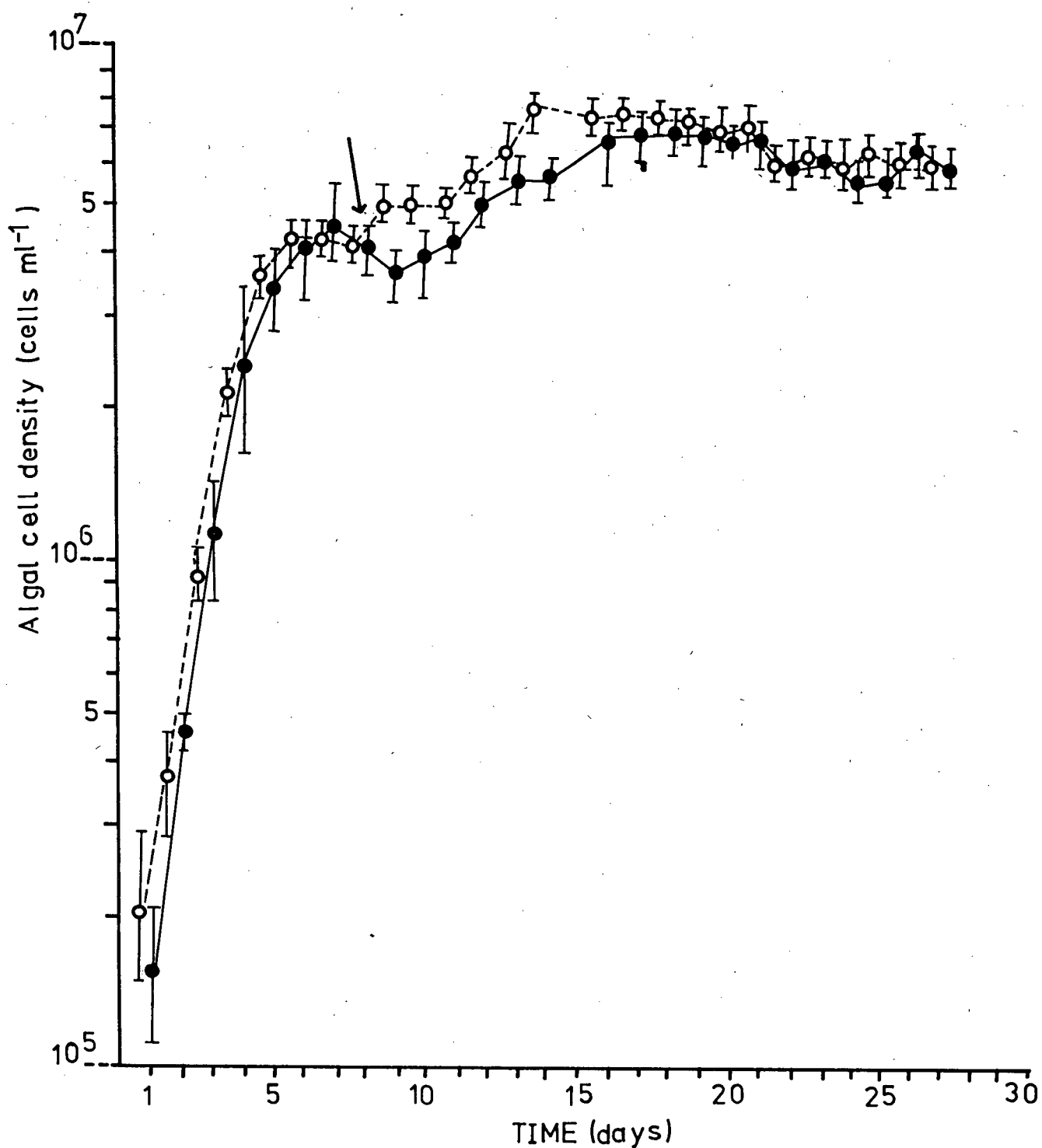
Figure 8. Daily cell density of D. tertiolecta grown in swine
waste-seawater (o) and defined inorganic media (●)
in continuous culture system using 20 l borosilicate
carboys (Expt 5). Arrow indicates start of
continuous culture. The above values are ranges
and means of replicates per treatment.
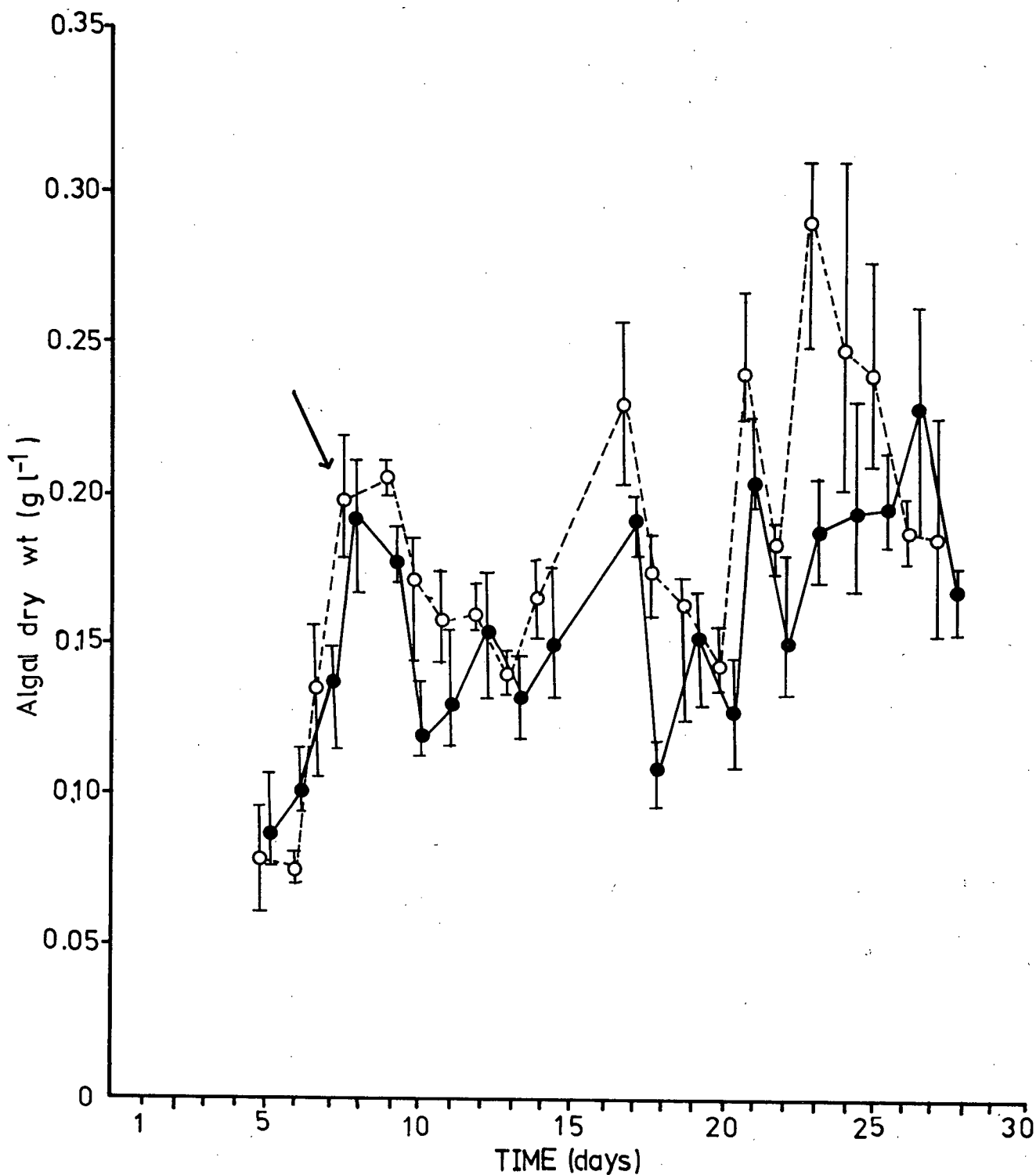
Figure 9. Daily measurement of dry wt of D. tertiolecta grown
in swine waste-seawater (o) and defined inorganic
media (●) in continuous culture system using 20 l
borosilicate carboys (Expt 5). Arrow indicates
start of continuous culture. The above values are
ranges and means of replicates per treatment.

Engineering Department Aquaculture Laboratory. Statistical analysis showed a highly significant difference (P<0.01) between treatments but it showed no significant differences for replicates. (Appendix 5a). The data analysed were from day 8 (onset of nutrient inflow) up to day 27. However, the results were different when data were analysed in segments (Appendices 5b, 5c, 5d). Thus, the following were obtained:

Days		F-Ratio
8-14	treatment	highly significant (P < 0.01)
	replicate	not significant
15-22	treatment	both not significant
	replicate	
23-27	treatment	both not significant
	replicate	

The variability of the above results was probably due to the very dynamic system. Statistical analysis for growth constant showed no significant differences for treatments and replicates (Appendix 6). However, for algal dry weight, significant differences for treatment (P<0.01) and for replicates (P<0.05) were observed (Appendix 7).

Nutrients in the form of nitrogen such as total Kjeldahl nitrogen, NH_3-N, Organic-N and NO_3-N + NO_2-N were measured in the algal effluent during the second continuous culture (Expt 5) to determine whether these nutrients were fully utilized (Table 2).

Table 2. Nitrogen (TKN, NH_3-N, Organic-N, NO_3-N + NO_2) measured in the algal effluent during the second continuous culture (Expt 5) in μg-at N l^{-1}.(SW, swine waste seawater medium; DM, defined inorganic medium).

Date	Sample No.	TKN	NH_3-N	Organic Nitrogen*	NO_3-N+NO_2-N
26 June (d9)	SW 1	3.8×10^2	3.2×10^2	6.0×10^1	undetectable
	2	3.6	3.5	1.0	"
	3	3.4	2.8	6.0	"
	DM 1	2.0	1.4×10^1	1.9×10^2	1.78×10^2
	2	1.7	1.4	1.6	1.85
	3	2.1	1.8	1.9	1.28
30 June (d13)	SW 1	3.2	2.1×10^2	1.1×10^2	undetectable
	2	2.8	1.4	1.4	"
	3	2.9	2.1	8.0×10^1	"
	DM 1	1.3	3.8×10^1	9.2	1.07×10^2
	2	1.6	2.1	1.4×10^2	1.28
	3	1.4	1.8	1.2	1.14
06 July (d19)	SW 1	2.5	1.8×10^2	7.0×10^1	undetectable
	2	2.1	1.3	8.0	"
	3	1.8	1.1	7.0	"
	DM 1	7.1×10^1	3.6×10^1	3.5	1.42×10^2
	2	1.4×10^2	2.6	1.1×10^2	1.64
	3	1.4	2.1	1.2	1.14
10 July (d23)	SW 1	2.7	1.4×10^2	1.3	undetectable
	2	2.3	1.4	9.0×10^1	"
	3	1.9	8.9×10^1	1.0×10^2	"
	DM 1	9.3×10^1	1.9	7.4×10^1	1.28×10^2
	2	3.6	1.9	2.0	1.50 x
	3	1.3×10^2	1.8	1.1×10^2	1.78

Sample nos 1, 2 and 3 were obtained from each of the culture vessels.

*Organic Nitrogen = (TKN) - (NH_3-N)

Brine Shrimp Feeding Experiments

Figure 10 shows the growth in total length of <u>Artemia</u> (freshly measured and preserved samples) during the 12-day culture period. In this preliminary experiment the algal density was maintained daily at the range 3.0 to 5.0 x 10^5 cells ml^{-1}. Figure 11 shows the percentage survival for this experiment.

Figures 12 and 13 show the total length measurement and percentage survival of the shrimp, respectively, during the 14-day culture period. In this particular experiment four replicates were used for each treatment.

Statistical analysis for total length measurement showed no significant differences for treatments and replicates (Appendix 8). However, statistical analysis for percentage survival showed highly significant differences ($P < 0.01$) for treatments and replicates. The overall mean length of <u>Artemia</u> in treatment 2 (<u>Artemia</u> fed with swine waste grown algae) was higher than treatment 1 (<u>Artemia</u> fed with defined inorganic medium grown algae). Furthermore, statistical analysis showed different results when percentage survival data were analysed in segments (days 1-7 and 8-14). The analysis for the first seven days showed highly significant differences ($P < 0.01$) in percentage survival for treatments and replicates. However, during the last seven days the percentage survival showed no significant differences for treatments and replicates (Appendices 9a, 9b, 9c).
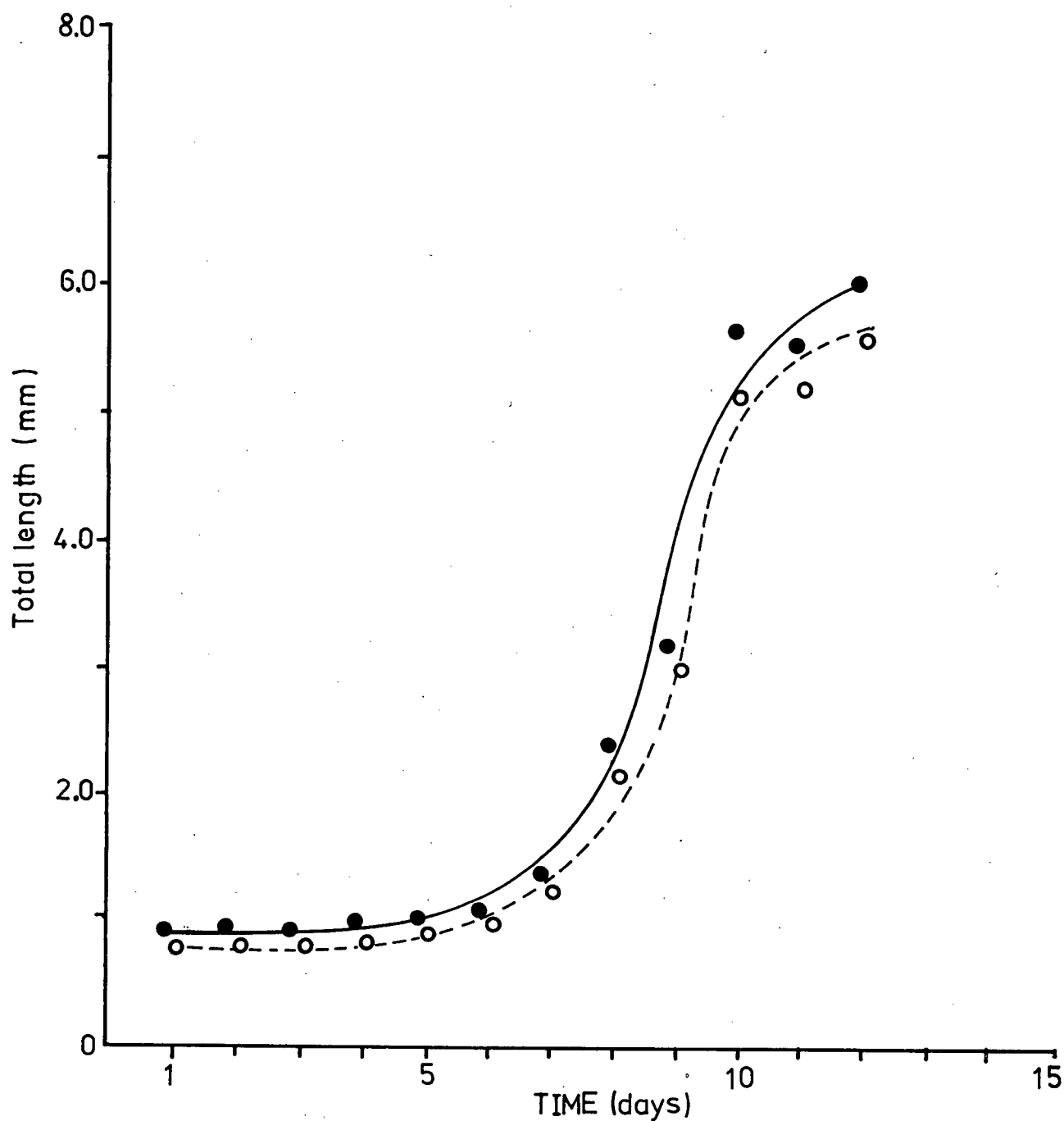
Figure 10. Growth in total length of _Artemia_ _salina_ L. over a
            12-day culture period fed with _D_. _tertiolecta_ grown
            in defined inorganic medium. The above values are
            average measurements of anesthesized samples (●)
            and 2-weeks old preserved samples (o).
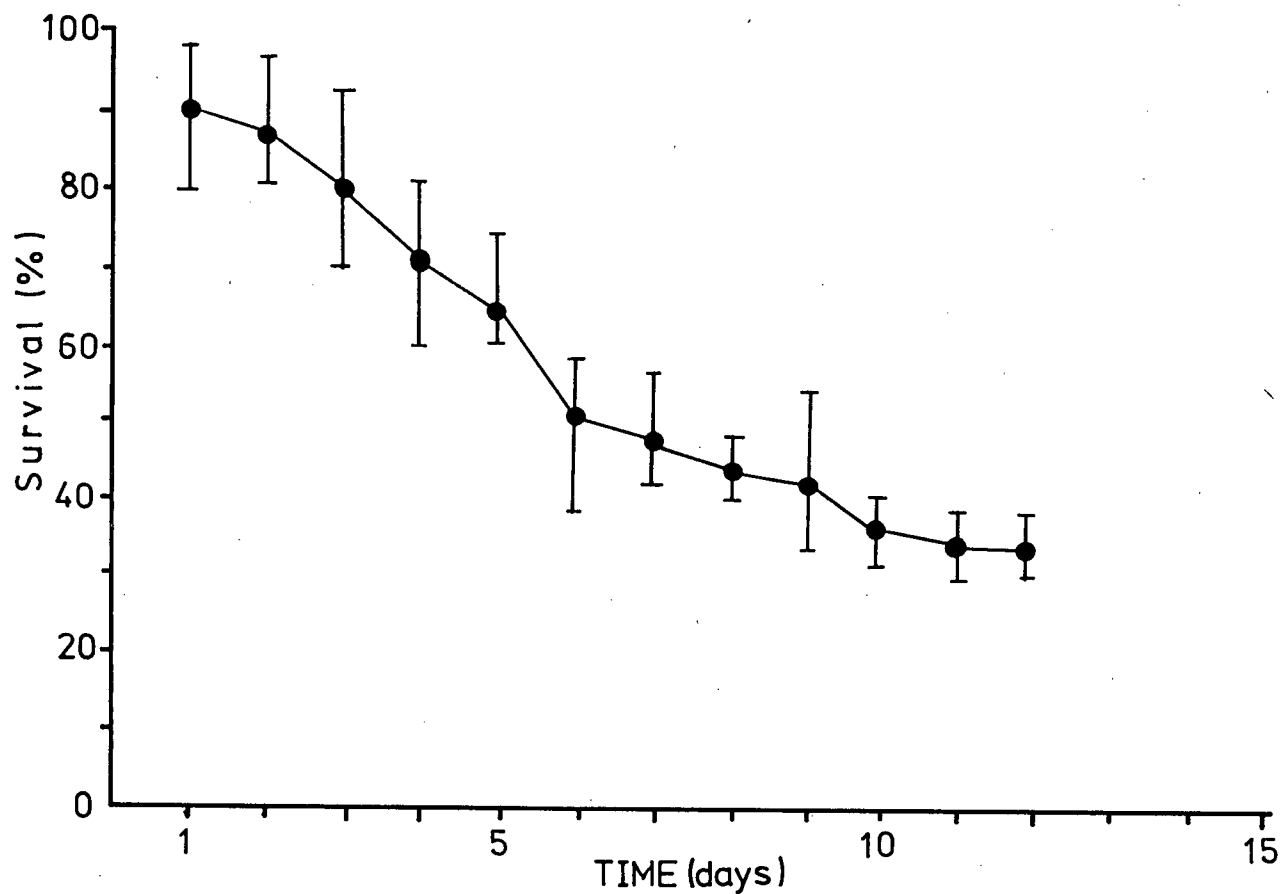
Figure 11. Percentage survival of <u>Artemia</u> <u>salina</u> L. fed with
           <u>D</u>. <u>tertiolecta</u> grown in defined inorganic medium.
           The above values are ranges and means of replicates.
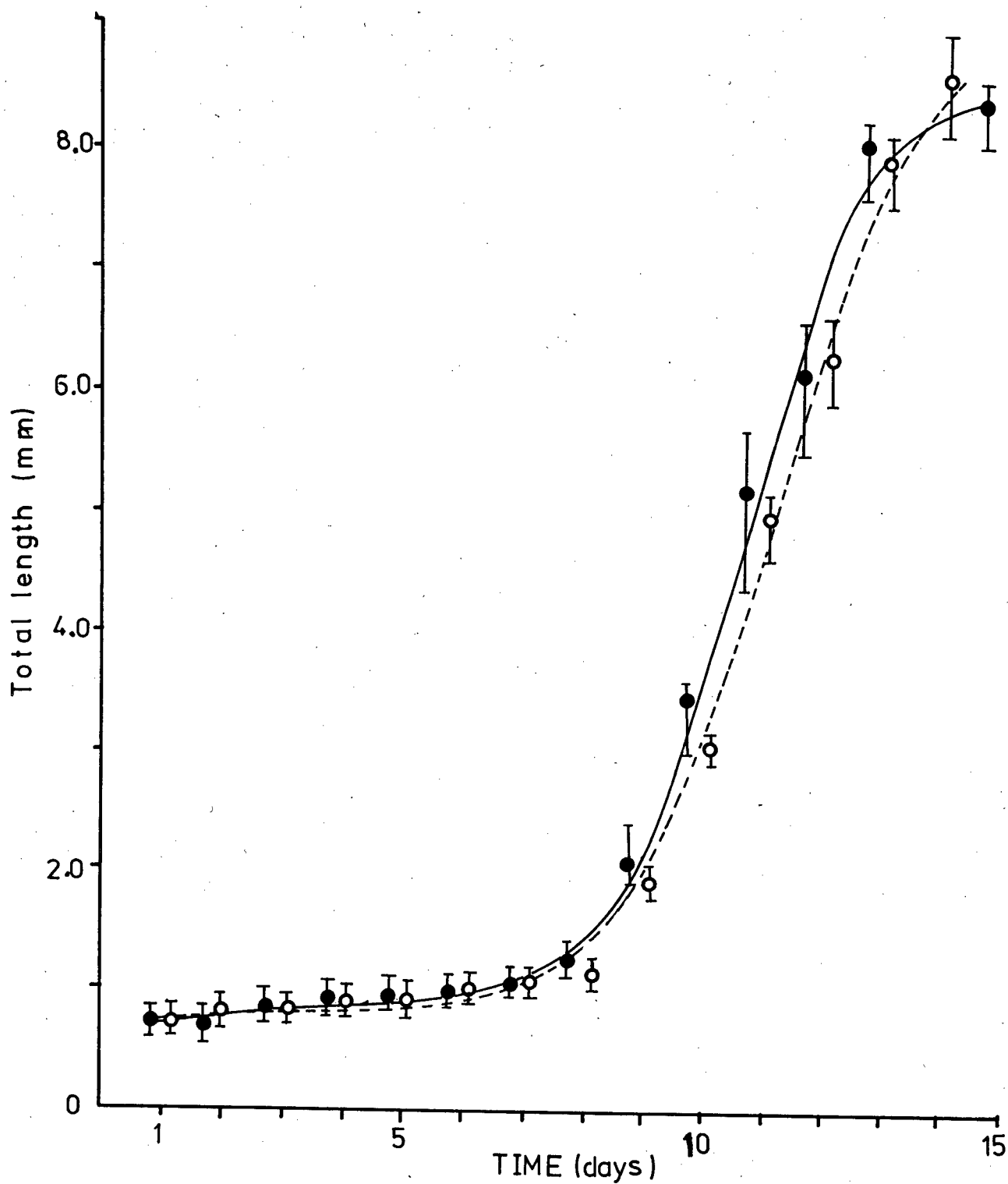
Figure 12. Growth in total length of _Artemia_ _salina_ L. over a
14-day culture period fed with swine waste-seawater
(o) and defined inorganic (●) media grown
D. tertiolecta. The above values are ranges and
means of four replicates per treatment.
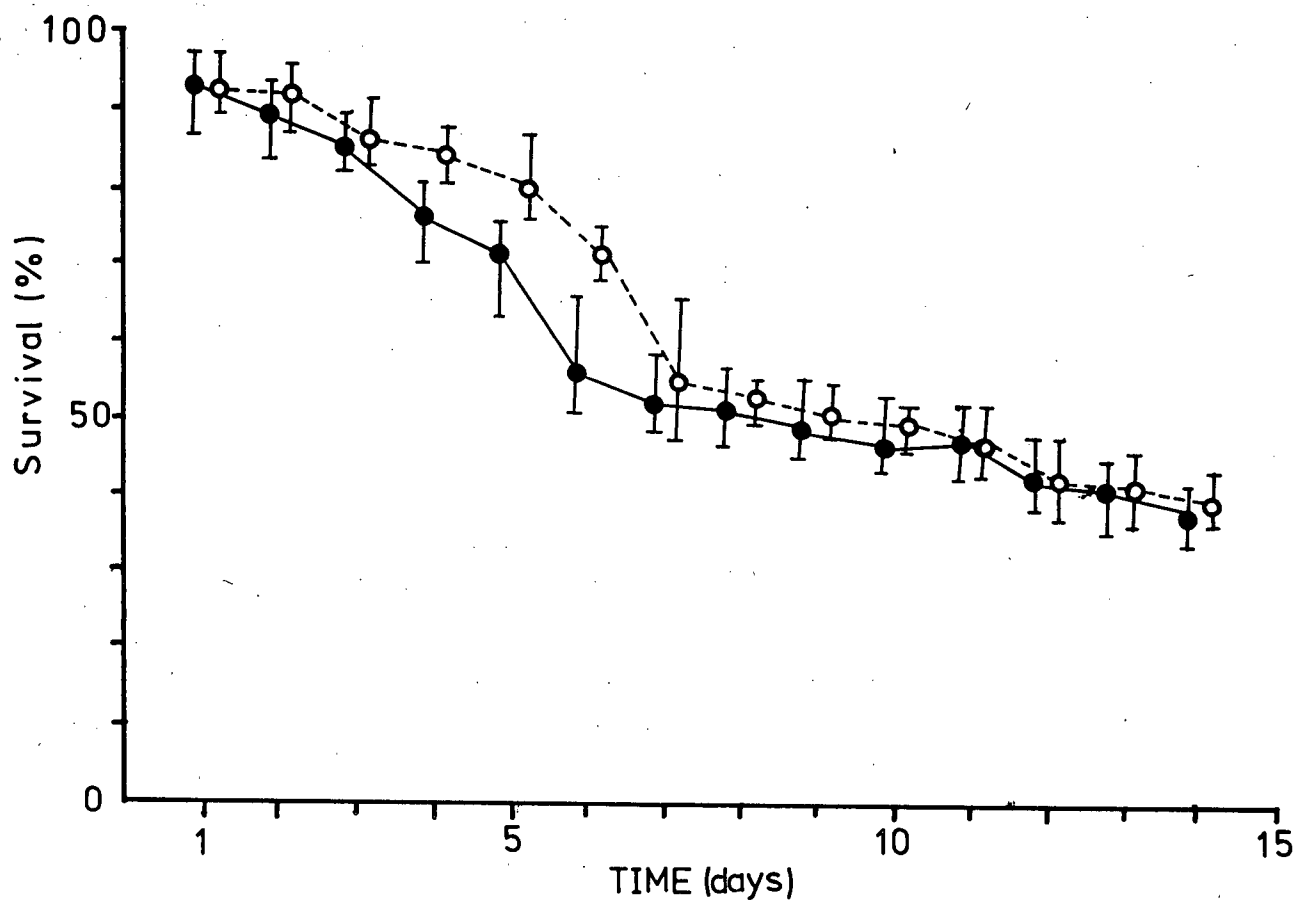
Figure 13. Percentage survival of _Artemia_ _salina_ L. over a
14-day culture period fed with swine waste-
seawater (o) and defined inorganic media (●)
grown _D._ _tertiolecta_. The above values are
ranges and means of four replicates.

Adult _Artemia_ produced in the system after the 14-day culture period were measured in wet and dry weight basis (Table 3). Statistical analysis for wet and dry weight of _Artemia_ showed no significant differences between treatments and replicates (Appendices 10a and 10b, respectively).

Table 3. Adult _Artemia_ biomass produced after the 14-day
          culture period in wet and dry weight basis.

Artemia Fed With Swine Waste-Seawater Grown Algae	Wet Weight (g)	Dry Weight (g)
1	18.82	1.82
2	19.46	2.13
3	19.43	2.06
4	20.47	2.40
$\overline{X}$	19.55 ± 0.68	2.10 ± 0.24
Artemia Fed With Defined Inorganic Medium Grown Algae		
1	16.00	1.50
2....	18.06	1.91
3	19.08	2.01
4	18.05	1.87
$\overline{X}$	17.80 ± 1.29	1.82 ± 0.22

DISCUSSION

Livestock Waste As A Nutrient Source
In Algal Culture

Analysis of total Kjeldahl nitrogen (TKN), NH_3-N, NO_3-N + NO_2-N PO_4-P and total suspended solids (TSS) of the swine waste samples collected during the course of the experiments showed that the nutrient content of the waste varied only slightly. The constancy of the manure nutrients concentration found in these experiments is significant (Table 1). It indicates that for a particular farm it maybe unnecessary to analyse each batch of waste and that analysing the waste periodically may be adequate to provide the necessary information for calculating the required nutrient loading rates. In this case, the samples were taken from the same batches of finishing hogs and just prior to pit flushing which may have increased the uniformity of the manure samples.

In the present study, the highest percentage of nitrogen found in livestock waste was in the form of ammonia nitrogen which is in agreement with the results of Dunstan and Menzel (1971) and Thomas et al. (1974) for the sewage. This form of inorganic nitrogen is readily utilized by phytoplankton as long as toxic levels are not attained. Although in the swine waste-seawater mixtures, ammonia nitrogen concentration was about 500 µg-at N l^{-1} and 50% higher than that of defined inorganic medium, this NH_3-N level did not appear to exhibit toxic effects on the algae.

Certain species of phytoplankton (<u>Hemiselmis</u> <u>virescens</u> and <u>Dunaliella</u> <u>tertiolecta</u>) can also fully utilize organic sources of nitrogen (Antia and Charney, 1968; Antia et al., 1975). If these phytoplankton were grown on livestock waste or sewage, they could have a great advantage in terms of nitrogen utilization and conversion efficiency since large amounts of organic nitrogen are found in these wastes. It should be noted, however, that in the present study very little organic nitrogen is present in the medium due to prefiltration.

Although animal wastes may contain toxicants from feed residues and growth stimulants such as copper added to the ration, these are generally more predictable and controllable than in the case of sewage (Dodd, 1979). Variability of nutrients found in sewage could also affect the species composition of phytoplankton in culture (Dunstan and Tenore, 1972) grown on this medium. Moreover, the problem of toxicants in livestock waste is considerably less in developing countries than in highly industrialized countries. The latter uses a highly intensive livestock culture system involving greater amounts of synthetic stimulants in their food ration. In the present study, the presence of toxicants in the swine is probably insignificant. However, further studies should be conducted on trace metals accumulation not only on the algae but also on the next trophic level.

Growth Constant, Cell Density And

Dry Weight of $\underline{D}$. $\underline{tertiolecta}$

In both batch and continuous algal culture systems, the growth constant ($\underline{K}_{(10)}$ hr^{-1}) and mean generation time ($\underline{t}g$ hrs) obtained in all experiments have similar values either in defined inorganic or swine waste-seawater media. The values obtained (Table 4) were similar to earlier studies (Antia and Kalmakoff, 1965; Jitts, et al., 1964) in which the values reported are considered to be close to optimal. These results indicate that the culture conditions in the present study were optimal in most cases and that neither the high levels of NH_3-N nor possible toxicants in the manure were having a detrimental effect on algal growth rate.

Algal cell densities attained after 7-8 days growth during batch culture were 5 to 7 x 10^6 cells ml^{-1} for both media. Similar values were attained during continuous cultures and were maintained for the duration of the experiments. This showed that the swine waste-seawater medium has neither inhibitory nor toxic effect on the growth of $\underline{D}$. $\underline{tertiolecta}$ when compared with the defined inorganic medium. In a similar study, using 15 liter volume cultures, it was demonstrated that secondary treated sewage effluent is an excellent medium for the growth of mixed populations of marine phytoplankton (Dunstan and Menzel, 1971).

The algal dry weight measured daily for swine waste-seawater and defined inorganic media during continuous culture (Figure 2) was not constant. It ranged from 0.12 to 0.25 g l^{-1}. It was observed that although the concentration of cells remained

Table 4. Growth Constant ($\underline{K}_{(10)}$)hr^{-1}) and Mean Generation Time ($\underline{t}$g hrs) from present and earlier studies on $\underline{D}.$ tertiolecta.

	Defined Inorganic Medium		Swine Waste-Seawater Mixtures	
	($\underline{K}_{(10)}$hr^{-1})	($\underline{t}$g hrs)	($\underline{K}_{(10)}$hr^{-1})	($\underline{t}$g hrs)
Present Study				
Expt 1	0.023	13.0	0.024	12.5
Expt 2	0.023 ± 0.0	12.92 ± 0.0	0.023 ± 0.001	12.95±0.34
Expt 3	0.023 ± 0.002	13.92 ± 0.36	0.0231 ± 0.001	13.02±0.05
Expt 5	0.023 ± 0.002	12.85 ± 0.49	0.024 ± 0.004	12.71±0.67
Antia and Kalmakoff (1965)	0.025	12.0		
Jitts et al. (1964)	0.030			

fairly constant, the algal biomass decreased at different times
(Figures 8 and 9). It was observed that the algal biomass tended
to increase shortly after the nutrient media being added to the
culture was replenished from the stock solution. Moreover, the
higher variability was observed in swine waste-seawater medium
due to considerable amount of suspended solids in the swine waste-
seawater mixture as compared to defined inorganic medium.
Significant difference was found in the algal dry weight produced
daily for both media. Further research should be carried out to
determine possible reasons why at the time of replenishing the
nutrientsolution an increase in algal biomass was observed.

Continuous Culture System In Algal Production

One of the major values of the present work was the
demonstration that the continuous culture system can produce far
more algal biomass than the batch system. This culture system
when compared to a batch system is far more conducive for optimal
productivity of the algae. Although the batch system was capable
of producing 0.015 g dry wt l^{-1} day^{-1} (0.10 dry wt l^{-1} in
6 days), the continuous culture system was capable of producing
0.2 to 0.25 g dry wt l^{-1} day^{-1}.

It should be pointed out that the nutrients in both media
were in excess and thereby the nutrients were not fully utilized
by the algae during continuous culture (Table 2). It is suggested
that additional culture vessels should be added in series to fully
utilize the nutrients, thus resulting to a higher biomass.

McAllister et al (1964) concluded that the maximum photosynthetic efficiency (P_{max}) for D. tertiolecta was achieved at 0.1 ly min $^{-1}$. In the present set-up, light was probably limiting. Since the light intensity from the fluorescent tubes was between 0.04 to 0.05 ly min^{-1}, increasing the light intensity will increase the photosynthetic production.

Furthermore, using continuous cultures, it is possible to provide a more uniform product daily by selecting growth conditions to obtain the desired composition of the product. For example, by selecting the flow rate, a product of high or low protein cells could be produced consistently (Ballard, 1972; Palmer et al., 1975; Lampert, 1976). On the other hand, it is a characteristic of batch cultures that their composition will vary continuously as they get older (Taub and Dollar, 1965).

Artemia Feeding Experiments

Total Body Length and Percentage Survival

Total body length and percentage survival measurements of the brine shrimp during the 14-day culture period showed no significant differences when fed swine waste grown algae with those fed defined inorganic medium grown algae. Daily observations on their swimming or feeding behavior gave no apparent differences between the two treatments.

The rapid growth of Artemia between 2 to 7 mm stage in the present study was remarkable. Mason (1963) observed a similar growth pattern. These lengths correspond to the periods when growth rate is most rapid and highly dependent upon food level

and with the time when food first shows its effect, days 4-6
(Provasoli et al, 1959).

Although the same number of algae are being fed and those
algae contain about 25% less organic nitrogen (Table 2), this
did not affect the subsequent growth rate of the Artemia.
The algae are still able to grow and reproduce while the Artemia
are feeding on them.

Scott and Middleton (1979) have shown that turbot larvae
with D. tertiolecta given during the rotifer feeding stage showed
poor animal growth and survival compared to larvae in tanks with
either Isochrysis galbana, Phaedactylum tricornotum or Pavlova
lutheri added. Since D. tertiolecta is known to be not toxic,
its effect on the turbot larvae is probably due to a dietary
efficiency. It has been pointed out that the nutritional state
of the algal food is important for the growth of herbivores
(Parsons et al., 1961). This is probably the cause of low
survival of Artemia in the present study. Tobias et al. (1979)
achieved 80% survival of Artemia fed with diatom Chaetoceros
curvisetus (clone STX-167) in a flow-through raceway system.

Biomass Conversion Efficiency

The biomass conversion efficiency (%) is defined here as a
measure of the amount of biomass of Artemia produced by a
given quantity of algae fed during the culture period. The formula
is given as:

$$B.\ C.\ E.\ \% = \frac{\text{dry weigth of Artemia produced}}{\text{dry weight of algae fed}} \times 100$$

where B. C. E. is biomass conversion efficiency

The biomass conversion efficiencies for Artemia fed with swine waste-seawater grown algae and defined inorganic grown algae were 57% and 58%, respectively (Table 5). The calculated algal-Artemia conversion efficiencies showed no statistically significant differences between treatments and replicates (Appendix 11).

Fifty percent protein-N conversion efficiency from D. tertiolecta to Artemia cultured in the laboratory was reported by Reeve (1963c). Helfrich (1963) indicated a 50% conversion efficiency (ash-free dry wt.) was attained in a feasibility study conducted on Christmas Island for a production scheme of Artemia eggs and adult. The present study cannot be directly compared with the above studies since the units used were in g dry wt. The present results, however, may be said to have reached a comparable conversion efficiency.

Reports on efficiency of growth of Artemia are quite varied in terms of units used such as wet weight, dry weight, ash-free dry weight, protein-N and mg C (Gibor, 1957, Mason, 1963; Helfrich, 1973; Sick, 1976 and Bossuyt and Sorgeloos, 1980). It is suggested that the use of such units should have common parameters such that results will be comparable and also applicable to aquacultural studies.

Combined Livestock Waste-Artemia Culture System

The present study shows that the use of swine waste as a source of nutrients in algal culture is highly comparable to defined inorganic media. The study also shows that Artemia fed

Table 5. Calculated Biomass Conversion Efficiency (BCE %)
          of _Artemia_ _salina_ L.

Parameters Measured	Fed With Swine Waste Grown _D_. _tertiolecta_	Fed With Defined Inorganic Medium Grown _D_. _tertiolecta_
Algal Production ($\overline{X}$ = g dry wt day^{-1})	0.17 ± 0.72	0.15 ± 0.61
Corresponding Algal Cell Density ($\overline{X}$ = cell ml^{-1})	$5.78 \pm 1.65 \times 10^{6}$	$5.31 \pm 1.62 \times 10^{6}$
Cell Weight ($\overline{X}$ = g dry wt cell^{-1})	$2.99 \pm 1.01 \times 10^{-11}$	$2.78 \pm 0.9 \times 10^{-11}$
Algal Cell Censity Maintained as Feed For _Artemia_ day $^{-1}$ ($\overline{X}$ = cell ml^{-1})	$5.0 \pm 0.50 \times 10^{5}$	$5.0 \pm 0.50 \times 10^{5}$
No. of Algal Cells Added As Feed For _Artemia_ day^{-1} ($\overline{X}$ = cells day^{-1}	$8.67 \pm 1.50 \times 10^{9}$	$9.97 \pm 1.0 \times 10^{9}$
Algal Dry Weight Added As Feed In 14-Day Culture Period ($\overline{X}$ = g dry wt)	3.63 ± 1.40	3.10 ± 1.09
Artemia Biomass Produced After 14 Days ($\overline{X}$ = g dry wt)	2.10 ± 0.42	1.82 ± 0.38
Biomass Conversion Efficiency (%)	57.93 ± 5.84	58.79 ± 7.19

on algae grown in swine waste-seawater mixture grow as well as those fed with algae grown in defined inorganic medium.

This is an important consideration, especially for an agriculture-aquaculture integrated system, and it shows, that Artemia has a high potential in converting algal biomass grown in agricultural wastewater into much needed protein, particularly in Third World countries. For example, Artemia can be used as a substitute for fish meal. In this regard, integrated livestock waste-Artemia production system can be most economically feasible in Third World countries. It should be pointed out, however, that there are still numerous areas of uncertainties in the said system, such as biological, engineering and economic problems.

It is suggested that future studies should be conducted on outdoors for algal-Artemia production scheme so that data, especially on engineering and economic aspect can be applied to aquacultural production ventures.

SUMMARY AND CONCLUSIONS

Analysis of total Kjeldahl nitrogen(TKN), NH_3-N, NO_3-N + NO_2-N, PO_4-P and total suspended solids(TSS) of the swine waste samples showed only slight variation during the course of the experimental work. In both batch and continuous culture systems, the algal growth constants obtained in all experiments were found to have similar values for both defined inorganic and swine waste-seawater media. The production parameters maintained during algal culture in both batch and continuous culture systems were as follows: temperature, $24 \pm 1^{\circ}C$; salinity, 32 ± 1 ppt; fluorescent light intensity, ca 0.04 cal min^{-1} cm^{-2} (or 0.04 ly min^{-1}). The algal cell density attained after 7-8 days growth for batch culture was 5 to 7 x 10^6 cells ml^{-1} in both media. Similar values were attained during continuous cultures and successfully maintained for the duration of the experiments.

The results show that the use of swine waste-seawater mixtures as a nutrient source in algal culture is comparable to defined inorganic medium. One advantage of using livestock waste as a nutrient source for algal culture is that nutrient loading such as nitrogen and phosphorus can be predicted. It is also cheap and available in many areas.

The total body length and percentage survival measurements of Artemia during the 14-day culture period showed no significant differences between Artemia fed swine waste-seawater grown D. tertiolecta and those fed defined inorganic medium grown

<u>D</u>. <u>tertiolecta</u>. The calculated biomass conversion efficiencies for <u>Artemia</u> fed with swine waste-seawater grown algae and defined inorganic medium grown algae were 57% and 58%, respectively. The algal-<u>Artemia</u> conversion efficiencies showed no statistically significant differences between treatments and replicates.

The study shows that <u>Artemia</u> fed on algae grown in swine waste-seawater mixture grow as well as those fed with algae grown in defined inorganic medium.

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APPENDICES

Appendix 1

Composition of Enrichment "f/2"

Major Nutrients

$NaNO_3$	75 mg (883 uM)
$NaH_2PO_4 \cdot H_2O$	5 mg (36.3 uM)

Trace Metals

Na_2EDTA^+	4.36 mg (ca 11.7 uM)
$FeCl_3 \cdot 6H_2O^+$	3.15 mg (0.65 mg Fe or ca 11.7 uM
$CuSO_4 \cdot 5H_2O$	0.01 mg (2.5 ug Cu or ca 0.04 uM)
$ZnSO_4 \cdot 7H_2O$	0.022 mg (5 ug Zn or ca 0.08 uM)
$CoCl_2 \cdot 6H_2O$	0.01 mg (2.5 ug Co or ca 0.05 uM)
$MnCl_2 \cdot 4H_2O$	0.18 mg (0.05 mg Mn or ca 0.9 uM)
$Na_2MoO_4 \cdot 2H_2O$	0.006 mg (2.5 ug Mo or ca 0.03 uM

Vitamins

Thiamin·HCl	0.1 mg
Biotin	0.5 ug
B_{12}	0.5 ug

Seawater   to one liter

Appendix 2

Composition of Artificial Seawater

FORTY FATHOMS BIO-CRYSTALS MARINEMIX

Assay Chart

Average solution of Forty Fathoms Marinemix hydrated to a density of 1.025 using distilled water. Figures cited from actual independent laboratory analysis.

	Concentration (ppm)
Aluminum	0.06
Antimony	0.0005
Argon	trace
Arsenum	0.01
Barium	0.12
Bicarbonate	174.0
Beryllium	0.0002
Bismuth	trace
Boron	2.1
Bromide	62.0
Cadmium	0.009
Calcium	410.0
Carbonate	10.0
Cerium	0.0007
Cesium	trace
Chromium	0.02
Chloride	18600.0
Copper	0.007
Cobalt	0.0025
Dysprosium	trace
Erbium	trace
Europium	trace
Fluoride	1.9
Gadolinium	trace
Gallium	0.0004
Germanium	0.00005
Gold	trace
Hafnium	trace
Helium	trace
Holmium	trace
Indium	trace
Iodine	0.03
Iron	0.03
Krypton	trace
Lanthanum	trace
Lead	trace
Lithium	0.24
Lutetium	trace
Magnesium	1290.0
Manganese	0.008

Composition (ppm)

Mercury	0.0007
Molybdenum	0.005
Neodynium	trace
Neon	trace
Nickel	0.009
Niobium	trace
Nitrogen	0.85
Palladium	trace
Phosphorus	0.04
Potassium	380.-
Praeseodymium	trace
Protactinium	trace
Radium	trace
Radon	trace
Rubidium	0.06
Ruthenium	trace
Samarium	trace
Scandium	trace
Selenium	trace
Silicon	4.5
Sodium	110400.0
Strontium	12.4
Sulfur (as SO_4)	2600.0
Tantalium	trace
Tellerium	trace
Terbium	trace
Thalium	0.00007
Thulium	trace
Tin	0.006
Titanium	0.004
Tungsten	0.004
Uranium	0.00005
Vanadium	0.0009
Xenon	trace
Ytterbium	trace
Yttrium	trace
Zinc	0.24
Zirconium	trace

ANALYSIS OF VARIANCE TABLE FOR GROWTH CONSTANT (EXPT 2A)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
PROBABILITY TEST TERM					
TREATMENT	0.16666E-08	1.	0.16666E-08	0.76920E-01	0.80755
RESIDUAL					
RFP	0.43333E-07	2.	0.21667E-07	1.0000	0.50000
RESIDUAL					
RESIDUAL	0.43333E-07	2.	0.21667E-07		
TOTAL	0.88333E-07	5.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
GROWTH	0.23283E-01	0.13292E-03

ANALYSIS OF VARIANCE TABLE FOR GROWTH CONSTANT (EXPT 3A)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	PROBABILITY	TEST TERM
TREAT	0.20167E-06	1.	0.20167E-06	7.5624	0.11070	RESIDUAL
REP	0.13334E-07	2.	0.66668E-08	0.25000	0.80000	RESIDUAL
RESIDUAL	0.53334E-07	2.	0.26667E-07			
TOTAL	0.26833E-06	5.				

	OVERALL MEAN	OVERALL STANDARD DEVIATION
GROWTH	0.22917E-01	0.23166E-03

ANALYSIS OF VARIANCE TABLE FOR CELL DENSITY

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT RESIDUAL	0.76163	1.	0.76163	3.9737	0.06160
REP RESIDUAL	4.2740	2.	2.1370	11.149	0.00071
DAY RESIDUAL	202.71	9.	22.524	117.51	0.00000
TREAT*REP RESIDUAL	3.9576	2.	1.9788	10.324	0.00103
REP*DAY RESIDUAL	5.6726	18.	0.31514	1.6442	0.15031
TREAT*DAY RESIDUAL	2.1127	9.	0.23475	1.2248	0.33986
RESIDUAL	3.4500	18.	0.19167		
TOTAL	222.94	59.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
DENSITY	3.5733	1.9439

ANALYSIS OF VARIANCE TABLE FOR ALGAL DRY WEIGHT (EXPT 3C)

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT RESIDUAL	0.47538E-03	1.	0.47538E-03	0.79947E-01	0.78060
REP RESIDUAL	0.20040E-01	2.	0.10020E-01	1.6851	0.21339
DAY RESIDUAL	0.59008	9.	0.65565E-01	11.026	0.00001
TREAT*REP RESIDUAL	0.16076E-01	2.	0.80382E-02	1.3518	0.28381
REP*DAY RESIDUAL	0.10690	18.	0.59387E-02	0.99874	0.50105
TREAT*DAY RESIDUAL	0.63669E-01	9.	0.70743E-02	1.1897	0.35845
RESIDUAL	0.10703	18.	0.59462E-02		
TOTAL	0.90240	59.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
DRY WEIGHT	0.18375	0.12367

ANALYSIS OF VARIANCE TABLE FOR CELL DENSITY (EXPT 5B)

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT RESIDUAL	6.6082	1.	6.6082	45.442	0.00000
REP RESIDUAL	0.93253	2.	0.46627	3.2063	0.05167
DAY RESIDUAL	294.11	19.	15.479	106.45	0.00000
TREAT*REP RESIDUAL	0.62585	2.	0.31293	2.1519	0.13023
REP*DAY RESIDUAL	5.5737	38.	0.14668	1.0086	0.48950
TREAT*DAY RESIDUAL	8.1307	19.	0.42793	2.9427	0.00227
RESIDUAL	5.5260	38.	0.14542		
TOTAL	321.51	119.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
DENSITY	5.5438	1.6437

ANALYSIS OF VARIANCE TABLE FOR CELL DENSITY (EXPT 5B1)

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE		F-RATIO
TREAT	8.1420	1.	8.1420	61.907	0.00001
RESIDUAL					
REP	0.63622	2.	0.31811	2.4187	0.13471
RESIDUAL					
DAY	31.404	6.	5.2340	39.796	0.00000
RESIDUAL					
TREAT*REP	0.62014	2.	0.31007	2.3576	0.14057
RESIDUAL					
REP*DAY	2.3135	12.	0.19279	1.4658	0.26708
RESIDUAL					
TREAT*DAY	2.2230	6.	0.37050	2.8170	0.06512
RESIDUAL					
RESIDUAL	1.4467	11.	0.13152		
TOTAL	47.840	40.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
DENSITY	4.9885	1.0936

ANALYSIS OF VARIANCE TABLE FOR CELL DENSITY (EXPT 5B2)

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE		F-RATIO
TREAT	1.1959	1.	1.1959	4.4383	0.05513
RESIDUAL					
REP	0.32929	2.	0.16464	0.61102	0.55767
RESIDUAL					
DAY	5.0913	6.	0.84854	3.1491	0.03924
RESIDUAL					
TREAT*REP	0.76389	2.	0.38194	1.4175	0.27741
RESIDUAL					
REP*DAY	1.3014	11.	0.11831	0.43908	0.91017
RESIDUAL					
TREAT*DAY	1.4670	6.	0.24450	0.90740	0.51899
RESIDUAL					
RESIDUAL	3.5029	13.	0.26946		
TOTAL	12.791	41			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
DENSITY	6.6921	0.55855

ANALYSIS OF VARIANCE FOR CELL DENSITY (EXPT 5B3)

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT	0.14963E-01	1.	0.14963E-01	0.15520	0.70391
RESIDUAL					
REP	0.16247E-01	2.	0.81233E-02	0.84255E-01	0.92000
RESIDUAL					
DAY	0.15365	4.	0.38412E-01	0.39841	0.80482
RESIDUAL					
TREAT*REP	0.40613	2.	0.20306	2.1062	0.18415
RESIDUAL					
REP*DAY	0.94345	8.	0.11793	1.2232	0.39130
RESIDUAL					
TREAT*DAY	0.22895	4.	0.57238E-01	0.59368	0.67726
RESIDUAL					
RESIDUAL	0.77131	8.	0.96413E-01		
TOTAL	2.5347	29.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
DENSITY	5.8537	0.29564

ANALYSIS OF VARIANCE TABLE FOR GROWTH CONSTANT (EXPT 5A)

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT. RESIDUAL	0.10667E-06	1.	0.10667E-06	0.26556	0.65763
REP RESIDUAL	0.32333E-06	2.	0.16167E-06	0.40249	0.71302
RESIDUAL	0.80333E-06	2.	0.40167E-06		
TOTAL	0.12333E-05	5.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
GROWTH	0.23533E-01	0.49666E-03

ANALYSIS OF VARIANCE TABLE FOR ALGAL DRY WEIGHT (EXPT 5C)

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT RESIDUAL	0.18800E-01	1.	0.18800E-01	36.241	0.00000
REP RESIDUAL	0.84485E-03	2.	0.42243E-03	0.81432	0.45052
DAY RESIDUAL	0.46410	19.	0.24426E-01	47.087	0.00000
TREAT*REP RESIDUAL	0.22687E-02	2.	0.11344E-02	2.1867	0.12621
REP*DAY RESIDUAL	0.11038E-01	38.	0.29048E-03	0.55996	0.96109
TREAT*DAY RESIDUAL	0.24594E-01	19.	0.12944E-02	2.4953	0.00808
RESIDUAL	0.19712E-01	38.	0.51874E-03		
TOTAL	0.54135	119.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
DRY WEIGHT	0.16005	0.67448E-01

ANALYSIS OF VARIANCE TABLE FOR TOTAL LENGTH OF <u>ARTEMIA</u>

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE		F-RATIO
TREAT	0.36363E-03	1.	0.36363E-03	0.14954E-01	0.90332
RESIDUAL					
REP	0.74867E-01	3.	0.24956E-01	1.0263	0.39183
RESIDUAL					
DAY	833.23	13.	64.094	2635.8	0.00000
RESIDUAL					
TREAT*REP	0.12452	3.	0.41508E-01	1.7070	0.18189
RESIDUAL					
REP*DAY	1.3383	39.	0.34315E-01	1.4111	0.14532
RESIDUAL					
TREAT*DAY	0.68787	13.	0.52913E-01	2.1760	0.03137
RESIDUAL					
RESIDUAL	0.92405	38.	0.24317E-01		
TOTAL	836.75	110.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
GROWTH	2.8957	2.7580

ANALYSIS OF VARIANCE TABLE FOR PERCENTAGE SURVIVAL OF <u>ARTEMIA</u>

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT	331.95	1.	331.95	39.365	0.00000
RESIDUAL					
REP	242.75	3.	80.917	9.5958	0.00007
RESIDUAL					
DAY	41120.	13.	3163.1	375.10	0.00000
RESIDUAL					
TREAT*REP	105.10	3.	35.035	4.1547	0.01200
RESIDUAL					
REP*DAY	395.58	39.	10.143	1.2028	0.28346
RESIDUAL					
TREAT*DAY	591.09	13.	45.468	5.3920	0.00002
RESIDUAL					
RESIDUAL	328.87	39.	8.4326		
TOTAL	43369.	111.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
SURVIVAL	61.393	19.766

ANALYSIS OF VARIANCE TABLE FOR PERCENTAGE SURVIVAL OR <u>ARTEMIA</u>

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE		F-RATIO
TREAT	631.86	1.	631.86	78.730	0.00000
RESIDUAL					
REP	134.34	3.	44.782	5.5798	0.00600
RESIDUAL					
DAY	10628.	6.	1771.4	220.72	0.00000
RESIDUAL					
TREAT*REP	115.01	3.	38.338	4.7769	0.01141
RESIDUAL					
REP*DAY	229.41	18.	12.745	1.5880	0.15837
RESIDUAL					
TREAT*DAY	330.71	6.	55.119	6.8678	0.00045
RESIDUAL					
RESIDUAL	160.51	20.	8.0257		
TOTAL	12311.	57.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
SURVIVAL	77.862	14.696

ANALYSIS OF VARIANCE TABLE FOR PERCENTAGE SURVIVAL OF <u>ARTEMIA</u>

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT RESIDUAL	12.532	1.	12.532	2.9323	0.10310
REP RESIDUAL	129.96	3.	43.320	10.136	0.00033
DAY RESIDUAL	1267.5	6.	211.246	49.426	0.00000
TREAT*REP RESIDUAL	66.274	3.	22.091	5.1689	0.00883
REP*DAY RESIDUAL	74.818	18.	4.1566	0.97255	0.52185
TREAT*DAY RESIDUAL	20.097	6.	3.3496	0.78373	0.59317
RESIDUAL	81.204	19.	4.2739		
TOTAL	1653.3	56.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
SURVIVAL	45.368	5.4335

ANALYSIS OF VARIANCE TABLE FOR <u>ARTEMIA</u> WET WEIGHT

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE		F-RATIO
TREAT RESIDUAL	6.1075	1.	6.1075	0.0	1.00000
REP RESIDUAL	4.5720	3.	1.5240	0.0	1.00000
TREAT*REP RESIDUAL	1.8382	3.	0.61272	0.0	1.00000
RESIDUAL	0.0	-0.	0.0		
TOTAL	12.518	7.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
WET WEIGHT	18.671	1.3373

ANALYSIS OF VARIANCE TABLE FOR <u>ARTEMIA</u> DRY WEIGHT

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE		F-RATIO
TREAT RESIDUAL	0.15680	1.	0.15680	0.0	1.00000
REP RESIDUAL	0.25965	3.	0.86550E-01	0.0	1.00000
TREAT*REP RESIDUAL	0.60300E-01	3.	0.20100E-01	0.0	1.00000
RESIDUAL	0.0	-0.	0.0		
TOTAL	0.47675	7.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
DRY WEIGHT	1.9625	0.26097

ANALYSIS OF VARIANCE TABLE FOR <u>ARTEMIA</u> BIOMASS CONVERSION EFFICIENCY (BCE)

SOURCE PROBABILITY TEST TERM	SUM OF SQUARES	DF	MEAN SQUARE	F-RATIO	
TREAT	1.5051	1.	1.5051	0.0	1.00000
RESIDUAL					
REP	230.91	3.	76.969	0.0	1.00000
RESIDUAL					
TREAT*REP	53.863	3.	17.954	0.0	1.00000
RESIDUAL					
RESIDUAL	0.0	-0.	0.0		
TOTAL	286.28	7.			

	OVERALL MEAN	OVERALL STANDARD DEVIATION
BCE	58.356	6.3950