AQUACULTURE STUDIES OF THE GIANT SCALLOP

PLACOPECTEN MAGELLANICUS (GMELIN):

CONDITIONING OF BROODSTOCK AND ENERGY

REQUIREMENTS OF THE LARVAE AND JUVENILES

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Aquaculture studies of the giant scallop
Placopecten magellanicus (Gmelin):
Conditioning of broodstock and energy requirements
of the larvae and juveniles.

by

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ABSTRACT

The giant scallop, Placopecten magellanicus, has proven very difficult to rear under laboratory conditions. This study aimed at optimizing the growth and survival of Placopecten magellanicus larvae and juveniles under laboratory or hatchery conditions.

Broodstock was successfully conditioned with algal diets equivalent to 3-5% of their body weight per day, on a dry weight basis. The effect of conditioning was estimated by measuring the protein, lipid and carbohydrate content of the eggs and larvae. Conditioned adults produced gametes with approximately 30% more energy reserves than starved females. Algal diets resulted in the production of eggs with the highest content of protein (26-30 mg per million eggs) and lipid (14-18 mg per million eggs). These equalled or exceeded the protein and lipid levels in the eggs of naturally conditioned females. In all cases, carbohydrate was a minor energy reserve (2-3 mg per million eggs).

Both protein and lipid are important energy reserves in the larvae of the giant scallop. The larvae utilized 60% of the energy reserves sequestered in the egg during develpment

to the D-shaped veliger stage, 3 days at 15°C, regardless of the total energy content of the eggs.

When the larvae were fed an appropriate diet, (Isochrysis galbana, Chaetoceros calcitrans and Thalassiosira pseudonana) the larvae which had higher energy reserves in the eggs exhibited greater rates of growth and accumulation of energy reserves over the first four weeks of pelagic life.

The effect of algal cell concentration and body size on filtration rates was studied using a static system. Juveniles ranged from 5 mg to 400 mg in tissue dry weight and the concentration of Isochrysis galbana was varied from 5×10^3 to 25×10^3 cells/ml. At the highest cell concentration tested the smallest juveniles ingested 10% of their body weight per day and the largest individuals' ingested approximately 5% of their body weight per day. Comparisons with other species indicate that this ration is sufficient for both maintenance and growth.

ACKNOWLEDGEMENTS

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A	Algal diet
AE	Absorption efficiency
C	Carbohydrate
ES	Modified Erd-Shreiber algal growth medium (Stein, 1973).
F	Filtration rate
FSW	Filtered seawater
f/2	Algal growth medium of Guillard and Ryther (Stein, 1973).
IR	Ingestion rate
L	Lipid
M	Mixed algal diet
MSRL	Marine Sciences Research Laboratory
P	Protein
R	Ration
S	Starch diet
SA	Starch and algal diet
ST	Starved females
TE	Total energy
W	Weight

Chapter I

INTRODUCTION

Bivalve larvae have been widely studied in recent years, especially since their hatchery culture may play an important role in providing seed for the commercial shellfish industry. The success in rearing _Placopecten magellanicus_ (Gmelin) larvae in the laboratory, however, has been extremely limited (Culliney, 1974). Although larvae are easily obtained, growing them through to settlement is difficult.

Two phases of care are commonly present in shellfish aquaculture: the hatchery and the nursery. The hatchery phase is concerned with rearing the larvae through to settlement. This is a less labour intensive method of obtaining spat, the alternative being the use of spat collectors. An intermediate nursery phase for rearing larvae settled in the laboratory is often introduced between the hatchery culture and transplantation of juveniles to the natural environment (Persoone and Claus, 1980). The nursery phase is desirable to avoid serious loss due to handling, predation, temperature shocks and shortage of food (DePauw, 1981). For successful commercial ventures, therefore, it is

necessary to understand the nutritional requirements of juveniles as well as those of the larvae.

Traditionally, the general condition of bivalve larvae (starved, stressed, or healthy) has been determined by monitoring shell growth and mortality rates, and by microscopic examination of feeding and swimming behavior, and morphology of the larvae. As Gallager and Mann (1981) pointed out these monitoring systems usually detect a problem too late to save the larvae. A more immediate warning system is needed, one which would allow adverse conditions to be corrected before irreparable damage is done. By determining the gross biochemical makeup of the eggs and planktonic larvae, it might be possible to predict the potential success of a given batch of larvae. Gallager and Mann (1981) elaborated a staining assay to determine the lipid levels in oyster larvae which gives an immediate indication of the condition of the larvae.

The development of successful spawning and rearing techniques for bivalve larvae (Loosanoff and Davis, 1963; Walne, 1966) and the development of microanalytical techniques (Holland and Gabbott, 1971; Holland and Hannant, 1973) led to studies on nutritional (Ukeles, 1970) and biochemical aspects of oyster and mussel larvae (Millar and Scott, 1967; Gabbott and Holland, 1973; Helm et al., 1973; Holland and Spencer, 1973; Bayne et al., 1975, 1978;

Holland, 1978). These studies sought to explain the unpredictability of larval success and to determine the factors which would ensure good larval growth and viability as well as successful metamorphosis and settlement.

Very little work has been done on the giant scallop, Placopecten magellanicus. Drew (1906) described the anatomy and embryology of this species. Thompson (1977) and Robinson et al. (1981) have described the storage cycle of nutrients accompanying gametogenesis for Newfoundland and Maine populations, respectively. Naidu (1970) reported the reproductive cycle of scallops in Port au Port, Newfoundland. MacDonald (1984) studied the partitioning of energy between growth and reproduction in the giant scallop. The only published report of the development of P. magellanicus through to settlement is that of Culliney (1974). As a result our knowledge of the reproductive and developmental biology of the giant scallop lags far behind that for mussels and oysters.

1.1   REPRODUCTION AND DEVELOPMENT

Males and females of this species are separate (Drew, 1906). Animals can be sexed by the colour of their gonad which is readily visible through the gaping shell. Mature gonads are large and plump with the male gonad being whitish in appearance and the ova giving a bright orange colour to

the female gonad. When the adults begin to spawn the gametes are passed through the kidneys and then expelled into the surrounding seawater. The resultant colour change of the kidneys can therefore serve as an early indicator of spawning.

Placopecten magellanicus exhibits a distinct annual reproductive cycle. Gametogenesis begins in March and is completed by May when temperatures are low and food supply is high (Thompson, 1977). During April and May gonad and somatic tissues of P. magellanicus begin to accumulate lipid and carbohydrate (Thompson, 1977). Spawning occurs during August and September in southeast Newfoundland. An earlier, minor spawning is common in most pectinids. Naidu (1970) reported that P. magellanicus has a minor spawn in June in Port au Port, Newfoundland. The importance of this spawn is not known. Animals held at Marine Sciences Research Laboratory (MSRL) have been successfully induced to spawn from June to February.

The average diameter of P. magellanicus eggs is 64.0 μm (Culliney, 1974). Analysis by light and scanning electron microscopy showed that the extrusion of the first polar body occurs within an hour of fertilization (Powell et al., 1985). As in other molluscs, cleavage is typically spiral and uneven (Drew, 1906). The stages of larval development of the giant scallop are shown in Figure 1. The blastula is

FIGURE I: The development of the larvae of
Placopecten magellanicus. A. blastula, 1 mm =
1.33μm B. ciliated gastrula, 1 mm = 1.05 μm C.
trochophore, 1 mm = 0.87 μm D. newly formed
veliger, 1 mm = 10.0 μm.
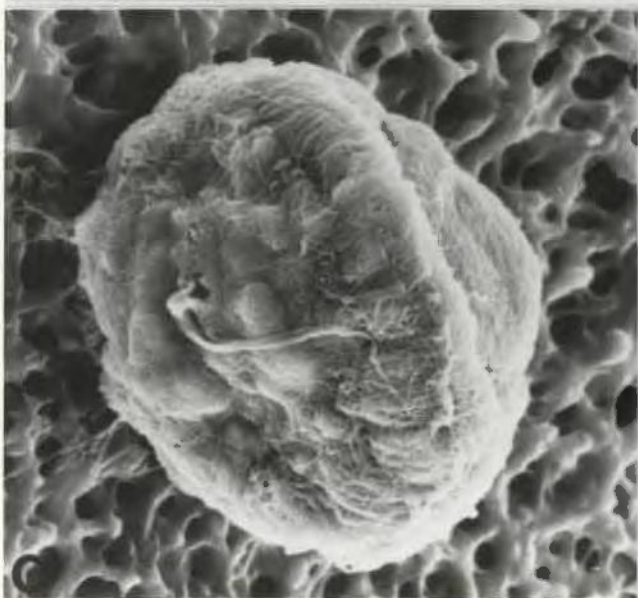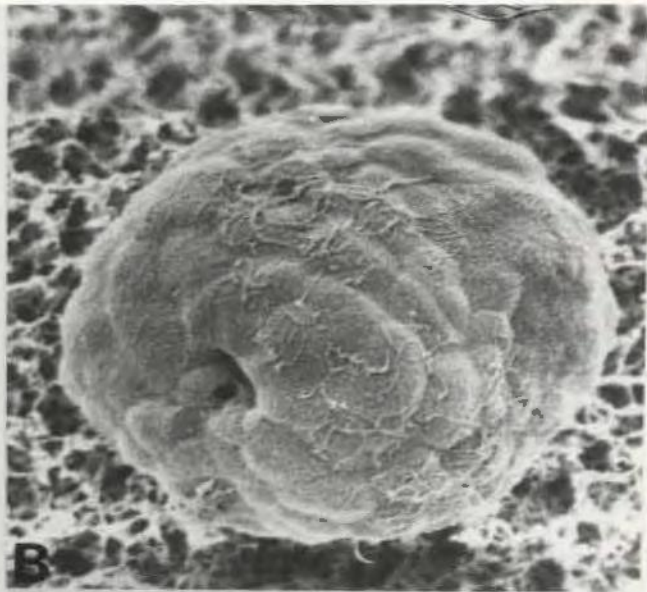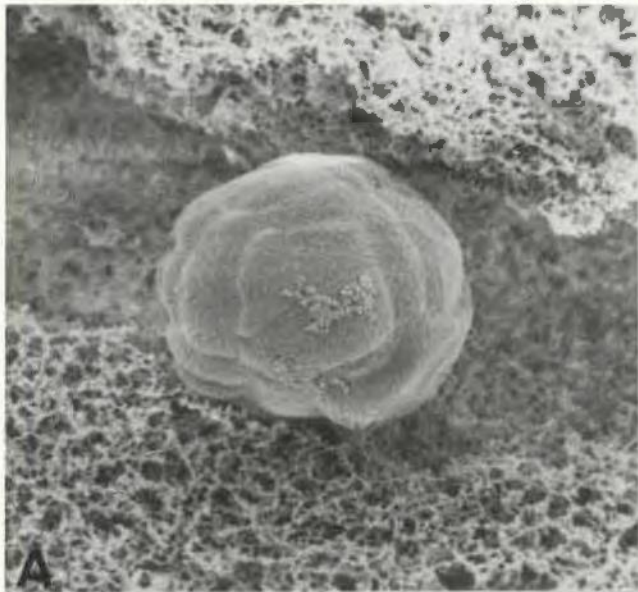
formed 12 to 14 hours after fertilization at 15°C. The embryos become motile as ciliated gastrulas sixteen to twenty-four hours after fertilization. However they do not exhibit directionality until they elongate into trochophore larvae, approximately 48 hours after fertilization (Powell, et al., 1985).

The trochophore is also ciliated and possesses a long compound apical flagellum (Powell et al., 1985). As the larvae enter the trochophore stage the shell gland appears on one side. This gland develops into the mantle which secretes the prodissoconch I shell. A ring of cilia, called the prototroch, appears on the animal pole of the larvae. The prototroch develops into the velum, the larval swimming and feeding organ, as the larva enters the veliger stage. At 15°C the embryos develop into D-shaped veligers in 72 hours.

According to Culliney (1974) the average size of straight-hinge larvae is 105 micrometres in length and 82 micrometres in height. As the veliger larvae develop, shell is deposited and the velum and visceral mass increase in size but very little morphogenesis takes place. Culliney (1974) reported that after 13 days the veliger larvae took on the more rounded appearance of the umbo. Eyespots were observed on the twenty-third day in larvae larger than 230 μm in length and 200 μm in height. The foot appeared on the

twenty-eighth day. At this stage the pediveliger begins to secrete the prodissoconch II shell.

Culliney (1974) reported a cluster of long active cilia at the tip of the foot of _Placopecten magellanicus_. During the pediveliger stage the larvae began to show an adhesive tendency, causing them to stick to the containers, to debris and to each other. This phenomenon may be associated with the first attempts at byssus formation.

At this time the bivalve larvae are ready to undergo metamorphosis to the adult form and settle. Competent mussel (_Mytilus edulis_) pediveligers have a large velum, a foot with retractor muscles, three to five paired gill filaments which are not yet functional, a simple mantle, an alimentary system, a sensory system, and a byssus system (Bayne, 1965).

During metamorphosis most bivalve larvae cease to feed since the larval feeding mechanism, the velum, is cast off and the adult feeding mechanism, the gill, is not yet functional (Hickman and Gruffydd, 1971). Settling involves a change from the pelagic larva to a benthic post-larva. This marks a critical stage in the life history of many marine invertebrates. Following settlement, the gills and oral palps become functional and take over the function of feeding. The inner fold of the mantle begins to add both proteinaceous and calcareous material to the larval shell, forming the dissoconch shell.

1.2   CONDITIONING OF BROODSTOCK

The life history of bivalve larvae can lead to the understanding of some of the energetic requirements of the veligers. The development of bivalve larvae can be separated into three distinct stages (Bayne, 1972). The first stage includes fertilization, cleavage, gastrulation, formation of a trochophore and development up to the prodissoconch I larva. With the appearance of the velum the second stage begins during which the larva increases in size. The third stage begins with the elaboration of the foot. The pediveliger undergoes metamorphosis and settles. As in the first stage, this is a period of intense morphogenesis and very little growth.

There is a greater reliance on stored food during stages I and III than during stage II since the larval feeding mechanism is completely functional only during stage II. Reserves for stage I larvae originate from the egg, whereas stage II larvae accumulate the reserves which are needed to carry stage III larvae through to settlement (Bayne, 1972).

During each of these periods the energetics of the larvae are likely to be quite distinct. The success of stage I larvae depends largely on reserves deposited in the eggs by the adult. Newly formed straight-hinge larvae will survive if the proper food and the optimum particle concentrations are supplied.

Recognition of these stages suggests that two important factors in obtaining healthy larvae are the condition of the adults during gametogenesis and the diet fed to the larvae during their pelagic stage.

Since the gametogenic cycle is intimately linked with a cycle of synthesis and storage of carbohydrate and lipid, followed by their utilization (see Sastry, 1975; 1979), the physiological condition of the adult females will have a profound effect on the larvae. Walne (1966), Bayne and Thompson (1970), Gabbott and Walker (1971), Gabbott and Bayne (1973), Bayne (1973, 1975) and Widdows (1978b) have shown that mussels and oysters tend to decline in condition under laboratory conditions of high temperature and low ration. The stress caused by such conditions results in the utilization of energy reserves which would otherwise be available for gametogenesis. An increase in temperature without the availability of sufficient quantities of food may lead to resorption rather than proliferation of gametes (Sastry, 1975). Bayne et al. (1978) found that as a result of environmental stress, the fecundity of mussels, Mytilus edulis, was reduced approximately in proportion to the decline in energy available for gamete production. The evidence suggests, however, that when a temperature increase is not so great as to bring about complete cessation of gametogenesis and subsequent resorption of gametes, and when

a certain minimum amount of food and nutrient reserves are present, the animal can continue gametogenesis and vitellogenesis (Bayne, 1976). Although gametogenesis may proceed, the quality of the gametes may be affected.

Adult bivalves, including Placopecten magellanicus, accumulate carbohydrates as their major energy store (Giese, 1969; Walne, 1970a; Thompson, 1977). Several studies have shown that gametogenesis in some bivalves, including Placopecten magellanicus, is predominantly sustained by the input of nutrients from feeding (Bayne, 1976; Griffiths, 1977; Thompson, 1977; Thompson, 1984; Hawkins et al., 1985).

Algae such as Tetraselmis suecica and Isochrysis galbana (Parke) have been used as standard diets for conditioning adults since these phytoplankters are easily cultured in the laboratory. Artificial diets have also been used experimentally as food for some bivalves, with limited success (Castell and Trider, 1974; Trider and Castell, 1980). Dupuy et al., (1977) reported some success in conditioning adult oysters using cornstarch as the sole component of the diet.

Under a stress regime fecundity is decreased and the viability of the gametes and the vigour of the larvae are impaired to some degree (Bayne, 1972). Bayne (1972) observed a high percentage of abnormal cleavages, trochophores and prodissoconch I stages in larvae from stressed adults of Mytilus edulis.

Helm et al. (1973) observed that oyster larvae liberated from adults which were held at low ration had a slower rate of growth than larvae from adults at high ration, and that the rate of growth of the larvae over the first 96 hours was predictive of spat yield. Helm et al. (1973) also found that more and earlier broods could be obtained from adult oysters (Ostrea edulis) when they were maintained in good condition. Evidence suggests that this may be at least partially attributable to reduced lipid synthesis in the developing ova (Helm et al., 1973).

In the hatchery, the maintenance of breeding stock in the best possible condition during gonad maturation, spawning and incubation of the larvae (in the case of larviporous species such as Ostrea edulis) is an important factor in obtaining healthy, vigorous larvae.

1.3   ENERGY RESERVES IN EGGS AND LARVAE

The most striking difference in the biochemical composition of the eggs produced by the adults is in their lipid. In most marine invertebrate larvae lipid is the major reserve material (see Holland, 1978). The fact that larvae feed on a carbohydrate rich diet and that it has been well established that the adults use glycogen as their major energy store (Giese, 1969) led some researchers to study carbohydrates in bivalve larvae. Collyer (1957) attempted

to relate the viability of oyster larvae, <u>Ostrea</u> <u>edulis</u>, to their glycogen content upon release. Although she found some carbohydrate, no correlation existed between carbohydrate and the survival of the larvae.

Millar and Scott (1967) were the first to notice the difference in the energy reserves between the larvae and the adults. They observed that lipid reserves were the most important in sustaining oyster larvae, <u>Ostrea</u> <u>edulis</u>, during periods of starvation. Holland and Spencer (1973) reported that neutral lipid accounted for 41% of the total organic matter lost during starvation, whereas protein accounted for 34% and carbohydrate only 25%. Bayne <u>et</u> <u>al.</u> (1975) found that, for <u>Mytilus</u> <u>edulis</u> larvae, greater losses of protein occurred but carbohydrate still played only a minor role.

Bayne <u>et</u> <u>al.</u> (1978) observed a greater decrease in lipid levels than in other components during development of the egg to the larva of <u>Mytilus</u> <u>edulis</u>. The amount of neutral lipid fell from 30% in the eggs to 11% in the larvae. The amount of phospholipid decreased from 22% in the egg to 8% in the larvae. This indicates that in energetic terms, both triacylglycerides and phospholipids can be considered important storage reserves. These results agree with those of Helm <u>et</u> <u>al.</u> (1973) for oysters, <u>Ostrea</u> <u>edulis</u>.

Although marine invertebrate larvae normally accumulate lipid reserves during their pelagic life stage, the

biochemical composition of early larvae seems to be important. The dependence of growth on neutral lipid reserves suggests that the best batches of larvae may be those which are provided with the most neutral lipid or, more specifically, triacylglycerides, in the eggs by the adult.

During the pelagic stage the diet is the most important factor in determining the success of larval growth and survival. Neutral lipid is accumulated by the larvae during the pelagic stage and reaches a peak just before metamorphosis. Since the larvae are unable to feed during metamorphosis and settlement these reserves are rapidly depleted during this period to cover the costs of metabolism and morphogenesis (Holland and Hannant, 1974). Indeed Holland and Spencer (1973) observed that the success of setting was highly correlated with the amount of lipid reserves accumulated by the larvae. Therefore the correct diet is required not only to ensure good growth of pelagic larvae but to accumulate enough energy reserves to sustain larvae through metamorphosis.

In the case of Placopecten magellanicus, the larval diet is very important because of the length of the pelagic stage. P. magellanicus larvae begin to settle in their natural environment in approximately 40 days (Merrill and Edwards, 1976) and in 35 day in the laboratory at 15°C

(Culliney, 1974). _Mytilus edulis_ will begin metamorphosis in 18 days at $16^{o}C$ (Bayne, 1965). _Crassostrea virginica_ completes metamorphosis in 18 days at $23^{o}C$ and _Mercenaria mercenaria_ larvae settle after 16 days at $18^{o}C$ (Loosanoff and Davis, 1963).

1.4 LARVAL AND JUVENILE DIETS

Bivalve larvae are normally present in the water column during times of high densities of phytoplankton, and, having high growth efficiencies, they are able to accumulate large reserves of energy (mostly lipid) in a short time period to be used subsequently during non-feeding stages. In this way they minimize the time spent in the plankton and the risk of predation but still have sufficient time for dispersal (Holland, 1978).

Certain algae are known to provide a better diet for bivalve larvae than others. The nutritional qualities of a large number of algal species have been tested for various larvae and juveniles (Walne, 1963; 1964; 1970b) and some algae have been found to provide a much better diet than others. _Isochrysis galbana_ (Parke), _Tetraselmis suecica_, _Thalassiosira pseudonana_ and _Chaetoceros calcitrans_ have long been recognized as good food for bivalve larvae (Imai and Hatanka, 1949; Davis and Guillard, 1958; Parsons _et al._, 1961; Loosanoff and Davis, 1963; Walne, 1974; Helm and

Millican, 1977; Helm, 1977; Pechenik and Fisher, 1979; Pechenik and Fisher, 1979; Ewart and Epifanio, 1981). Size and cellular complexity are two characteristics which can initially serve to determine the potential worth of a diet. The mean mouth diameter and the enzyme systems present in larvae are also critical factors. Walne showed that a given species of alga may not be equally acceptable as food for different broods of larvae. There are also differences among species of larvae. The food value of a single algal species may vary with the temperature (e.g. Chlorella sp.: Loosanoff and Davis, 1963), with the size of the larvae and with the age of the algal culture (Walne, 1966).

Mixtures of algae often support a faster rate of growth than single species (Davis and Guillard, 1958; Bayne, 1965; Walne and Spencer, 1968; Calabrese and Davis, 1970; Pilkington and Fretter, 1970; Helm, 1977; Kempf and Willows, 1977; Chia and Koss, 1978). Gruffydd and Beaumont (1972) reared the larvae of Pecten maximus on a mixture of Isochrysis galbana (mean cell diameter 3.8 μm) and Chaetoceros calcitrans (3.7 μm) up to 14 days after fertilization, after which Pyramimonas obovata (5.6 μm) and later still Tetraselmis suecica (6.9 μm) were added to the mixture to provide the larger larvae with larger cells in a more complex diet.

It has become apparent that lipid is very important to larval success. The overall digestibility and accessibility of neutral lipids in an algal species may determine its worth as a diet. Up until recently the food value of phytoplankters has been judged by larval growth rates, mortality rates, pediveliger production and setting success. Biochemical analyses of phytoplankters have been used to determine the value of one algal species over another. The amount of various macronutrients present in algal cells changes with the age of the culture. As the culture advances from the logarithmic phase to the stationary phase the cells change from containing large amounts of protein to mostly lipid and carbohydrates (Fogg, 1959). The importance of lipid as an energy source for bivalve larvae has led researchers to believe that a diet with a high proportion of accessible fatty acids could enhance this storage strategy and provide essential components to the larvae. Results from Helm (1977) showed that growth of Ostrea edulis larvae tends to be greatest when they are fed cells from dense cultures. Flaak and Epifanio (1979) observed that a ration of Thalassiosira pseudonana supported maximal growth of Crassostrea virginica when the algal cultures were in the stationary phase. Wilson (1979) concluded that the media of some cultures of Isochrysis galbana during late exponential and early stationary growth phases may contain a

substance or substances which stimulate larvae to increase their grazing rates. However, Cary et al. (1981) failed to show that there was any difference in growth rates of larvae fed exponential or stationary phase algae.

1.5 ENERGY REQUIREMENTS OF LARVAE AND JUVENILES

For aquaculture it is important to determine the optimum conditions for growth. Growth can be determined by measuring the increase in protein, carbohydrate and lipid content, or the total increase in dry tissue weight, of the organism over a period of time. Metabolic rates can be determined directly from measurements of oxygen consumption or from the caloric equivalents of the loss of energy during starvation. Since the necessary energy for maintenance is derived from reserves during periods of starvation, the total energy loss can be taken as an estimate of the metabolic energy demand. Thus the energy requirements of larvae can be estimated through indirect measurements of the biochemical contents of the larvae. However, as Crisp (1976) pointed out, these measurements might be depressed compared with the rates of actively feeding larvae since metabolic rates are lowered during starvation.

Measurement of the rates at which an individual filters the surrounding medium gives an indication of the amount of energy available to the animal. Only a fraction of this

energy is used for growth. The remainder is lost through incomplete digestion, excretion and metabolism.

The efficiency with which the ingested ration is converted into body tissue is termed growth efficiency. Gross growth efficiency (K1) is defined as growth per unit of ingested ration and net growth efficiency (K2) is defined as the growth per unit of absorbed ration. Since assimilation is never 100%, K2 is always greater than K1.

Bivalves are filter feeders. Veliger larvae process the water with their velum and juveniles and adults use the ciliated gills for feeding. Filtration rate is measured as the volume of water cleared of particles by the animal in a given period of time. Pumping rate is defined as the volume of water pumped past the gills of the animal. Filtration rate and pumping rate are equal when the retention efficiency for the alga is 100%.

Filtration rate in bivalves is a function of algal cell size and concentration (Winter, 1970, 1973, 1978; Walne, 1972; Wilson and Seed, 1974; Shulte, 1975; Epifanio and Ewart, 1977; Riisgard and Randlov, 1981). In general, filtration rates decrease with increasing cell concentration. Many bivalves have the ability to regulate feeding within a range of particle concentrations in order to obtain a constant ration (Thompson and Bayne, 1972, 1974; Winter, 1973; Foster-Smith, 1975a,b; Widdows, 1978a;

Griffiths, 1980a; Palmer and Williams, 1980; Navarro and Winter, 1982; Gerdes, 1983; Seiderer et al., 1984). Winter (1973) observed that Mytilus edulis regulated its filtering rate within the concentrations of 1×10^7 and 4×10^7 cells per litre to obtain a constant ration of algae.

Food uptake in a dense algal culture is limited by the capacity of the gut and the digestibility of the algae (McMahon and Rigler, 1965). Therefore the filtration rate decreases with an increase in algal concentration and ingestion rates remain constant. At dilute algal concentrations food uptake is characterized by a constant filtration rate and an increasing ingestion rate with increasing algal concentration.

In aquaculture it is important to determine the maintenance and optimum rations for a given species. Knowing the concentration of particles in the water and having determined the filtration rate of the animal it is a simple matter to compute the weight or number of ingested particles per unit time. Maintenance ration is the amount of food which results in zero growth. Optimum ration is reached at the minimum particle concentration which will result in maximum net growth. The ration fed to larvae and juveniles must be sufficient to cover maintenance costs but should not exceed the optimum ration because of the high cost of growing the algae.

The optimum ration will be affected by the assimilation efficiency of the diet, which is a function of the quality of the food (Corner and Davies, 1971). Therefore the ration at which growth is most efficient will depend on the digestibility of the food, the capacity of the gut and the algal concentration available.

The maintenance ration and the optimum ration are also a function of body size and temperature. When expressed as a percentage of dry weight these amounts are larger for smaller individuals although the actual amount of food ingested is lower. This is a reflection of the higher metabolic rates of smaller animals.

The determination of the minimum food concentration at which maximum net growth is realized is important in aquaculture since algal concentrations above this level will not result in faster growth. The optimum food concentration is close to the pseudofaeces-free cell density (highest algal concentration which does not cause the production of pseudofaeces) since filtration activity is reduced to low energy consuming filtration rates and all the cells filtered out are ingested (Winter, 1978).

1.6 OBJECTIVES

This study aims to determine the energetic requirements of scallop (Placopecten magellanicus) larvae and juveniles with a view to increasing the success in rearing the scallops under laboratory and hatchery conditions.

From an aquaculture point of view it is important to maximize the growth and survival rates of the larvae and juveniles. The literature indicated that maximizing the energy reserves in mussel and oyster eggs led to higher survival rates and better larval growth. Thus, I first conditioned adult P. magellanicus the laboratory to determine:

1.   whether the nutrients in the eggs can be increased beyond the levels of the energy reserves in the eggs of females in the natural environment

2.   whether higher levels of energy reserves will lead to better growth and survival of scallop larvae and

3.   whether any of the biochemical components of the eggs (protein, lipid or carbohydrate) can be used as a predictor of the potential success of a given batch of scallop larvae.

The second step in improving larval growth is determining the nutritional requirements of the larvae. The biochemical components of the larvae and algae were measured in order to determine:

1.   the condition of the larvae

2.   the rate of accumulation of protein, lipid and carbohydrate

3.   a simple energy budget.

Finally a study of filtration rates was undertaken to determine the optimum feeding ration for juvenile scallops.

Chapter II

MATERIALS AND METHODS

2.1 SOURCE OF BROODSTOCK

Divers collected adult scallops (<u>Placopecten</u> <u>magellanicus</u>) from Fox Harbour, Newfoundland on March 26, July 20 and August 11, 1983; from Sunnyside, Newfoundland on July 17, 1984; and from an aquaculture farm in Little Bay, Burin Peninsula, Newfoundland on May 30, June 28 and August 9, 1984. Animals were transported in large polyethylene tanks, by truck, to the Marine Sciences Research Laboratory (MSRL) on the day of collection. Scallops were sexed, and males and females were placed in separate tanks with flowing seawater at ambient salinity (32 ppt). Ambient temperatures were maintained as indicated in Figure 2. Fifteen to twenty animals were placed in each 200 litre tank. Each group of animals received eight litres of algal culture, <u>Isochrysis</u> sp. (Tahitian strain), at approximately 4×10^6 cells.ml^{-1}, five times a week. All the males used for spawning were obtained from this stock. Females used for conditioning experiments were separated from this stock within two weeks of arriving at MSRL. Throughout the season the remaining females were used for spawning when their gonads were ripe.

FIGURE 2: Mean monthly temperatures of ambient seawater at MSRL (o) and seawater in the wet benches (●) where the larvae of <u>Placopecten magellanicus</u> were reared.
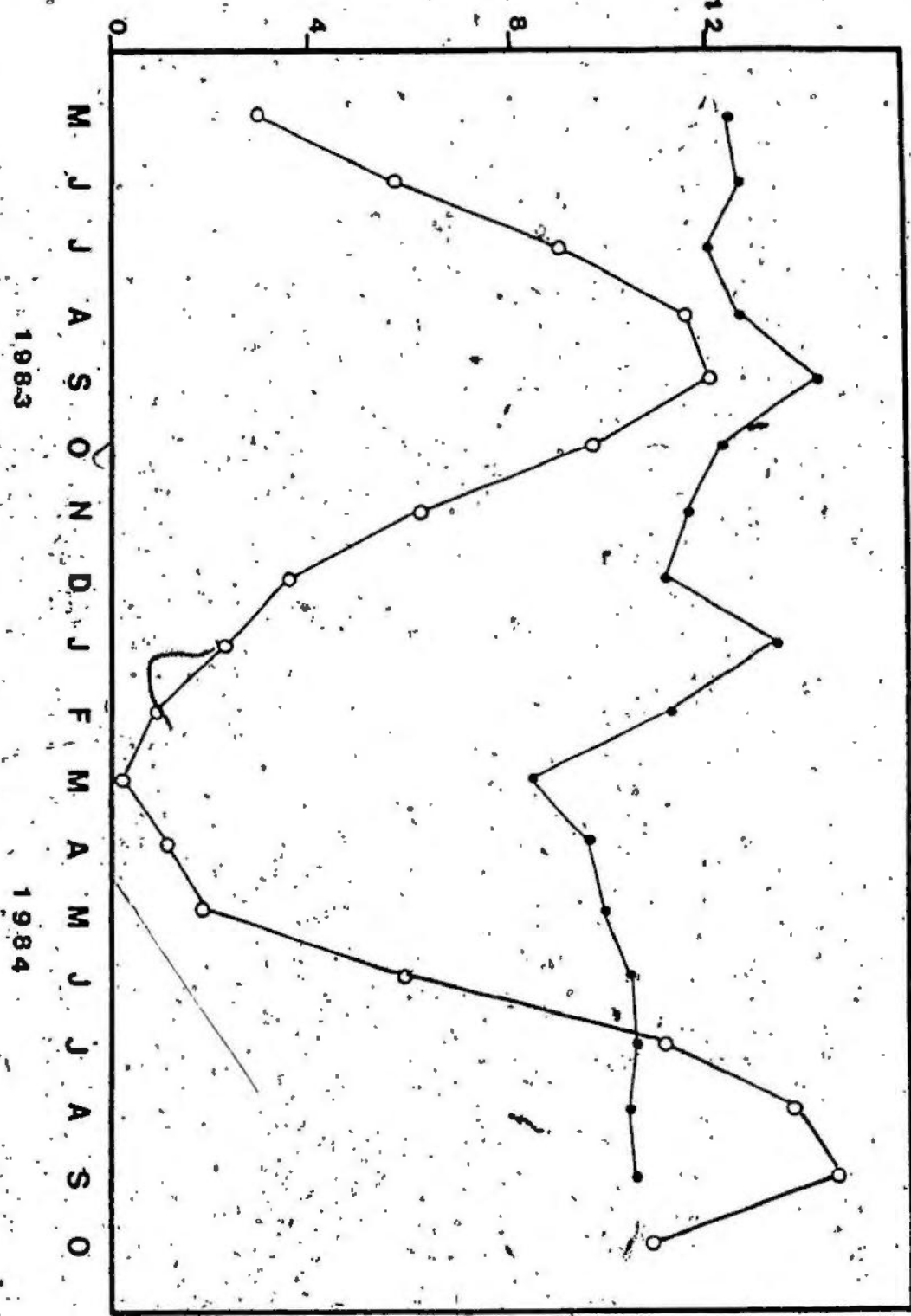
2.2   ALGAL CULTURE

All seawater used for the culture of algae and larvae was passed through a series of five filters: a prefilter designed to remove sediment and large particles; a series of three Gelman cartridge filters, pore sizes 10 μm, 1 μm and 0.45 μm; and a Millipore membrane filter, 0.2 μm pore size. When ambient seawater temperature fell below 9°C it was necessary to heat the filtered seawater (FSW) to 12-13°C. Air was bubbled through the FSW during heating to ensure proper mixing and avoid supersaturation.

A culture collection of various algal species was maintained at a constant temperature of 15°C. These cultures were maintained in 20 ml culture tubes with modified Erd-Shreiber algal growth medium (ES: Stein, 1973), and were transferred every month under sterile conditions. Although all of the cultures were unialgal, they were not all axenic. Algal species used in larval, juvenile or adult scallop diets are listed in Table 1.

Algal cultures used for maintaining broodstock and juveniles were grown in 20-litre glass carboys or 50-litre fibreglass tanks on ES or f/2 algal growth mediums (Stein, 1973). These were harvested every three or four days and fresh medium and FSW were added. These cultures were restarted from the culture collection periodically. Cultures were filtered through a 40 um nitex screen once a

TABLE 1

Source and date of purchase of algal species used in
diets of larvae and adults of _Placopecten magellanicus_.

ALGAL SPECIES	ISOLATOR	LOCATION	SOURCE PURCHASE	DATE PURCHASE
Isochrysis galbana	Parke	Plymouth, England	Bigelow*	02/83
Tetraselmis suecica	Guillard	Falmouth, MA	Bigelow*	07/83
Isochrysis sp.		Tahiti	Bigelow*	11/82
Pseudoisochrysis paradoxa	Kinchelae	Mass. Landing CA	Bigelow*	07/83
Pyramimonas parkeae		Catalina Island	Bigelow*	07/83
Thalassiosira pseudonana	Guillard	Forge River NY	Dalhousie**	06/84
Chaetoceros calcitrans	Ilmebayashi		Dalhousie**	06/84

*   Bigelow Laboratories, Maine, U.S.A.
** Dalhousie University, Halifax, Nova Scotia.

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week to remove clumped cells and debris. Daily counts of cultures were recorded and used to determine the amount required for feeding the adult and juvenile scallops.

All counts on algal cultures were done using Model Z_F Coulter Counter with a 100 μm pore size Coulter tube.

Cultures for feeding the larvae were grown in two or four litre Erlenmeyer flasks on ES medium. These cultures were also harvested every three or four days. The remaining culture, after harvesting, was filtered through a 50 μm nitex screen and transferred to a clean autoclaved flask. Fresh medium and FSW were added. Daily cell counts verified that the algal cultures were continually maintained in the exponential phase of growth. Fresh cultures were started from the algal collection every month to ensure vigorous, healthy unialgal cultures.

Algae harvested for feeding larvae were centrifuged in 250 ml sample bottles in an IEC International centrifuge (Model CS) at 300 g for fifteen minutes. The supernatant was discarded and the cells were resuspended in FSW. In 1983, harvested cells were washed twice in this manner, but in 1984 they were only washed once. This procedure helped to remove chelating agents, toxic metabolites and excess bacteria. It also had the advantage of concentrating cells and thus it was not necessary to add large volumes of algal cultures to the larval cultures. The algal cell count was determined and the appropriate ration was fed to the larvae.

Dry weights of the algal species used for feeding larvae, juveniles and adults were determined. Five counts were performed on each of three separate aliquots of algal culture to determine cell concentration. Measured volumes of the algal solution, in the range of ten to fifty millilitres, were filtered through preweighed, ashed, Whatman GFC filters using suction filtration. The cells in the filtrate were counted on the Coulter Counter, and the total number of cells caught on the filter was determined. Filter papers were then placed in aluminium dishes and dried to constant weight at 80°C (24 hours).

2.3 CONDITIONING OF BROODSTOCK

Females for conditioning experiments were removed from holding tanks and placed in twenty litre plastic trays (35cm x 55cm x 10cm) with flowing seawater at temperatures indicated in Figure 2.

The conditioning of broodstock was undertaken in an attempt to maximize the energy reserves in scallop eggs. I wished to determine if it was possible to successfully condition females in the laboratory by feeding them unialgal, multialgal or carbohydrate diets such that the energy reserves deposited in the eggs would be equal to or greater than those present in the eggs of females in the natural environment. I also wished to determine if totally

spent animals could be conditioned in the laboratory over the winter.

The ration and the method of delivering the diet were varied between the different experiments in a further attempt to increase the energy reserves available to the scallops. The biochemical content of the eggs and the larvae was used as an indication of the success of the conditioning regimes.

Four experiments were performed between May 1983 and September 1984. In each experiment females were maintained at seawater temperatures indicated in Figure 2 and fed various diets as outlined in Table 2. The groups of scallops which received no supplemental feeding are termed "starved". Additional animals brought into the laboratory at the end of the conditioning period were induced to spawn along with conditioned females and are termed "naturally conditioned" females. These two groups served as a negative and a positive control, respectively.

In Experiment 1, females obtained from Fox Harbour, Newfoundland in March 1983 were removed from the holding tanks on May 2, 1983. These animals were batch fed every day by dripping the ration into the trays over a period of two hours. During this time the flow of seawater was cut off. Once the animals had cleared the food particles, approximately three hours after feeding had begun, the seawater flow was reestablished.

TABLE 2

List of conditioning routines for broodstock of <u>Placopecten</u>
<u>magellanicus</u>.

EXPT	BEGINNING OF CONDITIONING D/M/Y	DATE OF SPAWNING D/M/Y	CONDITION FEMALES	NUMBER FEMALES CONDITIONED	NUMBER FEMALES SPAWNED
1	02/05/83	15/07/83	ST	6	6
			S	6	6
			SA	6	6
			A	6	6
			NC		6
2	15/08/83	09/09/83	ST	6	4
			S	6	4
			SA	6	4
			A	6	4
			NC		2
3	01/11/83	24/07/84	5% T-Iso	5	2
			3% T-Iso	5	1
4	29/06/84	03/08/84	ST	6	6
			A	6	6
			M	6	6
			NC		4

ST   Starved females
S   Females fed cornstarch: Expt 1- 300 mg/day/scallop
     Expt 2- 900 mg/day/scallop
SA   Females fed cornstarch + <u>T-Isochrysis</u> sp.
     Expt 1- 150 mg + 13.5 x 10^9 cells per day per animal
     Expt 2- 450 mg + 13.5 x 10^9 cells per day per animal
A   Females fed <u>T-Isochrysis</u> sp.
     Expt 1 & 2- 27.0x10^9 cells/day/scallop (3%b.w./day)
     Expt 4-   50.0x10^9 cells/day/scallop (5%b.w./day)
M   Females fed a mixture of algae: 5 x 10^{10} cells/day
     <u>I. galbana</u>, <u>P. paradoxa</u>, <u>P. parkeae</u>,
     <u>T-Isochrysis</u>, <u>T. suecica</u>
NC   Naturally conditioned females
3% T-Iso Females fed 3% T-<u>Isochrysis</u> sp. per day on
        a tissue dry weight basis ($3x10^{10}$ cells/day)
5% T-Iso Females fed 5% T-<u>Isochrysis</u> sp. per day on
        a tissue dry weight basis ($5x10^{10}$ cells/day)

The time frame for spawning was determined by the facilities available. Males from holding tanks were induced to spawn at the same time as females to permit fertilization of the eggs. Additional females from Fox Harbour were brought into the laboratory on July 20, 1983. Six of these naturally conditioned females were induced to spawn.

Animals in subsequent experiments were fed continuously by supplying algae to the adults with the use of a peristaltic pump. The concentration of the feeding suspension and the speed of the pump were adjusted to deliver the food over a twenty-four hour period.

Females obtained from Fox Harbour, Newfoundland on August 11, 1983 were used for the second conditioning experiment. They were placed in 20-Litre trays in a constant temperature room at 15°C. The flow rate of seawater was 5.3 +/- 0.7 litres/hour.

Larvae obtained by fertilizing eggs in Experiments 1 and 2 were fed 25 cells/ul of Isochrysis sp. (Tahitian strain) three times per week.

In the third experiment, ten female and five male scallops from Fox Harbour, Newfoundland, which had been spawned during one of the previous two conditioning experiments, were placed in 20-litre trays in a constant temperature room at 15°C. Males were placed in one tray and females were divided amoung three other trays. The flow rate of seawater through the trays was 128.3 +/- 28.3 1/h.

Each tray of animals received 9 litres of Isochrysis sp. (Tahitian strain), at a cell concentration of 3×10^6 cells.ml^{-1}, five times a week from November 1983 until June 25, 1984. On June 25 the ration of five females was increased to 5×10^{10} cells per day per animal. Larvae subsequently obtained from these scallops were maintained on a diet of 25 cells.μl^{-1} of Isochrysis galbana (Parke) fed three times a week.

Scallops conditioned in Experiment 4 were obtained from an aquaculture farm in Little Bay, Newfoundland on June 28, 1984. On August 9, 1984 additional females were obtained from Little Bay, Newfoundland. These animals were the naturally conditioned controls. Larvae from this group were fed 25 cells.μl^{-1} of Isochysis galbana (Parke), 5 cells.μl^{-1} Thalassiosira pseudonana (Guillard) and 10 cells.μl^{-1} of Chaetoceros calcitrans, three times per week.

In all experiments samples of the eggs were taken for counting and sizing with an electronic particle counter, Model Z_B Coulter Counter with channelizer and plotter. A Coulter tube of 280 μm pore size was used for counting eggs. Two samples of forty to fifty thousand eggs were taken for biochemical analyses. These samples were kept in a minimum of FSW and frozen at -20°C until ready for analysis. Total protein was determined using a micro-Lowry assay (Lowry et al., 1951). The charring method of Marsh and Weinstein (1966) was used to measure total lipid.

Carbohydrates were measured spectrophotometrically using the phenol-sulfuric acid reaction (Dubois et al., 1956).

Samples of one to six million eggs were used for dry weight determination. Whenever the number of eggs available was large enough, duplicate or triplicate determinations were performed. A measured volume of eggs was placed in a glass beaker and mixed thoroughly with a perforated plunger. A sample was taken and counted. The eggs were then allowed to settle. The supernatant was removed with a pipette and an aliquot of supernatant was counted such that the number of eggs present in the sample could be determined. The eggs were washed twice with isotonic ammonium formate (2.5% w/v), and allowed to settle between washings. The excess ammonium formate was removed by pipette. The eggs were then poured into preweighed 25 or 50 ml aluminium weighing pans and dried to constant weight (48 hours) at $80^{\circ}C$. Eggs were weighed to 0.1 mg accuracy.

2.4 SPAWNING AND REARING

The standard methods for growing bivalve larvae (Loosanoff and Davis, 1963) were modified for _Placopecten magellanicus_ (Gmelin). Conditioned females were induced to spawn at the end of the conditioning period. Males and females which were not selected for feeding experiments were spawned when their gonad was full and plump in appearance.

In all cases methods for spawning, rearing the larvae, and sampling were the same.

To provide information on the growth and survival of larvae during starvation, groups of larvae from Females 53, 54, 55, and 56 (Table 3) were maintained in FSW without food. Additional groups of larvae from these females were fed 25 cells.μl^{-1} of _Isochrysis galbana_.

Adults were removed from the tanks and their shells were scrubbed. The scallops were measured and their age was estimated by counting the number of growth rings on the left valve. They were then placed in 10-litre pans half-filled with FSW. A recirculating pump was used to play a jet of water over the adults. The water temperature was maintained at 14-16^{0}C.

Adults about to spawn could be identified since the kidneys take on the colour of the gametes immediately prior to spawning. They were placed in separate 4-litre containers with approximately two litres of FSW and allowed to spawn completely. Males and females which released a large number of gametes were transferred to clean containers several times before they finished spawning.

The orange coloured eggs were filtered through a 20 μm nitex screen and mixed thoroughly with a perforated plunger. A sample was taken for counting and sizing.

TABLE 3

Spawning data for females of <u>Placopecten</u> <u>magellanicus</u> from Fox Harbour, Newfoundland, not selected for Conditioning Experiments in 1984. (Groups of larvae from these females were starved.)

FEMALE	SIZE ht(cm) x l(cm)	AGE (yrs)	DATE DD/MM/YY
53	11.4 x 11.4	6	15/11/83
54	13.6 x 14.3	10	13/12/83
55	16.0 x 17.0	8	13/01/84
56	15.1 x 16.3	11	20/01/84

Eggs were fertilized in 4-litre glass beakers with 280,000 eggs per litre of FSW. Sperm was added to the eggs until the active sperm to egg ratio was approximately 3:1 as observed under a Zeiss binocular microscope using dark field microscopy. Fertilization was allowed to proceed for ten minutes.

One litre flat glass pyrex dishes were used as containers for the early development of Placopecten magellanicus larvae. Seventy thousand eggs suspended in 750 ml FSW provided a density of 100 eggs.cm^{-2} on the bottom of the flat dishes. An antibiotic, neomycin sulfate, was added at a concentration of 25 mg/l. The dishes were placed in a constant temperature room at 15°C and the larvae were allowed to develop to the straight-hinge stage, approximately 72 hours.

When larvae first became D-shaped veligers they were gently poured through a 150 μm prefilter and caught on a submerged 50 μm nitex screen. These larvae were rinsed gently with FSW and placed in 4-litre plastic containers at a density of 10 larvae per ml. Neomycin sulfate was added to give a concentration of 50 mg/l. The larvae were fed the algal diets indicated in Table 2. The containers were placed in a wet bench where the seawater temperature was maintained at 12 +/- 1°C (Figure 2) by a Neslab cooling unit.

Two aliquots of newly formed straight-hinge larvae were taken. Five separate counts were performed on each sample. The larvae still remaining after the counts (20,000-25,000) were used for analysis of protein, lipid and carbohydrate. Samples were obtained by filtering these larvae onto a 50 um nitex screen, washing them with isotonic ammonium formate (2.5%), and then rinsing them into 20 ml culture tubes. Once the larvae had settled, the excess supernatant was removed by pipette and samples were capped and frozen at -20°C until analysis.

Another sample of at least 20 larvae was taken for sizing. The height of larvae was measured using an ocular scale in a Zeiss monocular inverted microscope.

The protocol for rearing the larvae during their pelagic stage involved filtering them through a 150 μm prefilter onto a 50 μm nitex screen, placing them in clean containers with fresh FSW, and feeding them. This routine was performed three times a week. Weekly samples for measurement and determination of total protein, lipid and carbohydrate were taken as described above for newly formed D-shaped larvae with the exception that only one sample was taken for biochemical analysis and the number of live larvae present in this sample was determined by two 1-ml counts with a counting cell on a Zeiss binocular microscope using darkfield microscopy.

As the larvae grew the mesh size of the prefilter was increased from 150 μm to 200 μm and then 250 μm. When larvae exceeded 200 μm in height the 50 μm filter was replaced by a 100 μm screen.

2.5   BIOCHEMICAL ANALYSES

The protein, lipid and carbohydrate content of algae, eggs, larvae and juveniles were determined to obtain the relative importance of each biochemical component and as an estimate of the total energy content of an individual. The energy content was estimated by transforming the various constituents into energetic equivalents (P x 23.54 joules/mg; L x 39.54 joules/mg; C x 18.17 joules/mg). The caloric equivalents were those used by Crisp (1971). To obtain the energy equivalents in joules/mg a conversion factor 4.184 joules/calorie was used (Weast et al., 1984).

All analyses were done on samples stored at -20°C. Both samples of scallop eggs and both samples of newly formed larvae from each female were analysed in duplicate thus giving four replicates for the eggs and for the larvae (Appendix I; Tables A-5 to A-11). Samples of juveniles and algae were also analysed in duplicate (Appendix I; Tables A-15, A-16 and A-17). The algal samples were taken from the exponentially growing algal cultures used in the culture of scallop larvae.

All glassware used was washed in chromic-sulfuric acid cleaning solution (Chromerge, Fisher Laboratories) and rinsed several times in glass distilled water before use.

2.5.1 Fractionation and extraction

The method used was modified from Holland and Gabbott (1971)..

The frozen samples were thawed, the total volume was determined and the samples were homogenized with a 7ml Broeck model ground glass homogenizer (Pyrex brand, Corning). A 400 µl aliquot of the sample was placed in a 15 ml conical, stoppered test tube for lipid extraction. Another 400 µl aliquot for protein and carbohydrate extraction was placed in a 15 ml culture tube.

Lipid was extracted by the Bligh-Dyer method (Bligh and Dyer, 1959). A volume of 1.5 ml of a 1:2 (v:v) mixture of chloroform-methanol was added to the lipid samples. Glass distilled methanol and chloroform were obtained from Caladon Laboratories. Samples were mixed by vortexing for one minute. Five hundred microlitres of chloroform and five hundred microlitres of distilled water were added and samples were mixed thoroughly for one additional minute. Samples were then centrifuged at 1000 g in an International Clinical Centrifuge (Model CL) for ten minutes and the top phase was discarded. The tubes were placed in a water bath at 60°C and the lower phase dried under nitrogen. The lipid

residue was redissolved in chloroform. The sides of the tube were rinsed well with chloroform to ensure all lipid was dissolved. These samples were topped with nitrogen, stoppered and stored at $4^{\circ}C$ if analysis was not done immediately.

Protein and carbohydrate were extracted from the same sample. Protein was precipitated by addition of 200 μl of cold ($4^{\circ}C$) 15% trichloroacetic acid (TCA). The samples were mixed thoroughly on a vortex mixer for five minutes, cooled at $4^{\circ}C$ for 10 minutes and then centrifuged at 800g for twenty minutes. The supernatant, containing soluble carbohydrates, was removed by pipette and placed into 25 ml glass test tubes. The protein precipitate was washed with 200 μl of 5% TCA and centrifuged for another fifteen minutes at 800g. The supernatant was removed by pipette again and added to the previous supernatant. The precipitated protein was dissolved in 500 μl of 1.0 N NaOH and heated at $56^{\circ}C$ for thirty minutes.

To the combined supernatants 100 μl of 6N HCl was added and samples were heated at $95^{\circ}C$ for two hours to break down all polysaccharides. Once the sample had cooled, 100 μl of 6N NaOH was added to neutralise the solution. Total carbohydrate was determined on this sample.

2.5.2 Lipid determination

Total lipid was measured by the charring method of Marsh and Weinstein (1966). The conversion of lipid to carbon is a rapid, sensitive and reproducible process. A solution of 2 mg/ml tripalmitin (Sigma) in chloroform was used as a standard. The tripalmitin standard was added to test tubes as described in Table 4. A set of standards and two reagent blanks were run with each set of samples analysed.

The lipid samples were first dried under nitrogen. A buret was then used to deliver 2 ml of concentrated, reagent grade sulfuric acid (Fisher Chemicals) to reagent blanks, standards and test samples. The unstoppered test tubes were placed in a Tecam Dri Block heater (Model DB 3H) at 15 second intervals and heated to 180 +/- 3°C for 15 minutes. Glass beads were placed on the test tubes to prevent debris from contaminating the solutions. Test tubes were removed at fifteen second intervals, placed in a water bath at room temperature for fifteen seconds and then on ice until cool. Three millilitres of distilled water was added very carefully along the side of the test tubes and the solutions were mixed partially. The tubes were cooled on ice again, another 3 ml of distilled water was added and solutions were mixed thoroughly by vortexing. The absorbance was read at 375 nm on a SP6-500 Pye Unicam spectrophotometer.

TABLE 4

Standards used in the determination of protein, lipid and carbohydrate.

STANDARD	ALBUMIN (μg)	TRIPALMITIN (μg)	GLUCOSE (μg)
1	10	50	12.5
2	20	100	25.0
3	50	200	37.5
4	100	300	50.0
5	150	400	62.5
6	200	500	75.0
7	250	600	87.5

2.5.3 Protein determination

A modified Lowry method was used for determination of crude protein (Lowry et al., 1951). The standard used was albumin (crystallised and lyophilised, Sigma Chemicals) dissolved in 1.0N NaOH, at a concentration of 2 mg/ml.

Three solutions were required for protein determination. All solutions were stored at room temperature. Lowry A solution contained 1 g sodium potassium tartarate and 50 g sodium carbonate dissolved in 250 ml of 1.0N NaOH and further diluted to 500 ml with distilled water. Lowry B contained 1 g of sodium potassium tartarate and 0.5 g cupric sulfate ($CuSO_4.5H_2O$) dissolved in 45 ml distilled water and 5 ml 1.0N NaOH. The third solution, Lowry C, was prepared by diluting 1ml of 2N phenol reagent solution (Folin-Ciocalteau reagent from Fisher Laboratories) with 14 ml distilled water.

Required amounts of standards (Table 4) were added to 10 ml test tubes. Volumes in the range of 50-200 μl of the test solution were added to marked test tubes. The volume in all test tubes was adjusted to 500 μl with distilled water. The reagent blank contained 0.5 ml distilled water.

In the first step 0.5 ml Lowry A was added to all tubes and they were incubated for 10 minutes at 50°C. After cooling for 10 minutes, 50 μl of Lowry B was added to each tube, mixed and left at room temperature for ten minutes.

The final step involved adding 1.5 ml Lowry C to each tube, mixing thoroughly and incubating at 50°C for 10 minutes. The absorbance was read at 650 nm on a Gilford Spectrophotometer 240 using a reagent blank to set the zero. Disposable cuvettes (Bio Rad) were used for the measurement to avoid the problem of accumulated dye staining the cuvettes.

2.5.4 Carbohydrate determination

Total carbohydrate was measured using the phenol-sulfuric acid reaction (Dubois et al., 1956). This assay is rapid, sensitive and reproducible. The only reagents required were 80% phenol and reagent grade concentrated sulfuric acid (Fisher Chemicals). The phenol reagent was made by adding 10 ml distilled water to 90 ml of 90% liquified phenol from Fisher Chemicals. An aqueous solution of glucose (Analar), 0.5 mg/ml, was used as a standard as outlined in Table 4.

The total sample was used for analysis of carbohydrate in eggs, larvae and juveniles. When algae were assayed only 200 µl of the solution was analysed. The volume in all test tubes was adjusted to 2 ml with distilled water. 50 µl of 80% phenol was added to each test tube and mixed thoroughly. Five millilitres of concentrated sulfuric acid was delivered directly to the solution, not along the sides of the test tube. The heat created was necessary for the condensation of the monosaccharides with phenol in acidic solution which

yields a coloured compound. The samples were allowed to cool for thirty minutes. The orange colour produced is stable for several hours (Dubois et al., 1956). Absorbance was read at 485 nm on a Gilford Spectrophotometer 240 using disposable cuvettes (Bio Rad).

4.6   FILTRATION RATES

In aquaculture it is important to determine the optimum ration. Filtration rates of juveniles were measured in an attempt to determine the algal concentration which would provide the maximum ingested ration without the production of pseudofaeces.

Filtration rates of juvenile scallops were measured using a static system. Isochrysis galbana (Parke) was added at concentrations ranging from 5×10^3 cells.ml^{-1} to 25×10^3 cells.ml^{-1}.

Juveniles were obtained from spat collectors at an aquaculture farm in Little Bay, Newfoundland, in July and August 1983 and held in incubator trays with flowing seawater until needed. They were fed continuously on diet of Isochrysis sp. (Tahitian strain). In May and June 1984 juveniles from the same year class were obtained from Little Bay, Newfoundland. These scallops, although the same age as those held at MSRL for one year, were two to three times larger. Thus a wide range of sizes was available.

Juveniles used ranged in size from 9mm in height to 45mm in height. The scallops were placed in 2-litre containers of FSW twelve to fifteen hours before each experiment began. All containers were placed in a wet bench to control the temperature.

Filtration rates were determined by measuring the decrease in cell concentration with time. Filtration rate was calculated acccording to the equation of Coughlan (1969).

$$F=[(M.t^{-1} \times \ln C_O/C_t) - (M.t^{-1} \times \ln C_O'/C_t')].n^{-1}$$

where, F = Filtration rate (ml/h/animal)

   M = volume of FSW (ml)

   C_O = Initial cell concentration I. galbana

   C_t = Concentration I. galbana at time t

   t = time (hours)

   C_O' = Initial concentration I. galbana of control

   C_t' = Concentration I. galbana at time t in
      control

   n = number of scallops per group.

All algal counts were done on a Model Z_F Coulter Counter using a 100 μm pore size tube.

The ingestion rate (cells.h^{-1}) was calculated by multiplying the filtration rate with the appropriate algal concentration. At the end of each day the faeces produced

were examined under the microscope for the presence of pseudofaeces. Pseudofaeces were identified as being green in colour and loosely held together whereas faeces were brownish and formed long compact strings.

2.6.1 Experiment 1

Filtration rates of individuals 15-17 mm and 13-14 mm in height were determined on groups of 10-15 animals taken from a pool of 230 juveniles. These scallops were placed on wire mesh screens in 2-litre plastic containers and the FSW was bubbled gently. Two controls were run along with eight groups of experimental animals. Twenty-five millilitre samples were removed by means of a pipette every half hour for 7-10 hours and counted. The samples were replaced after counting. The volume loss was negligible (30 ml over 10 hours). Isochrysis galbana cells were added whenever the cell concentration fell below 40% of the starting concentration.

The filtration rate was determined for each group on two consecutive days. Scallops were left in 2-litre containers overnight in between these two determinations and transferred to clean containers at the beginning of the following day. The animals were then returned to the trays and different groups were chosen.

2.6.2   Experiment 2

For scallops 9-11 mm in height, filtration rates were determined on individual juveniles in 25 ml plastic beakers. These beakers were placed directly in the Coulter Counter. Hourly readings were taken for 3 hours. Then the scallops were transferred to clean containers and the experiment was repeated. When calculating filtration rates the volume was adjusted to compensate for loss during counting on the Coulter Counter.

At the end of each day the scallops were returned to the incubator trays and new juveniles were placed in 2-litre containers with FSW for the following day.

2.6.3   Experiment 3

Yearlings for this experiment were obtained from an aquaculture farm in Little Bay, Newfoundland. They were held at MSRL and their natural diet from the ambient seawater was supplemented with _Isochrysis galbana_ three times a week.

Individual animals were used to determine filtration rates in 2-litre containers. The FSW was not stirred or bubbled during the experiment except directly after addition of algae. Two controls as well as eight to ten experimental animals were maintained throughout each experiment.

Samples of 25 ml were taken every half hour, for seven to ten hours, to determine cell concentrations. At the end of

the experiment juveniles were returned to the stock and not used again.

2.6.4   Dry weight determination of juveniles

The total organic dry weight of juveniles was determined as the difference in dry weight before and after treatment with a 5% solution of sodium hypochlorite (Javex). The dry weight of body parts alone was also determined.

The dry weights of scallops were determined on 82 scallops of 8.0 mm to 20.0 mm in height and 33 scallops 30 mm to 45 mm in height from the 1983 year class and 41 juveniles or groups of juveniles of 4.5 mm 10.0 mm in height from the 1984 year class. The regression equations of shell height versus dry weight were used to estimate the tissue dry weight of scallops used for filtration rate studies.

The juveniles were anaesthetised in 0.2% phenoxyethanol. They were washed with 2.5% (w/v) ammonium formate to remove excess salts and blotted dry. Animals were then removed from the shell and both the soft tissues and the the shell were placed in aluminium weighing pans. They were dried to constant weight at $80^{o}C$ (48 hours). The shells and soft tissues were weighed separately to 0.1 mg accuracy.

Both the body parts and the shell were then placed in glass vials containing 5% sodium hypochlorite solution (Javex), for 24 hours. After this time the shells were removed and washed with distilled water, dried for another

24 hours and reweighed. The weight of total organic matter was calculated from the difference (Jespersen and Olsen, 1982).

2.7   STATISTICAL ANALYSES

All statistical analyses were performed with the use of SPSS (Statistical Package for the Social Sciences) by McGraw and Hill Ltd.

The data on the biochemical composition of the eggs and larvae of conditioned females were subjected to a two-level nested analysis of variance (see Appendix II). The first level comprised the conditioning treatments and the second the females or replicates within a conditioning treatment. This analysis tested for differences among conditioning treatments and between replicates within a treatment. Where conditioning differences appeared, mean values were analyzed by the Tukey-Kramer multiple comparison test to indicate where significant differences among the conditions existed.

TUKEY-KRAMER MULTIPLE COMPARISON TEST

$$MSD = Q_{0.05} \quad (t,df) \times SE_{ij}$$

MSD: Minimum significant difference
$Q_{0.05}$ Studentized range at the 0.05 level
t: number of treatments
df: degrees of freedom of MSE
$$SE_{ij} = \sqrt{MSE \frac{(1/n_i + 1/n_j)}{2}}$$

The data on the number of eggs spawned, the egg size and dry weight, and the success of fertilization (as shown by the rate of recovery of larvae from the eggs) were subjected to a oneway analysis of variance to determine differences among the conditions. Where differences were detected the mean values were further analysed by the Tukey-Kramer multiple comparison test to detect where the significant differences occurred.

Regression analysis was performed on the filtration and ingestion rate data to determine the relationship between size, algal concentration and filtration rates of juvenile scallops. Regression analysis was also used to determine the relationship between the total energy content of the juveniles and their dry weight.

Chapter III

RESULTS

3.1 CONDITIONING OF BROODSTOCK

3.1.1 Biochemical composition of eggs

Females were conditioned at various stages of gonadal development. The females brought in from the field for the first conditioning experiment had immature gonads and were difficult to distinguish from the males. The females used for conditioning during the winter had completely empty gonads at the beginning of the treatments and were only identifiable as females because they had been marked as such during previous experiments. The gonads of females brought into the laboratory for the second conditioning experiment were nearly full with ripe gametes. The last group of conditioned scallops were readily sexed at the beginning of the experiment although their gonads were not full and plump.

During the spawning of females from the first conditioning experiment it was noted that the gonads of starved (ST) and starch fed (S) females had only a slight tinge of pink colour instead of being a bright orange

colour. Although the eggs from the starch and algae fed (SA) and algae fed (A) females were more coloured they were still paler than the eggs released by the naturally conditioned females. The eggs from ST and S females in Experiment 2 as well as the eggs from ST females in Experiment 4 were a dull pink rather than the bright orange colour of the eggs produced by the females of the other conditioning treatments. The eggs produced by the females conditioned during the winter (Experiment 3) also had only a slight tinge of pink and the gonads were not full. The colour of the eggs is the first indication of the success of a conditioning treatment.

Most females were readily induced to spawn (one to two hours of stimulation) at the end of the conditioning period (Table 2). Females which would not spawn after five or six hours of stimulation were rejected. With the exception of the females conditioned during the winter, the inability to induce certain females to spawn was due to their having previously spawned in the tanks during the conditioning period.

Conditioning of the broodstock did cause an increase in the organic content of *Placopecten magellanicus* eggs. Protein and lipid levels in the eggs increased significantly when the adult females were fed a diet supplemented by cultured phytoplankton (Figures 3-6). The

FIGURE 3: Protein, lipid and carbohydrate levels in the eggs and newly formed veliger larvae of Placopecten magellanicus females from Conditioning Experiment 1.

SIGNIFICANT DIFFERENCES AT 0:05 LEVEL

EGGS: P ST S SA NC A

L ST S SA NC A

LARVAE: P ST S SA A NC

L ST S SA NC A

mg protein per million

Protein

Lipid

Carbohydrate
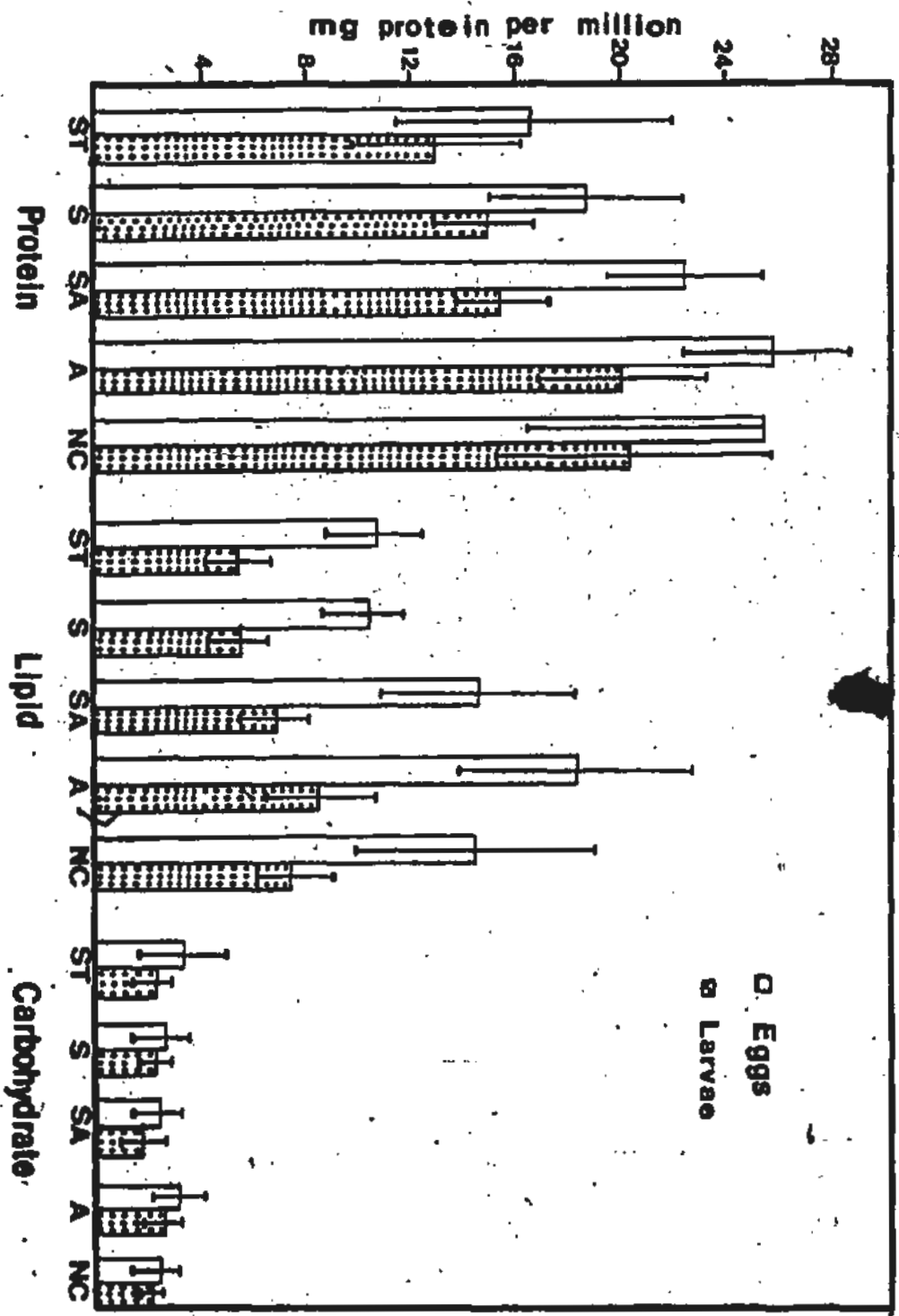
□ Eggs
▣ Larvae

FIGURE 4: Protein, lipid and carbohydrate levels in the
eggs and newly formed veliger larvae of
<u>Placopecten</u>   <u>magellanicus</u>   females   from
Conditioning Experiment 2.

SIGNIFICANT DIFFERENCES AT 0.05 LEVEL:

EGGS: P <u>ST   S SA   NC A</u>

       C <u>ST   S SA</u>   A NC

mg per million

Protein

Lipid

Carbohydrate
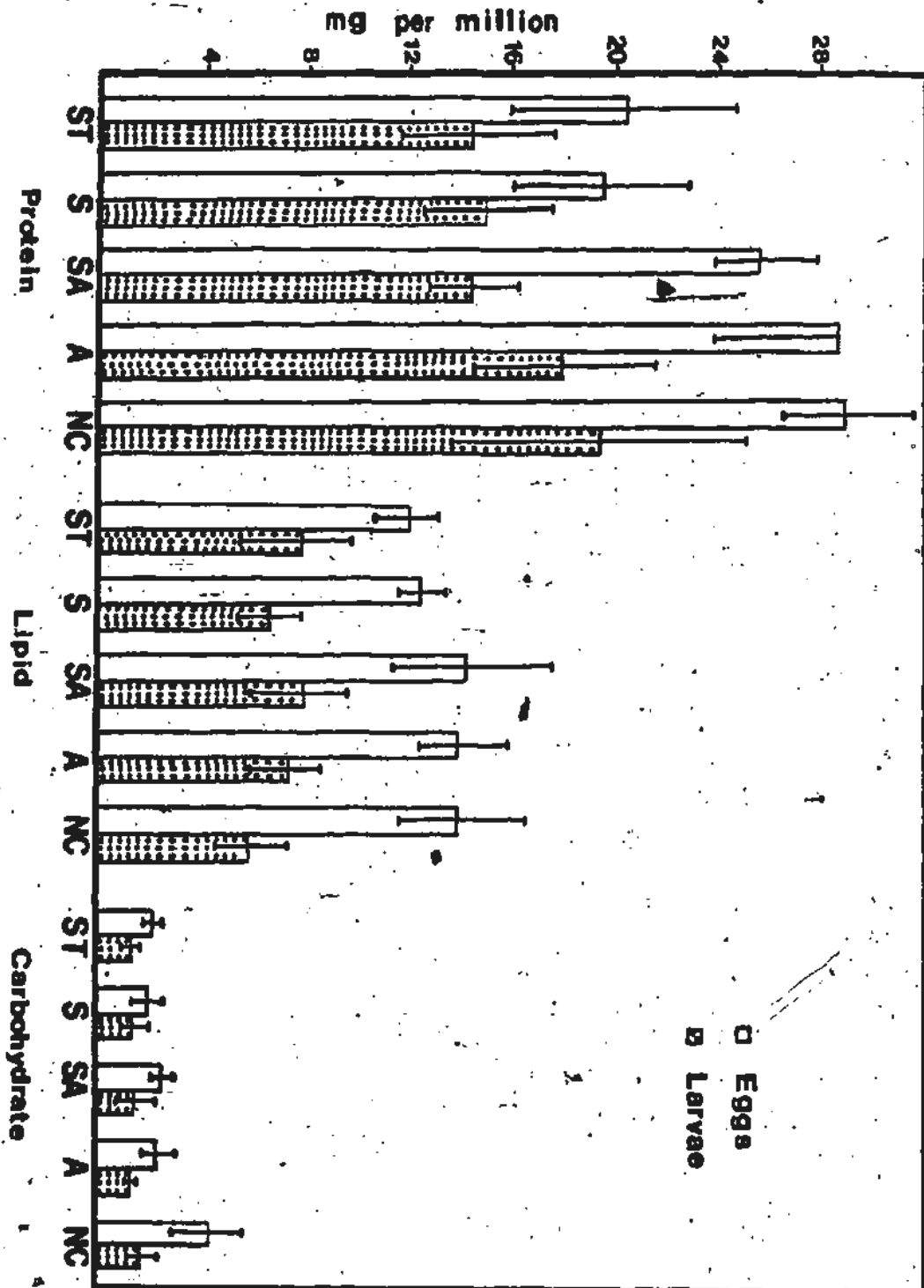
□ Eggs
▨ Larvae

FIGURE 5: Protein, lipid and carbohydrate levels in the eggs and newly formed veliger larvae of *Placopecten* *magellanicus* females from Conditioning Experiment 3. (3%,5%: Females fed *Isochrysis* sp. (Tahitian strain), 3% or 5% of their body weight per day.)

mg per million

Protein   Lipid   Carbohydrate
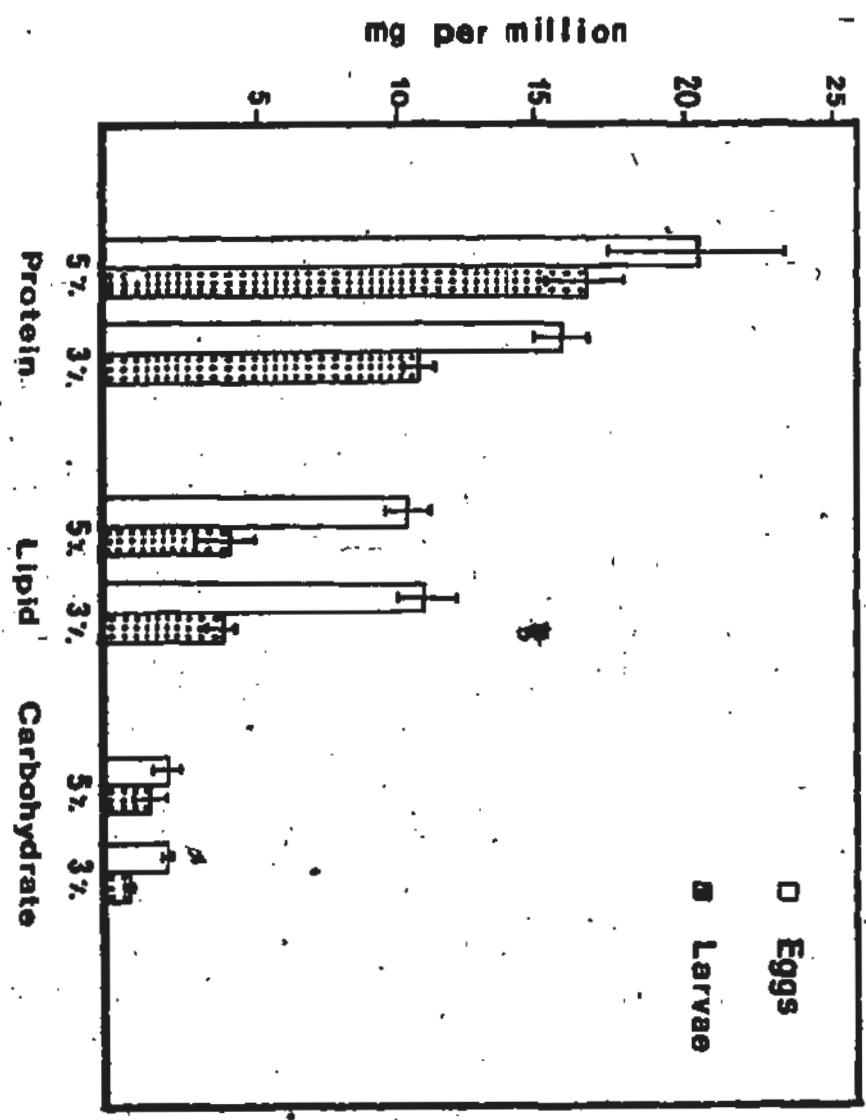
□ Eggs
■ Larvae

FIGURE 6: Protein, lipid and carbohydrate levels in the eggs and newly formed veliger larvae of _Placopecten_ _magellanicus_ females from Conditioning Experiment 4.

SIGNIFICANT DIFFERENCES AT 0.05 LEVEL:

EGGS: P ST NC A M

L ST NC A M

LARVAE: L ST NC A M

y-axis: mg per million

x-axis groups: ST A M NC (under Protein), ST A M NC (under Lipid), ST A M NC (under Carbohydrate)

Legend:
□ Eggs
▨ Larvae
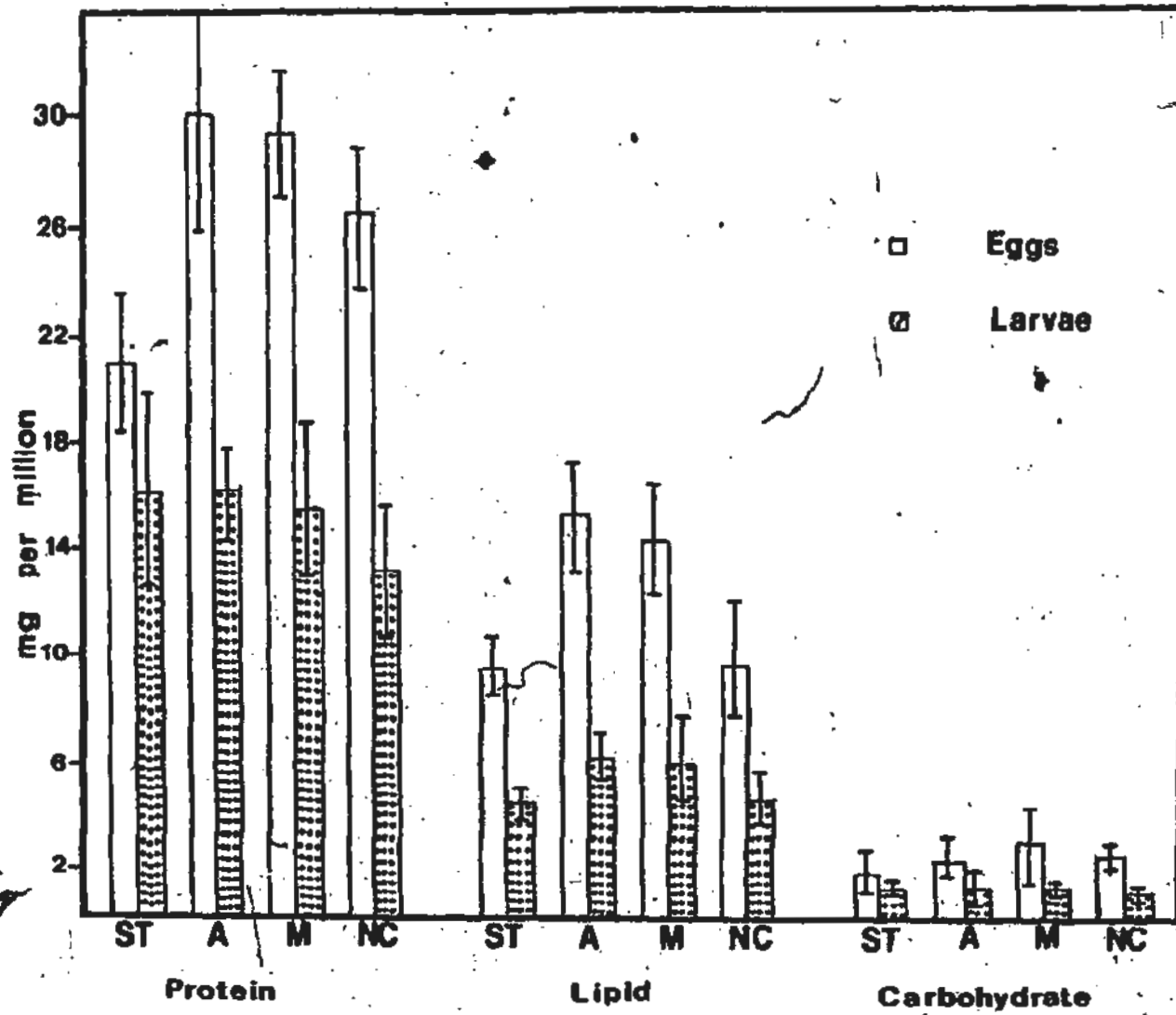
proportion of protein to lipid, however, did not vary irrespective of the conditioning treatments (Table 5). The level of carbohydrate in the eggs was very low, accounting for 5 to 10 percent of the total energy reserves (Table 5). Carbohydrate reserves were not affected by the various conditioning treatments in any of the experiments.

In Experiment 1 females fed a diet of 300 mg of cornstarch per day produced eggs with levels of protein and lipid which were comparable to those present in the eggs spawned by ST females. The eggs obtained from A females, however, had ~40% more lipid and 33% more protein than the eggs of ST and S females and 20% more lipid and 15% more protein than the eggs spawned by SA females. These differences are significant at 0.05 level (Figure 3). The eggs obtained from naturally conditioned females had 20% less lipid than those obtained from A females but the same amount of protein and carbohydrate (Figure 3).

In Conditioning Experiment 2 there were no significant differences, at the 0.05 level, in the lipid levels in the eggs among the different conditioning treatments, even though the eggs from the SA females, the A females and the naturally conditioned females had 13% more lipid than in those from S females and the starved controls (Figure 4). The biochemical composition of the eggs from the S scallops still did not differ from that present in the eggs from

TABLE 5

Biochemical composition of eggs and three day old larvae of
Placopecten magellanicus expressed as a percent of the total
energy reserves.

EXPT.	CONDITION FEMALES	PROTEIN eggs %	larvae %	LIPID eggs %	larvae %	CARBOHYDRATE eggs %	larvae %
1	ST	53.4	62.9	35.2	26.1	11.5	11.0
1	S	58.9	66.1	30.1	24.2	8.0	9.7
1	SA	57.3	64.6	36.6	28.4	6.0	7.0
1	A	54.7	64.5	38.4	27.1	6.9	8.5
1	NC	56.9	67.9	34.4	25.0	5.3	7.0
2	ST	58.0	62.4	35.4	32.2	6.6	6.4
2	S	56.5	64.7	37.2	28.5	6.3	6.8
2	SA	60.3	61.1	33.5	32.5	6.2	6.3
2	A	62.9	67.2	31.5	27.1	5.6	5.7
2	NC	61.0	70.6	29.7	21.6	9.3	7.8
3	5% T-Iso	62.7	74.8	30.9	18.5	6.3	6.7
3	3% T-Iso	54.3	70.1	38.1	25.5	7.6	4.4
4	ST	64.9	73.9	29.4	20.6	5.7	5.4
4	A	63.2	68.5	31.9	26.4	4.9	5.1
4	M	63.4	67.5	30.6	27.2	5.9	5.3
4	NC	68.6	69.8	25.0	24.8	6.4	5.4

starved controls even though the ration of cornstarch had been increased threefold to 900 mg per day. The amount of protein present in the eggs spawned by naturally conditioned females and algae fed scallops was 30% greater than in those from the starved and the S females.

In these two experiments it was shown that a diet of T-Isochrysis sp. equivalent to 3% of their body weight per day, on a dry weight basis, was sufficient to significantly increase the level of protein and lipid in the eggs compared to the starved controls. The diet was also sufficient to produce eggs which had the same levels or slightly higher levels of protein and lipid than the naturally conditioned females. ...

Feeding carbohydrates, which is the main energy reserve in the adults, in the form of cornstarch, to the adults did not produce an increase in the protein, lipid and carbohydrate components of the eggs over that present in the eggs spawned by the ST females. Neither the increased ration (300 mg to 900 mg per day) nor the change in the method of delivering the food to the adults (batch fed vs continuous flow) had any effect on the gross biochemical composition of the eggs produced by S females.

The third experiment was carried out to determine whether scallops spawned in the laboratory the previous year could be fully conditioned in the laboratory and induced to spawn

again. Three of the ten females conditioned spawned. Two of these females had received an increased ration for four weeks prior to spawning. Although the ration was increased from 3% of their body weight on a dry weight basis, to 5% per day four weeks before they were induced to spawn this was not sufficient to cause any differences in the levels of lipid and carbohydrate of the eggs. However, the amount of protein was 26% higher in the eggs spawned by females fed the increased ration (Figure 5). The levels of protein, lipid and carbohydrate in the eggs spawned by the females conditioned during the winter were similar to the levels found in the eggs of starved females in Experiments 1 and 2.

In the last conditioning experiment the mixed algal diet (M) females produced eggs with a similar biochemical composition as those spawned by the females fed a unialgal diet of <u>Isochrysis</u> sp. (Tahitian strain). There were significant differences, at the 0.05 level, among the protein and lipid levels of the starved controls and the A and M females (Figure 6). The algal diets fed to the females resulted in eggs with 10% more protein and 35% more lipid than the eggs spawned by naturally conditioned females, and 30% more protein and 35% more lipid than the starved controls. This showed that the increased ration, of 5% body weight per day, on a dry weight basis, (Table 2), provided the laboratory-held females with enough energy to

increase the reserves in their eggs above the levels present in the naturally conditioned females (Figure 6).

The different conditioning treatments did not produce any clear effect on egg size, weight and number over the four experiments (Table 6). No differences were detected in the egg-dry weights among the different conditioning treatments in any of the experiments. Only in the first experiment was there a difference in the number of eggs spawned by the females of the various treatments. The naturally conditioned females and the females which received an algal supplemented diet released two to three times more eggs than the starch fed females and the starved controls. In Experiment 2 the eggs spawned by naturally conditioned females were significantly larger than the eggs spawned by females from other conditioning treatments. In Experiment 4 the females fed <u>Isochrysis</u> sp. (Tahitian strain) produced significantly larger eggs.

3.1.2   Development of veligers

The initial phase of development for bivalve larvae is lecithotrophic and the larva must use the energy reserves present in the egg to develop into a veliger.

In all the conditioning experiments the eggs spawned by females fed algal-supplemented diets and the eggs from naturally conditioned females contained approximately 30% more energy reserves than the eggs of starved controls and 8

TABLE 6

The size, number and dry weight of eggs spawned by
conditioned females of <u>Placopecten magellanicus</u>,
and the larval recovery rates.

EXPERIMENT (CONDITION)	NUMBER EGGS SPAWNED ($\times 10^6$)		EGG SIZE (μm)		DRY WEIGHT $g/10^6$ eggs		% RECOVERY	
	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
1 (ST)	6.2	5.2	68.24	1.38	0.08	0.01	38.45	4.27
1 (S)	4.3	4.0	67.18	1.73	0.08	0.02	50.82	22.42
1 (SA)	17.0	10.0	67.57	1.15	0.08	0.01	66.33	5.23
1 (A)	21.0	9.4	67.40	0.68	0.08	0.01	74.95	15.80
1 (NC)	14.0	9.2	67.39	1.21	0.07	0.01	62.78	7.32
	<u>ST S SA NC A</u>*						<u>ST S SA A NC</u>*	
2 (ST)	19.0	26.0	68.7	0.91	0.10	0.01	61.47	5.41
2 (S)	13.0	4.6	67.63	0.52	0.09	0.01	57.05	15.65
2 (SA)	7.6	6.8	68.04	0.70	0.10	0.01	76.90	15.86
2 (A)	12.0	6.2	67.48	1.34	0.10	0.01	79.52	19.38
2 (NC)	9.8	4.1	71.40	2.11	0.11	0.00	66.50	28.00
			<u>ST S SA A</u> NC*					
3(3%T-Iso)	0.6		74.24				14.23	
3(5%T-Iso)	4.2		72.63		0.05		41.36	
4 (ST)	7.8	8.5	68.02	1.58	0.07	0.01	8.92	2.67
4 (A)	10.0	6.4	71.50	0.97	0.07	0.01	33.03	13.59
4 (M)	14.0	13.0	68.76	0.62	0.08	0.02	38.48	26.19
4 (NC)	4.8	3.6	69.52	0.50	0.07	0.00	24.63	15.96
			<u>ST M NC</u> A*				<u>ST A NC</u> M *	

* Indicates significant differences at the 0.05 level
with the Tukey-B test after oneway analysis of variance.

females. Since lipid is a more efficient energy source, protein reserves provided more energy for development than lipid reserves only for the larvae obtained from naturally conditioned females in Experiment 4 (Table 8).

In Experiments 1 and 3 50-60% of the reserves required for development of the egg to the straight-hinge stage and approximately 70% of the total energy required were supplied by the lipid fraction (Tables 7 and 8). In Experiments 2 and 4, however, more protein reserves were used during early development although 50-60% of the total energy requirements were met by the lipid fraction.

The total amount of energy used during development of the egg to the newly formed D-shaped larvae increased significantly as the total energy reserves in the eggs increased (Figure 7). Almost 60% of the total reserves in the eggs were used to cover the cost of development regardless of the amount of reserves present. The amount of energy supplied by the lipid fraction was similar in all instances. However the amount of energy supplied by the protein fraction increased as the total amount of protein deposited in the eggs increased (Table 7). Therefore the greater the amount of protein reserves in the eggs the greater the amount of energy used during development of the egg to the veliger larva.

TABLE .7

Amounts of protein, lipid and carbohydrate and percent contribution to the early development of _Placopecten magellanicus_ larvae (development of eggs to newly formed veliger larvae).

EXPT.	CONDITION FEMALES	PROTEIN mg/10^6	%	LIPID mg/10^6	%	CARBOHYDRATE mg/10^6	%
1	ST	3.68	35.8	5.37	52.2	1.22	11.9
1	S	3.81	42.5	4.82	53.8	0.33	3.7
1	SA	6.93	45.5	7.98	50.4	0.62	4.1
1	A	5.49	34.8	9.70	61.5	0.58	3.7
1	NC	4.95	40.7	6.92	56.9	0.28	2.3
2	ST	5.68	52.5	4.36	40.2	0.79	7.3
2	S	4.35	39.9	5.99	55.0	0.54	4.9
2	SA	11.18	59.1	6.64	35.1	1.10	5.8
2	A	10.55	57.1	6.98	37.8	0.97	5.2
2	NC	9.58	47.0	8.33	40.9	2.47	12.1
3	5% T-Iso	4.77	40.8	6.35	54.3	0.58	4.9
3	3% T-Iso	4.91	36.2	7.11	52.4	1.53	11.3
4	ST	4.82	45.6	5.09	48.1	0.66	6.2
4	A	13.97	58.3	9.04	37.7	1.14	4.7
4	M	14.18	59.0	8.18	34.0	1.66	6.9
4	NC	13.28	67.0	5.10	25.7	1.43	7.2

TABLE 8

Protein, lipid and carbohydrate in energetic equivalents and the percent contribution of each component to the early development of the eggs of _Placopecten magellanicus_.

EXPT.	CONDITION FEMALES	Mean loss of energy per million eggs					
		PROTEIN		LIPID		CARBOHYDRATE	
		joules	%	joules	%	joules	%
1	ST	86.8	27.1	212.4	66.4	20.9	6.5
1	S	89.6	31.3	190.5	66.6	5.7	2.0
1	SA	163.2	34.2	303.6	63.6	10.7	2.2
1	A	129.2	24.7	383.6	73.4	10.0	1.9
1	NC	116.4	29.5	273.6	69.3	4.9	1.2
2	ST	133.6	41.8	172.3	53.9	13.5	4.2
2	S	102.4	29.4	237.0	68.0	9.4	2.7
2	SA	263.1	48.3	262.4	48.2	8.9	3.5
2	A	248.2	45.9	275.8	51.0	16.6	3.1
2	NC	225.5	37.7	329.6	55.2	42.4	7.1
3	5% T-Iso	112.1	30.3	251.4	67.3	9.9	2.7
3	3% T-Iso	115.7	27.3	281.3	66.5	26.3	6.2
4	ST	113.4	34.8	201.4	61.7	11.4	3.4
4	A	329.0	47.1	350.1	50.1	19.6	2.8
4	M	333.7	40.0	471.7	56.6	28.6	3.4
4	NC	312.5	57.0	201.8	37.4	24.5	4.5

FIGURE 7: The relationship of the total energy content of the eggs to the amount of energy lost during development of the eggs to veliger larvae.

- Experiment 1
▲ Experiment 2
■ Experiment 3
○ Experiment 4

ENERGY LOSS (JOULES)

700

50.0

300

100

800   900   1000   1100   1200   1300
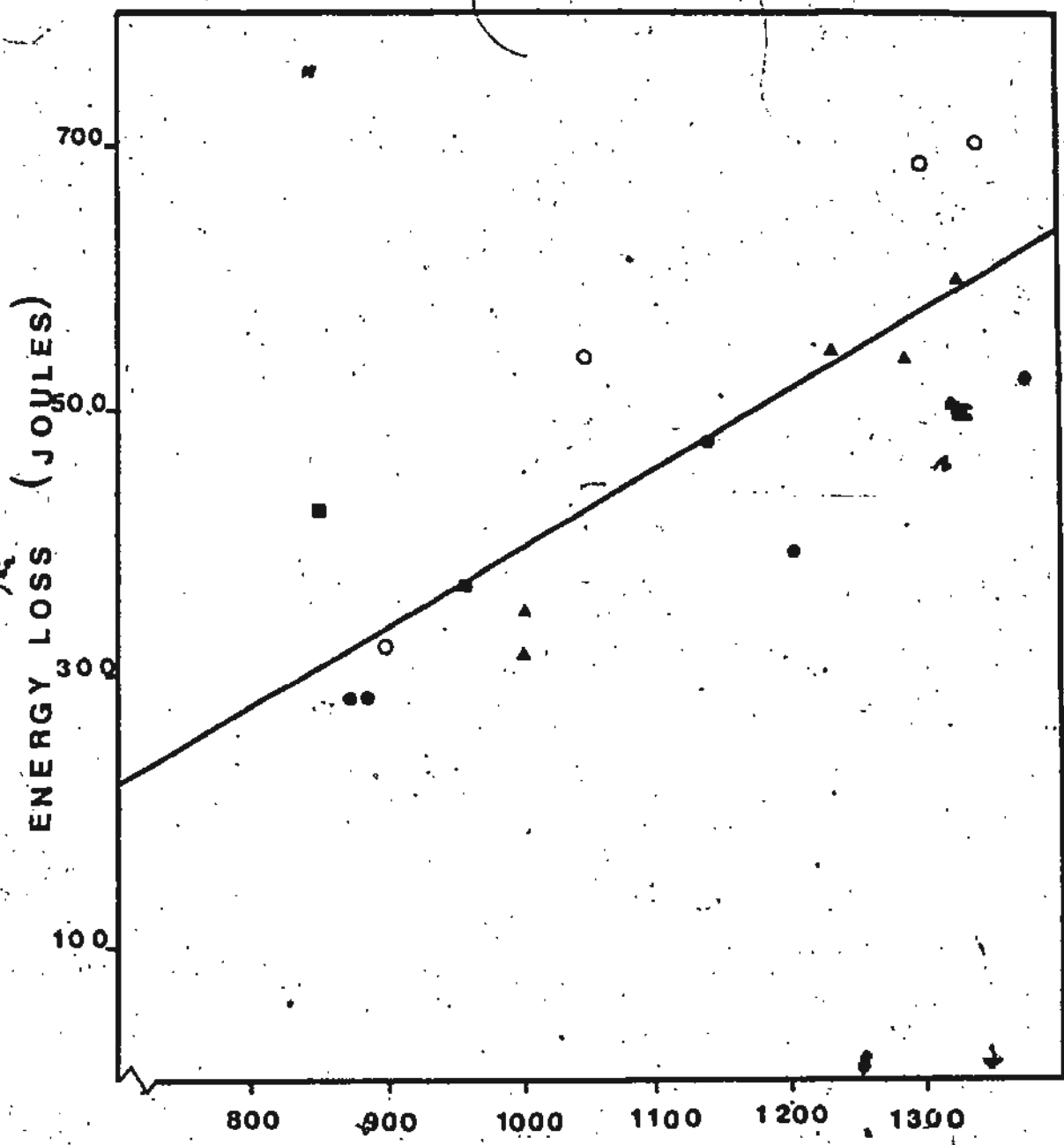
TOTAL ENERGY IN EGGS

JOULES PER MILLION EGGS

It is possible to estimate the conversion efficiency of each biochemical component from the eggs to the larvae using the ratio:

$$\frac{\omega \ \text{larvae}}{\omega \ \text{eggs}}$$

where ω is the weight (or energy content) of a given biochemical constituent (Holland, 1978). The conversion efficiencies give an estimate of the relative amount of each constituent used for structural purposes and for metabolism. The higher conversion efficiencies for protein indicate that protein is conserved (Table 9). The comparatively lower efficiencies for lipid indicate its importance as an energy source in covering metabolic costs.

The success of fertilization and early development of the larvae in the various experiments ranged from 5-95% (Appendix I: Table A-6, Table A-8, Table A-9, Table A-11; Table 6). The percent of straight-hinge larvae recovered from fertilized eggs was not different among any of the conditions in Experiment 2, and in all the treatments the recovery rates were high. In the first conditioning experiment the recovery rate of the larvae from SA, A and naturally conditioned females was significantly higher than the recovery rate of larvae from starved controls. The recovery rate of larvae from A females was also

TABLE 9

Conversion efficiencies of protein, lipid and carbohydrate
reserves during development from the egg to the veliger.

EXPERIMENT	CONDITION FEMALES	CONVERSION EFFICIENCIES (%)		
		PROTEIN	LIPID	CARBOHYDRATE
1	ST	78	50	64
1	S	80	52	84
1	SA	69	47	74
1	A	78	47	
1	NC	80	52	83
2	ST	72	65	65
2	S	77	52	76
2	SA	60	54	61
2	A	63	48	62
2	NC	67	42	44
3	5%T-Iso	81	40	68
3	3%T-Iso	68	36	34
4	ST	76	47	67
4	A	53	41	54
4	M	52	42	43
4	NC	50	47	42

significantly higher than the recovery rate of larvae from S females. In Experiment 4, the recovery rates of larvae from females of both phytoplankton fed groups and of the naturally conditioned females were three to five times higher than the larval recovery rates of starved females. However, only the females fed a mixture of algae showed a significantly greater larval recovery rate. The larval recovery rates in the last experiment were lower than in the first two experiments. Very few larvae (8.9%) were recovered from fertilization of the eggs of starved females. In the third experiment the recovery rate of larvae from the eggs of females fed an increased ration (5% of their body weight per day) was significantly higher than the recovery of larvae from the eggs of females fed only 3% of their body weight per day.

There were other factors apart from the condition of the females which affected the recovery rate of larvae. The various conditioning treatments, however, did have a significant affect on the success of early development.

3.1.3 Larval growth

The diet fed to the larvae was very important in determining the success of larval growth. Starved larvae of Females 53, 54 and 55 survived four to five weeks in filtered seawater. During this period of starvation very little growth occurred and a steady decline in the total energy reserves was noted (Table 10).

TABLE 10

Change in the protein, lipid and carbohydrate levels and the total energy content of the larvae of _Placopecten_ _magellanicus_ over a period of four weeks.

EXPT	FEMALE (CONDITION)	GROWTH RATE	PROTEIN	LIPID	CARB.	ENERGY GAIN
		μm/day	\multicolumn{3}{c}{mg/day/10^6 larvae}	joules/day 10^6 larvae
1	1 (ST)	0.22	-0.18	-0.01	-0.04	-5.32
1	2 (A)	0.39	-0.38	-0.09	-0.10	-14.22
1	3 (SA)	0.40	-0.42	-0.14	-0.03	-15.94
1	4 (S)	0.38	-0.24	-0.13	-0.13	-11.30
1	5 (A)	0.38	-0.47	-0.26	-0.08	-22.72
1	6 (ST)	0.57	-0.24	-0.11	-0.08	-11.37
1	7 (ST)	0.54	-0.28	-0.08	-0.02	-10.10
1	8 (SA)	0.17	-0.40	-0.17	-0.01	-16.31
1	9 (S)	0.53	-0.32	-0.18	-0.05	-15.51
1	10 (A)	0.26	-0.35	-0.26	-0.04	-19.21
1	13 (NC)	0.39	-0.60	-0.24	-0.04	-24.30
1	22 (S)	0.21	-0.52	-0.25	-0.04	-22.81
1	23 (SA)	0.13	-0.40	-0.17	-0.03	-16.65
1	24 (NC)	0.24	-0.50	-0.15	-0.05	-18.22
1	26 (NC)	0.21	-0.30	-0.18	-0.04	-14.87
2	31 (ST)	0.36	0.10	-0.19	-0.01	-5.33
2	32 (S)	0.35	-0.34	-0.02	-0.02	-9.14
2	33 (A)	0.26	-0.47	-0.15	-0.03	-17.15
2	35 (S)	0.45	-0.42	-0.14	-0.01	-15.59
2	36 (ST)	0.32	-0.30	-0.13	-0.04	-12.89
2	37 (A)	0.22	-0.57	-0.04	-0.01	-15.17
2	40 (SA)	0.10	-0.43	-0.16	-0.04	-17.14
2	43 (ST)	0.20	-0.59	-0.33	-0.03	-27.45
2	44 (A)	0.62	-0.71	-0.19	-0.03	-24.74
2	45 (SA)	0.53	-0.49	-0.13	-0.07	-17.88
2	46 (S)	0.76	-0.58	-0.20	-0.10	-21.73
2	47 (SA)	0.28	-0.51	-0.10	-0.01	-16.13
	53* (ST)	0.11	-0.18	-0.09	-0.002	-7.83
	53* (Iso)	1.11	1.01	-0.09		20.22
	54* (ST)	0.11	-0.23	-0.19	-0.03	-13.49
	54* (Iso)	0.21	-0.20	-0.16	-0.03	-11.55
	55* (ST)	0.11	-0.31	-0.10	-0.05	-12.11
	55* (Iso)	0.55	-0.12	-0.00	-0.03	-3.42

*Diet in parenthese refers to larvae, ST:Starved larvae, Iso: Larvae fed _Isochrysis_ _galbana_.

TABLE 10: CONTINUED

EXPT	FEMALE (CONDITION)	GROWTH RATE µm/day	PROTEIN	LIPID mg/day/10^6 larvae	CARB	ENERGY GAIN joules/day 10^6 larvae
3	7 (5% A)	0.26	0.36	0.05	0.03	10.97
3	8 (5% A)	0.55	0.35	0.01	0.06	9.96
3	9 (3% A)	0.63	0.31	0.06	0.05	10.53
4	15 (A)	3.16	3.13	0.73	0.42	109.76
4	16 (A)	4.10	10.13	2.56	0.78	353.08
4	17 (S)	3.00	0.42	0.22	0.18	21.68
4	19 (NC)	3.21	4.32	0.35	0.11	117.42
4	20 (NC)	2.96	4.13	0.94	0.41	141.43
4	21 (NC)	3.19	4.04	0.74	0.26	128.83
4	31 (M)	2.85	3.97	0.92	0.74	142.54
4	32 (M)	2.88	6.11	1.46	0.42	208.77

In Experiments 1 and 2 the larvae were fed 25 cells/µl of Isochrysis sp. (Tahitian strain) three times a week. Growth on this diet ranged from 0.1 µm per day to 0.7 µm per day and was not significantly different from the growth rates of starved larvae from Females 53, 54, 55 (Table 10). A steady decrease in all energy reserves was also seen in these larvae (Figure 8). Although the larvae were ingesting the algae fed to them in the first week or two of their pelagic life it was doubtful that they were capable of assimilating the ration. After two weeks the larvae ceased to feed altogether. The larvae remained on or near the bottom of the containers instead of actively swimming in the water column after the first week of pelagic life. The larvae did not live any longer than the starved larvae. On average the larvae lost 0.41 ng of protein, 0.16 ng of lipid and 0.04 ng of carbohydrate per day per larva (Table 10). During the starvation period protein reserves were the main source of energy for metabolism providing 50 to 65% of the total energy required.

The growth rates of larvae from unconditioned females (53, 54 and 55; Table 3) which were fed Isochrysis galbana (Parke), varied. Larvae from Female 53 had an average growth rate of 1.11 µm/day but did not grow past 130 µm in height. Although there was a loss of 0.09 ng of lipid per day per larva the increase in protein content of 1.01 ng per

FIGURE 8: Protein, lipid and carbohydrate content of the larvae of _Placopecten magellanicus_ from females in Conditioning Experiments 1 & 2.
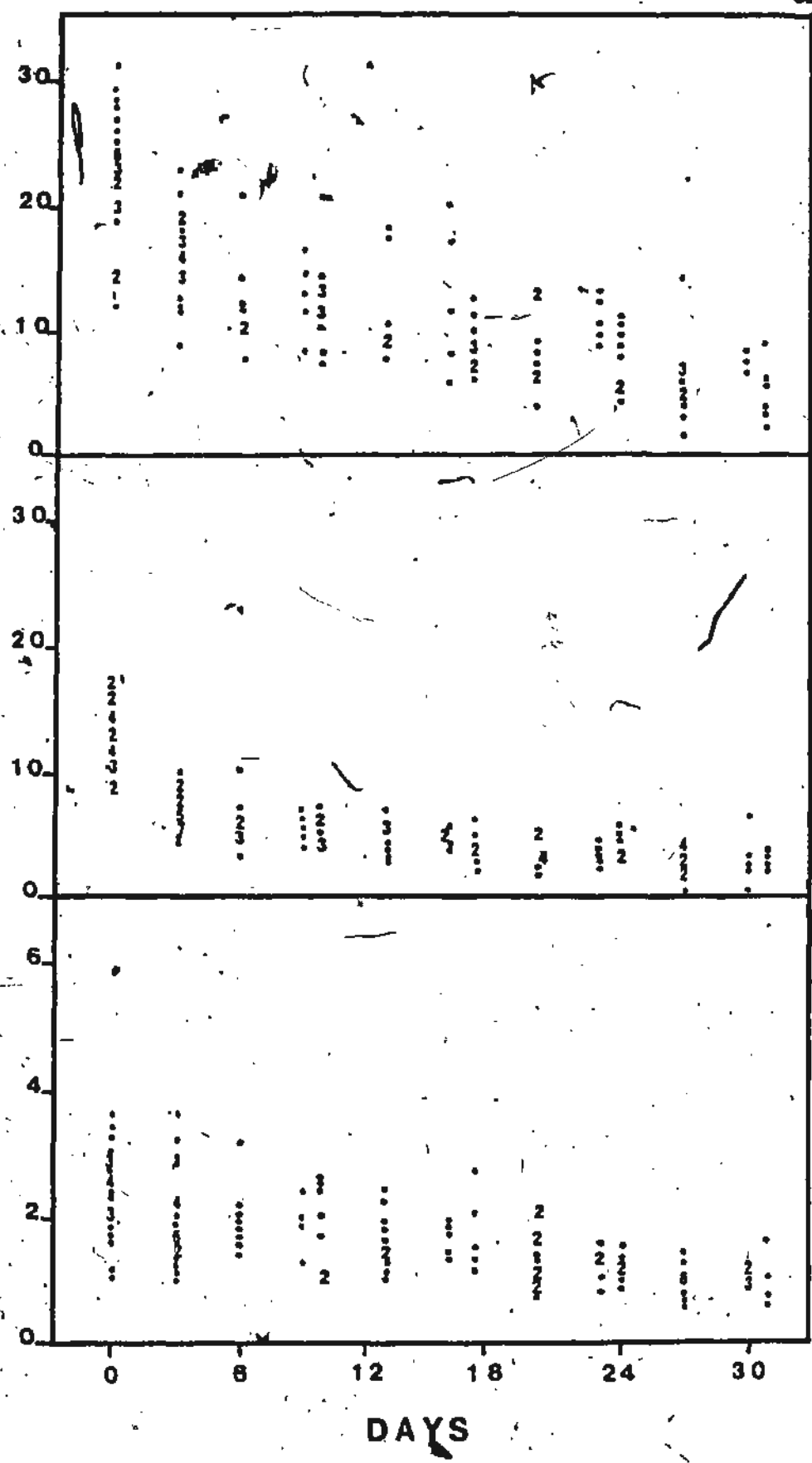
day per larva indicated a definite increase in body structure.

Unfortunately the larvae of the other unconditioned females, although they were also fed 25 cells per µl of I. galbana three times a week, did not grow and followed the same pattern of loss of energy reserves as larvae fed T-Isochrysis.

In 1984 the larvae from Experiment 3 were again fed on a diet of 25 cells per µl of Isochrysis galbana three times a week. Although growth rates were negligible the larvae did accumulate some energy reserves (Table 10).

In the last conditioning experiment the larvae were fed Isochrysis galbana, Thalassiosira pseudonana and Chaetoceros calcitrans three times per week. This diet was very successful in promoting growth. An average growth rate of 3-4 µm per day was maintained by the larvae over the first four weeks of pelagic life (Figure 9). This growth rate is sufficient to allow the larvae to reach, in 40 days, the size (250 µm in height) at which they settle in nature.

The increase in protein ranged from 0.42 ng per day per larva for larvae obtained from a starved female to 10.13 ng per day per larva for larvae obtained from females which had been fed T-Isochrysis sp. (Table 10). The lipid content of the larvae increased as well with 2.56 ng per day per larva being the greatest increase (Table 10).

FIGURE 9: Growth and the content of protein, lipid and carbohydrate of the larvae of Placopecten magellanicus from females in Conditioning Experiment 4. a. Female 15; b. Female 16; c. Female 17; d. Female 19; e. Female 20; f. Female 21; g. Female 31; h. Female 32.

- HEIGHT

- PROTEIN

▲ LIPID

▫ CARBOHYDRATE

HEIGHT (μm)

mg per million larvae
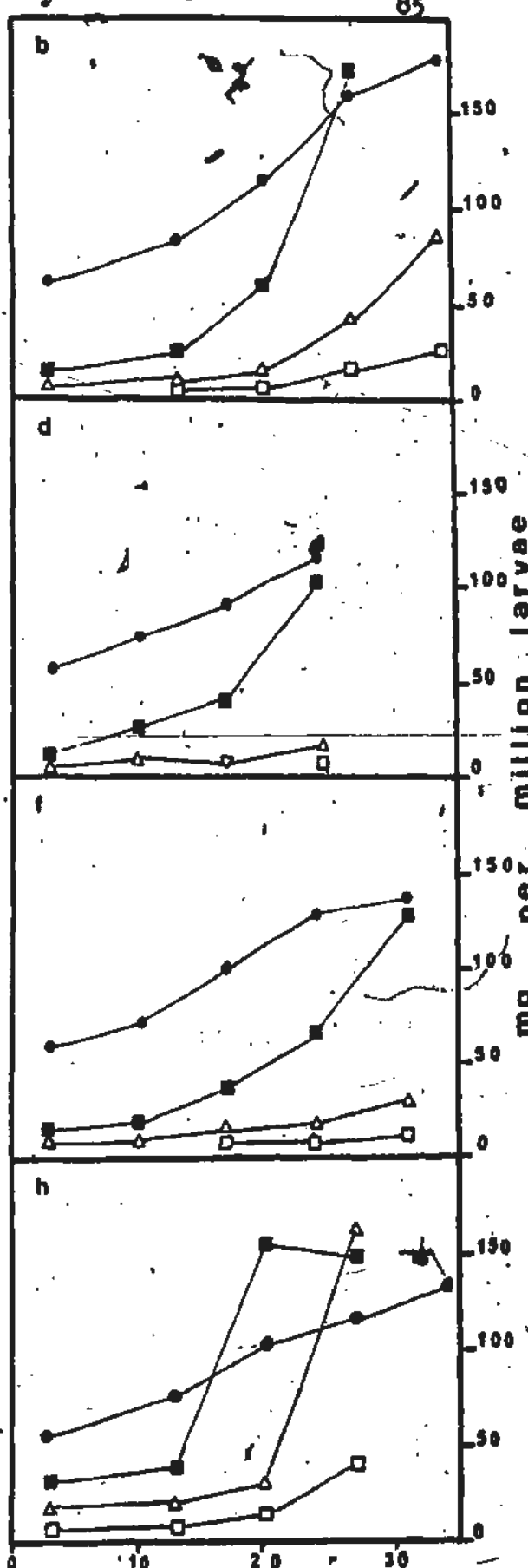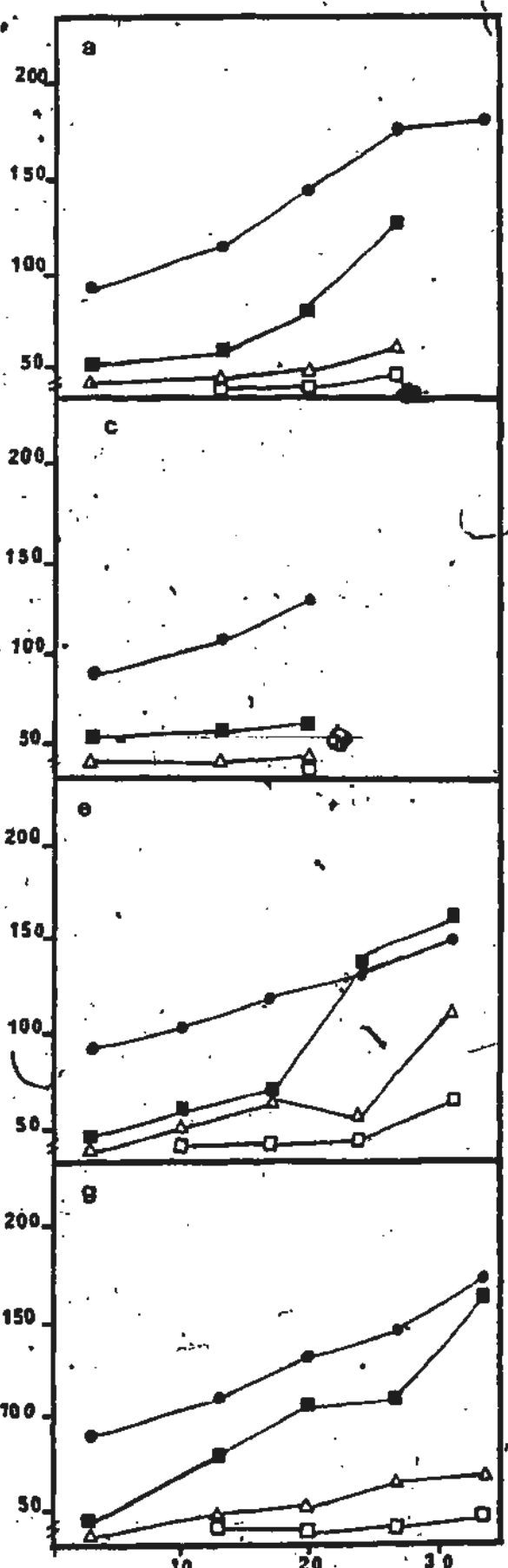
DAYS

The increase in protein, lipid and carbohydrate of the larvae from A, M and naturally conditioned-females was greater than in the larvae of ST females (Figure 9).

At the beginning of larval growth protein levels increased very rapidly. The levels of lipid reserves started to increase dramatically in the third or fourth week of pelagic life. This is consistent with the thought that lipid is essential for successful metamorphosis.

In the fourth conditioning experiment the differences in growth rates and the rates of accumulation of protein, lipid and carbohydrate were related to the energy present in the eggs. When the total energy in the egg was maximized and an adequate phytoplankton diet was supplied, survival, growth and the accumulation of energy reserves were enhanced.

Therefore maximizing the energy reserves in the eggs will not only increase the number of straight-hinge larvae produced but will also improve the chance of successful metamorphosis by increasing the rate of accumulation of energy reserves by planktonic larvae.

3.2 ENERGY REQUIREMENTS OF LARVAE

The algal species used in larval or juvenile diets varied significantly in their biochemical composition (Table 11). The most notable difference was the high carbohydrate content of Chaetoceros calcitrans. Isochrysis galbana

TABLE 11

Biochemical composition of phytoplankters used as diets for larval and adult <u>Placopecten</u> <u>magellanicus</u>.

ALGAL SPECIES (n)	DRY WEIGHT $\mu g.10^6$ cells		PROTEIN		LIPID ug per million cells (% of total organic matter)		CARBOHYDRATE	
	MEAN	S.D	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Iso(10)	24.2	2.9	10.79 (62.2)	1.94	5.32 (30.7)	1.37	1.23 (7.1)	0.43
			TE 0.485 joules per million cells					
T-Iso(6)	30.8	5.9	13.55 (46.6)	2.41	8.61 (29.6)	1.71	6.91 (23.7)	1.39
			TE 0.778 joules per million cells					
Tetra(4)	161.3	25.1	33.13 (60.2)	4.24	10.63 (19.3)	3.21	11.29 (20.5)	3.27
			TE 1.394 joules per million cells					
3H(6)			11.52 (35.6)	2.30	8.13 (25.1)	0.93	12.74 (39.4)	1.44
			TE 0.811 joules per million cells					
Chaet(6)			39.4 (36.8)	19.60	15.69 (14.7)	5.35	52.02 (48.6)	11.63
			TE 2.442 joules per million cells					

Iso   <u>Isochrysis</u> <u>galbana</u>
T-Iso <u>Isochrysis</u> sp. (Tahitian strain)
Tetra <u>Tetraselmis</u> <u>suecica</u>
3H   <u>Thalassiosira</u> <u>pseudonana</u>
Chaet <u>Chaetoceros</u> <u>calcitrans</u>

n   Number of samples
TE Total energy content

(Parke) and Isochrysis sp. (Tahitian strain) had relatively higher protein and lipid levels than the other algal species but had very low carbohydrate levels. Tetraselmis suecica also had high protein levels but lipid and carbohydrate levels were moderate. Chaetoceros calcitrans and Thalassiosira pseudonana both had high carbohydrate levels and moderate protein levels. C. calcitrans was especially low in lipid content.

The energetic requirements of the larvae can be divided into three categories: metabolism, growth and swimming. Metabolic costs are covered by energy reserves in the larvae when they are not feeding. A measure of the decrease in energy reserves during starvation therefore gives an estimate of metabolism.

The decrease in the energy content of scallop larvae of Placopecten magellanicus from Conditioning Experiments 1 and 2 and starved larvae from unconditioned Females 53, 54, and 55 was used to estimate the metabolic costs to the larvae. The following evidence suggests that the larvae obtained from conditioning experiments 1 and 2 were essentially starving:

1. shell growth was negligible

2. the decrease in protein, lipid and carbohydrate levels during pelagic life was equal to or greater than that of starved larvae obtained from Females 53, 54 and 55 (Table 10)

3.   the larvae ceased to feed altogether after one or two weeks of pelagic life

4.   larval activity decreased dramatically during the second week of pelagic life.

These larvae lost an average of 36.8 joules per day per million larvae during the first three days of pelagic life (Table 12a). During the following three weeks the metabolic costs decreased to only 15 joules per day per million larvae. In the 'fourth week the energy requirements decreased even further to 9.3 joules per day per million larvae.

The energy content of the tissue incorporated into growth can also be determined by analysis of protein, lipid and carbohydrate. Table 12b indicates the energy gains of Placopecten magellanicus larvae over the first four weeks of pelagic life. Accumulation of energy reserves increased exponentially with time.

Zeuthen (1947) estimated the swimming costs of mussel larvae (Mytilus edulis) at twice the metabolic costs. Using this estimate the total energy requirements for 3-13 day old scallop larvae, P. magellanicus, would be 166.2 joules per day per million larvae (Table 12c).

The net growth efficiency (K2) oan be estimated as:

$$K2 = \frac{\text{energy in growth}}{\text{total energy requirements}} \times 100,$$

TABLE 12

Energy required for growth and metabolism the larvae of
Placopecten magellanicus.

a. Energy loss by larvae of P. magellanicus from
Conditioning Experiments 1 and 2.

Age (days)	Sample size	Energy Loss (joules/day/10^6 larvae)
3-6	16	36.8
6-16	16	15.9
13-20	16	15.8
20-27	13	16.8
27-31	12	9.3

b. Energy gain by the larvae of Placopecten magellanicus
from Conditioning Experiment 4.

Age (days)	Sample size	Energy Gain (joules/day/10^6 larvae)
3-13	8	55.8
13-20	6	190.4
20-27	4	304.1
27-31	4	2549.3

c. Total energy requirements for 3-13 day old larvae of
Placopecten magellanicus.

	(joules/day/10^6 larvae)
Resting metabolism	36.8
Swimming costs	73.6
Growth	55.8
Total	166.2

which would be 33.6% for 3-13 day old larvae of <u>Placopecten</u> <u>magellanicus</u>.

3.3   ENERGY REQUIREMENTS OF JUVENILES

The biochemical content of juveniles was determined by analysis of protein, lipid and carbohydrate. The juveniles were obtained from Little Bay, Newfoundland, in July, 1984. Protein levels in the eggs, three day old veligers, twenty-eight day old pediveligers, 9 month old juveniles and 23 month old scallops were all very high, accounting for 60-70% of the total organic matter (Figure 10). In the twenty-three month old juveniles carbohydrate accounted for almost 25% of the total organic material and lipid reserves accounted for only 6%. The reverse was true in all younger scallops. Lipid is the more important energy reserve in scallop larvae whereas carbohydrate is more important in the adults.

The organic content of the shell was calculated to be 10.61 +/- 3.29% of the total organic material (dry tissue weight plus organic content of the shell) for juvenile scallops <u>Placopecten</u> <u>magellanicus</u>.

Regression of weight (W:mg) against total energy (TE:joules) gave the relationship indicated in Equation 1.

$$TE \text{ (joules)} = 14.30 \text{ } (W)^{0.979} \text{ } (mg) \quad (1)$$
$$R^2 = 0.825$$

FIGURE 10: Protein, lipid and carbohydrate levels in the eggs, larvae and juveniles of _Placopecten magellanicus_, expressed as a percent of the total energy reserves.

% TOTAL ORGANIC MATTER

100 —

75 —

50 —

25 —

N=77   N=77   N=8   N=10   N=59

P L C   P L C   P L C   P L C   P L C

EGGS   3 days   28 days   9 month   23 month
        VELIGERS   JUVENILES
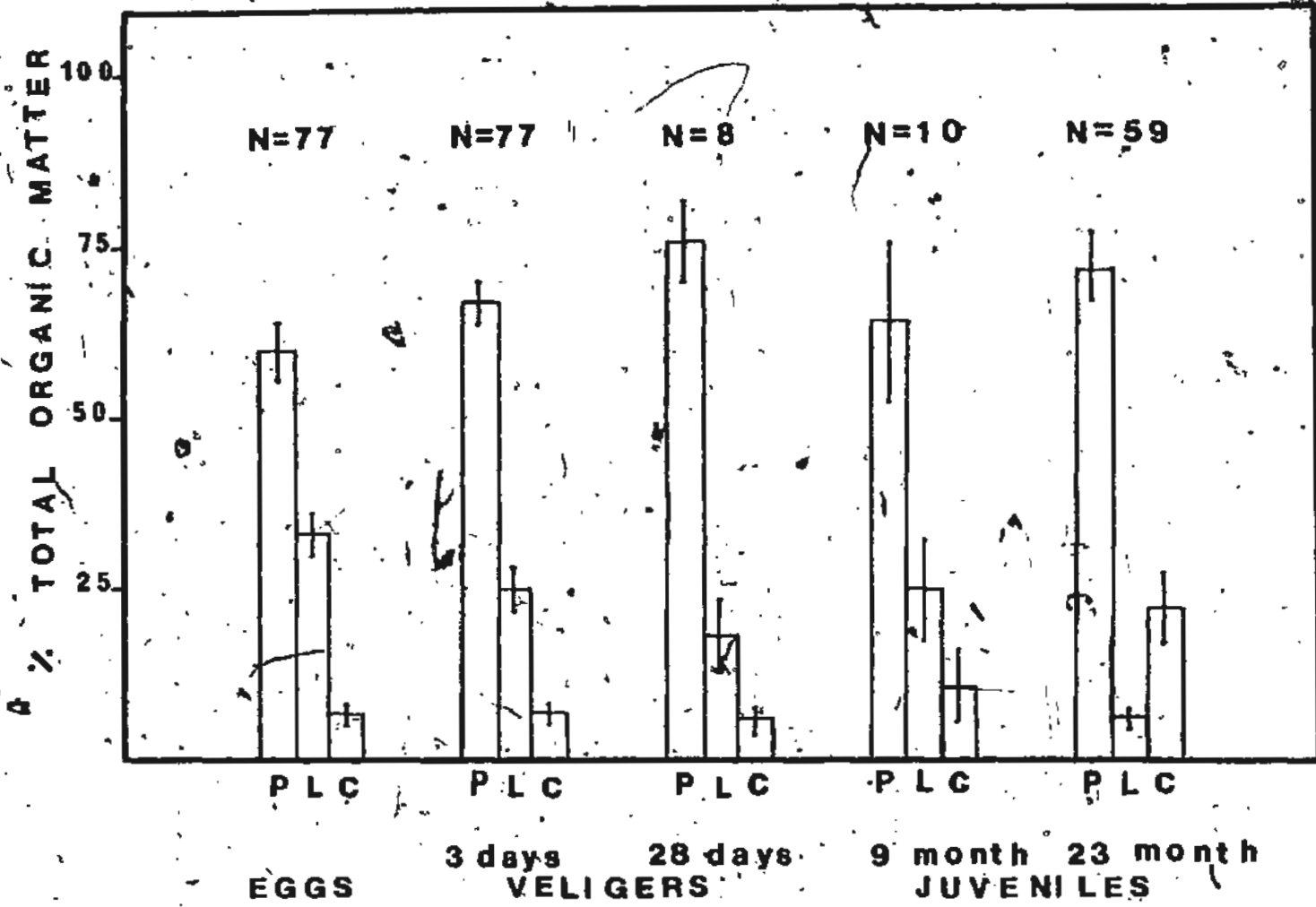
The filtration rate of juvenile <u>Placopecten</u> <u>magellanicus</u> was significantly affected by the size of the individuals and by the algal concentrations. The filtration rates of juveniles increased with the tissue dry weight (Figure 11). Filtration rates increased more rapidly at the lower algal concentrations as indicated by the slopes of the regression lines. The same trend is present in the ingestion rate data (Figure 12). Within the range of concentrations studied the ingestion rate increases rapidly with size.

The group of the smallest scallops studied, 5-8 mg tissue dry weight, increased their filtering rate rapidly as the concentration of <u>Isochrysis</u> <u>galbana</u> increased (Figure 13). The largest individuals decreased their filtering rates as concentrations increased from 5×10^3 cells/ml to 25×10^3 cells/ml. In scallops weighing 20-40 mg tissue dry weight there was no relationship between filtration rates and algal cell concentration.

Ingestion rates increased significantly with increasing cell concentration (Figure 14) at all scallop sizes studied. The ingestion rate increased more rapidly for smaller scallops than for larger ones.

The daily ingested ration (Table 13) increased from 0.27% tissue dry weight per day at 5×10^3 cells <u>Isochrysis</u> <u>galbana</u> per ml to 5.68% tissue dry weight per day at 2×10^4 cells.ml^{-1} for juvenile scallops weighing 5-8 mg tissue dry

FIGURE 11: Regression equations demonstrating the relationship of tissue dry weight to the filtration rate of juveniles of Placopecten magellanicus at various cell concentrations of Isochrysis galbana (see Table 13).

(———) 5×10^3 cells/ml
$F = 0.505\ W^{1.245}$, $R^2 = 0.854$
(- - -) 10×10^3 cells/ml
$F = 2.513\ W^{0.972}$, $R^2 = 0.882$
(—··—) 15×10^3 cells/ml
$F = 8.182\ W^{0.730}$, $R^2 = 0.646$
(———··) 20×10^3 cells/ml
$F = 4.383\ W^{0.831}$, $R^2 = 0.817$
(-···-) 25×10^3 cells/ml
$F = 3.435\ W^{0.855}$, $R^2 = 0.570$

FIGURE 12: Regression equations demonstrating the relationship of tissue dry weight to the ingestion rate of juveniles of Placopecten magellanicus at various cell concentrations of Isochrysis galbana (see Table 13).

(———) 5×10^3 cells/ml
$IR = 3.75 \times 10^3\ W^{1.190}$, $R^2 = 0.840$
(- - -) 10×10^3 cells/ml
$R = 2.973 \times 10^4\ W^{0.943}$, $R^2 = 0.859$
(—··—) 15×10^3 cells/ml
$IR = 1.206 \times 10^5\ W^{0.728}$, $R^2 = 0.634$
(———··) 20×10^3 cells/ml
$IR = 8.08 \times 10^4\ W^{0.854}$, $R^2 = 0.790$
(-···-) 25×10^3 cells/ml
$IR = 9.87 \times 10^4\ W^{0.837}$, $R^2 = 0.600$
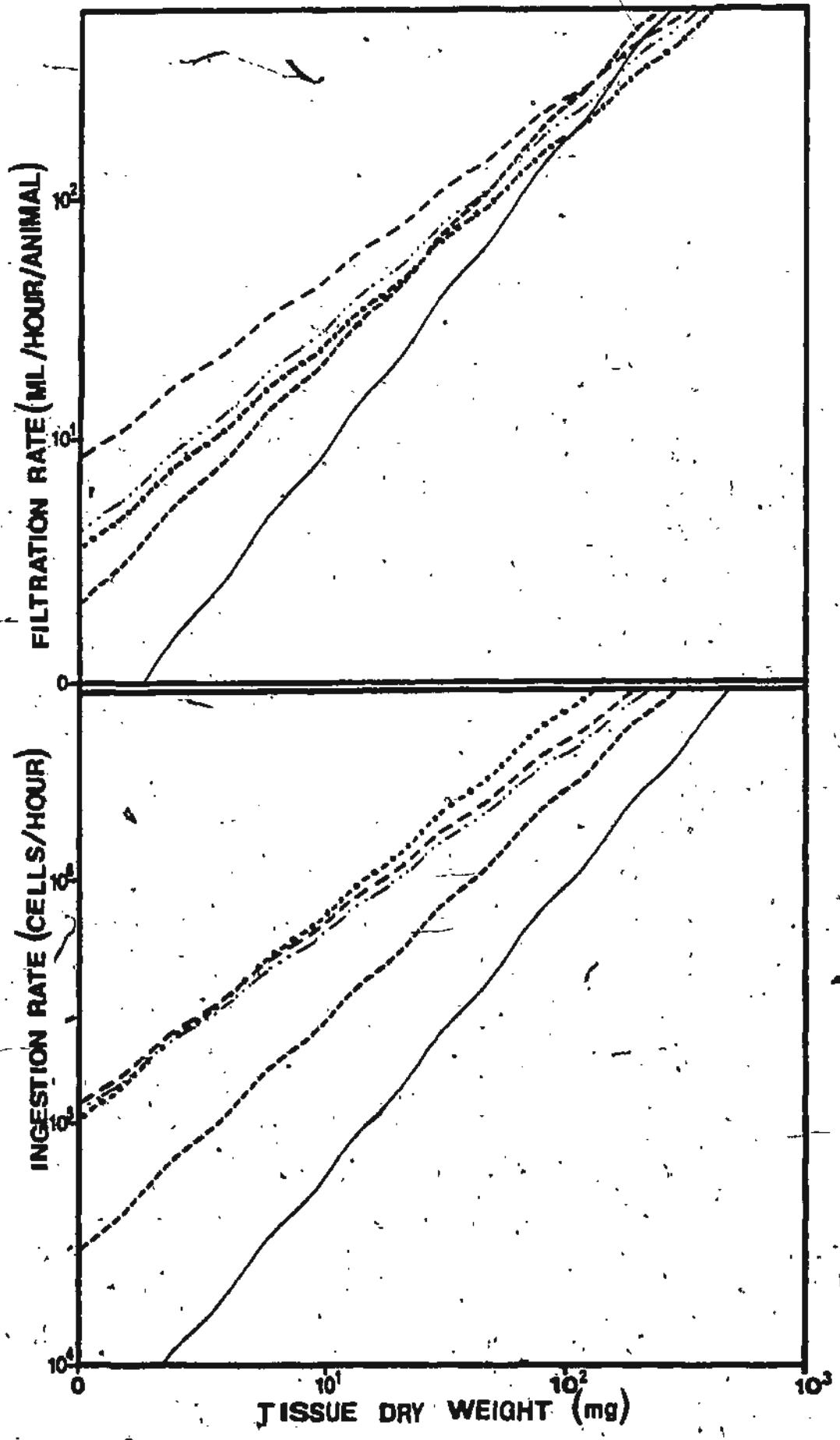
FIGURE 13: Regression equations demonstrating the relationship of the cell concentration of Isochrysis galbana to the filtration rate of juveniles of Placopecten magellanicus of various sizes (see Table 13).

(----) 5-8 mg tissue dry weight
$F = 2.2 \times 10^{-6} \ (\text{Conc})^{1.66}, \quad R^2 = 0.561$
(---) 20-40 mg tissue dry weight
$F = 33.82 \ (\text{Conc})^{0.889}, \quad R^2 = 0.025$
(——) 200-600 mg tissue dry weight
$F = 9.78 \times 10^{5} \ (\text{Conc})^{-0.856}, \quad R^2 = 0.404$

FIGURE 14: Regression equations demonstrating the relationship of the cell concentration of Isochrysis galbana to the ingestion rate of juveniles of Placopecten magellanicus of various sizes (see Table 13).

(----) 5-8 mg tissue dry weight
$F = 2.3 \times 10^{-6} \ (\text{Conc})^{2.66}, \quad R^2 = 0.767$
(---) 20-40 mg tissue dry weight
$F = 33.78 \ (\text{Conc})^{1.09}, \quad R^2 = 0.272$
(——) 200-600 mg tissue dry weight
$F = 2.21 \times 10^{3} \ (\text{Conc})^{0.818}, \quad R^2 = 0.492$
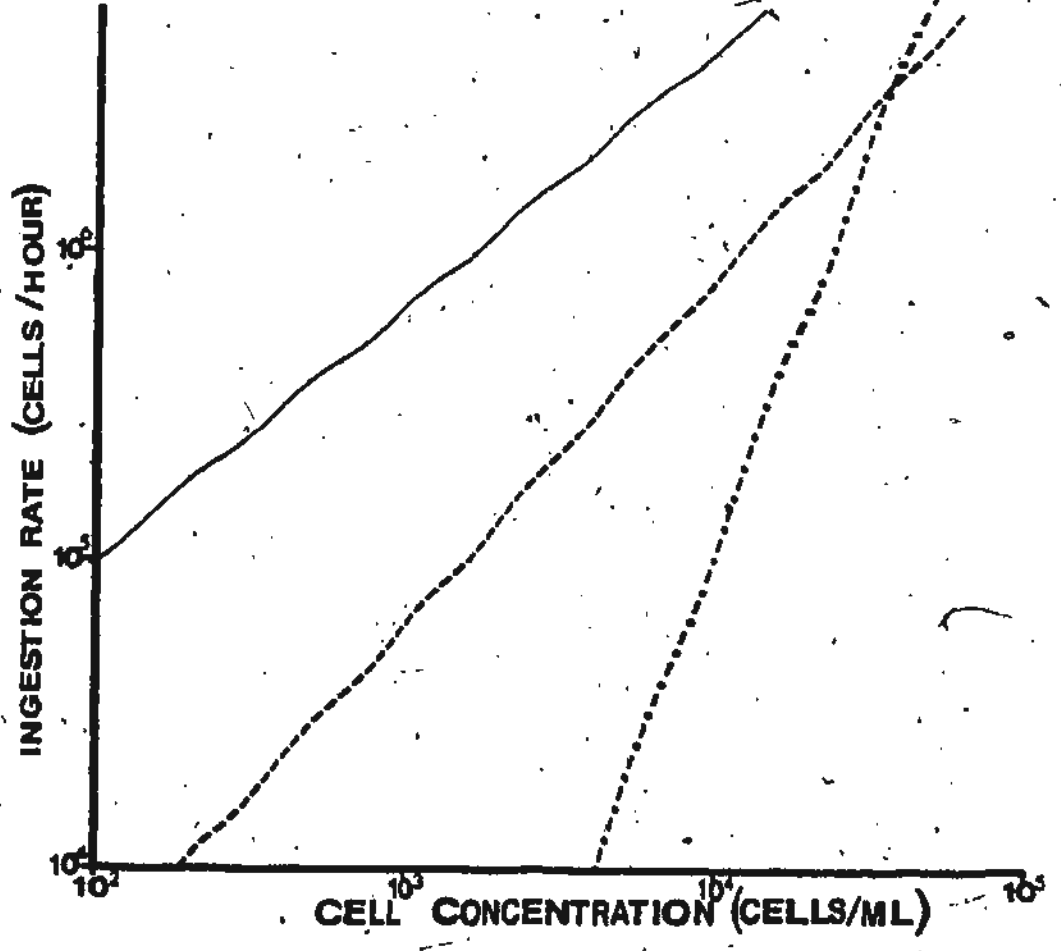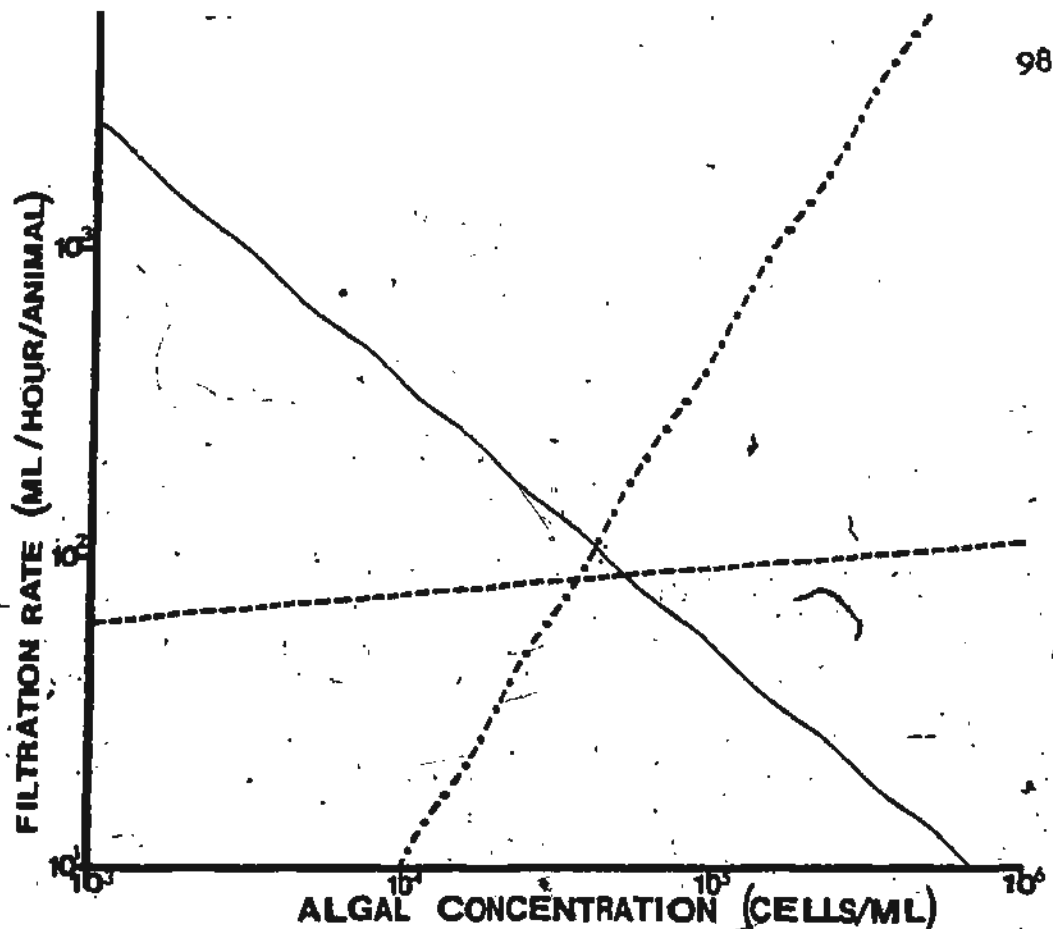
weight. Pseudofaeces production was observed at this cell concentration when the scallops were filtering at the maximum rate observed. The other groups ingested a maximum ration of 3-5% of their body weight per day, on a dry weight basis, at 2×10^4 cells.ml^{-1} of Isochrysis galbana. Pseudofaeces production was noted in juveniles scallops of 20-40 mg tissue dry weight at a cell concentration of 2.5×10^4 cells.ml^{-1}. Pseudofaeces production was not observed in the scallops weighing over 200mg at the cell concentrations tested.

The ingestion rate (IR) for the scallops studied can best be determined from the following regression equation:

$$IR = 0.066 \ (conc)^{1.389} \quad (weight)^{0.932} \quad (2)$$
$$R^2 = 0.797$$

where the algal concentration is in cells/ml and the weight is measured as mg of tissue dry weight and ingestion rate is measured in cells.h^{-1}.

Using this equation it is possible to determine the ingested ration for Placopecten magellanicus juveniles at a given concentration of algal culture.

Juveniles held in the laboratory all year had a mean tissue dry weight of 28 mg (Table 13). Using Equation 2 it is possible to estimate the ingested ration necessary for a 5 mg scallop to grow to 28 mg tissue dry weight in one year at a cell concentration of 2×10^4 cells/ml.

TABLE 13

Mean filtration rates and daily ingested ration at various concentrations of the alga _Isochrysis galbana_ for juveniles of _Placopecten magellanicus_.

TISSUE DRY WEIGHT (mg)		ALGAL CONCENTRATION (cells/ml)	n	FILTRATION RATE ml/h/animal		DAILY INGESTED RATION %tissue dry wt	
MEAN	S.D.			MEAN	S.D.	MEAN	S.D.
6.58	1.13	5×10^3	40	3.9	2.5	0.27	0.21
5.93	1.42	10×10^3	32	17.2	5.9	2.20	1.05
6.75	1.09	15×10^3	30	30.4	18.4	4.98	3.10
6.58	0.85	20×10^3	20	25.8	16.1	5.68	3.52
28.30	9.32	5×10^3	32	111.3	88.2	1.25	0.77
28.09	10.07	10×10^3	32	73.8	40.4	1.94	0.85
28.89	8.89	15×10^3	32	185.0	98.5	6.96	2.96
27.67	9.42	25×10^3	32	106.8	75.5	5.38	3.36
479.58	210.21	5×10^3	30	914.4	674.0	0.82	0.47
357.37	153.56	10×10^3	26	913.8	770.9	1.88	1.20
359.35	73.50	15×10^3	30	850.4	946.1	2.56	2.65
306.74	91.01	20×10^3	20	674.0	484.6	3.47	2.55
357.13	126.55	25×10^3	20	647.9	612.6	3.42	3.91

$$IR \text{ (cells/year)} = 0.066 \times (2 \times 10^4)^{1.389} \times 24 \times 365 \times (\text{weight})^{0.932}$$

If we assume that the juveniles filter 50% of the time (Rodhouse, 1979) then -

$$R \text{ (cells/year)} = 2.72 \times 10^8 (\text{weight})^{0.932}.$$

For a scallop which increased from 5 to 28 mg in tissue dry weight the ingested ration (R) would be determined by the integral:

$$R \text{ (cells/year)} = \int_5^{28} 2.72 \times 10^8 (\text{weight})^{0.932}.$$

Thus the ration ingested by the juveniles would be 8.48×10^{10} cells/year. Furthermore if we assume that the energy content of the ingested cells is 0.4 joules per million cells (approximately that found for Isochrysis galbana) we can determine the total amount of energy ingested as 3.41×10^4 joules.

The total energy put into growth can be estimated from Equation (1). The increase in energy will be equal to the total energy in juveniles at 28mg minus the total energy in the juveniles at 5 mg.

The gross growth efficiency can be determined now that we have estimates of the energy put into growth and the total energy ingested over a year. For the juveniles held at MSRL for a year the gross growth efficiency is 0.9%.

Chapter IV

DISCUSSION

4.1 CONDITIONING OF BROODSTOCK

Maintenance of the broodstock in good physiological condition has been shown to be important for the growth and survival of mussel and oyster larvae (Helm et al., 1973; Bayne et al., 1975; Bayne et al., 1978). For females of the giant scallop a daily ration of 3-5% of their body weight on a dry weight basis was required to provide the eggs with levels of protein and lipid equal to or greater than those present in naturally conditioned animals and offset the detrimental effects of holding the scallops in the laboratory at low rations.

The four experiments performed indicated that the condition of females can be ameliorated at most times during the gametogenic cycle. The first experiment was started just as gametogenesis began. These conditioning treatments produced the greatest increase in the levels of lipid reserves although the protein levels were slightly lower than in other experiments. The second conditioning experiment demonstrated that the reserves in the eggs differ with the amount of food supplied to the females even when

gametogenesis was nearly complete. This might be partially due to the beginning of resorption of the gametes in starved or stressed females.

The third experiment was important in showing that females and males can be held in the laboratory all year round and still produce viable gametes. The gonads of these females were examined every month. They appeared fuller in February and March than at the time of spawning in late July. Greater success might have been attained had these scallops been induced to spawn in March rather than July.

Resorption of the gametes appeared to have taken place after three of four months of conditioning, or the females may have spawned in the holding tanks. This is consistent with the work of other researchers. Helm et al. (1973) and Lannan et al. (1980) observed a decline in the overall condition of oyster (Ostrea edulis and Crassostrea gigas) females and the resulting larvae with increasing length of the conditioning period. Therefore the most efficient method of conditioning the adults would be to feed the adults intensely for a short period of time in order to bring them into optimum spawning condition as quickly as possible rather than conditioning them over longer periods of time at lower rations.

The length of the conditioning period will depend upon the stage of gonadal development when conditioning is

started. This requires detailed knowledge of the gametogenic cycle of the species such as that available for Placopecten magellanicus (Thompson, 1977; MacDonald, 1984).

Although we did not feed the males as intensely as the females their physiological condition also seems to be important. Lannan et al. (1980) noted that when both females and males were induced to spawn when fully ripe they produced maximally viable gametes. When either the males or females were induced to spawn outside of their optimum conditioned period the viability of the gametes and success of the larval settlement was reduced. Conditioning regimes would be most successful when both males and females were brought into optimum spawning condition together.

The various conditioning experiments clearly indicated that the algal diets were the most successful in providing the energy required for gamete production. It is evident that the nutrient content of the raw seawater at MSRL was insufficient for the energy requirements of the adults. Because of the greater success of mixed diets in promoting larval growth (Helm, 1977), it was originally thought that a mixed algal diet might prove more effective in covering the energy demands of the adults. However, a unialgal diet of Isochrysis sp. (Tahitian strain) was equally successful in conditioning the females as the multialgal diet in Conditioning Experiment 4 (Figure 6). The nutrient

requirements of the adults may not be as stringent as those of the larvae and as long as the correct balance of protein, lipid and carbohydrate is provided the adults can ingest and assimilate the diet.

The best non-algal feeds for bivalves have been carbohydrates (Haven, 1965; Gillespie et al., 1964, 1966; Sayce and Tufts, 1967; Dunathan et al., 1969; Castell and Trider, 1974; Trider and Castell, 1980). Dunathan et al. (1969) reported that diets containing 60% carbohydrates resulted in maximum glycogen production in oysters. Caestell and Trider (1974) also observed greater glycogen production with increasing levels of glycogen in the diet. However Castell and Trider (1974) found that lower levels of starch and higher levels of lipid in the diet resulted in more rapid weight gains followed by enlargement of the gonads.

The cornstarch diet was not successful in increasing the levels of protein, lipid and carbohydrate above the levels in the eggs of starved controls. This could be due to the fact that the cornstach tended to settle out of suspension very rapidly and would therefore be unavailable to the adults. It could also be that the particles in the raw seawater and the added cornstarch formed a diet deficient in other nutrients such as protein, essential amino acids, fatty acids, minerals and vitamins.

The mediocrity of the combined cornstarch and _Isochrysis_ sp. (Tahitian strain) diet would also seem to indicate that the cornstarch was either not being ingested or was lacking in essential nutrients. The females under this conditioning treatment might have been receiving only the algal portion of their diet, approximately 1.5% of their dry body weight per day.

Maximizing the energy reserves in the eggs results in greater larval recovery rates. However, the presence of high protein and lipid levels in the eggs was not, by itself, sufficient to ensure good larval growth and survival. However, when an adequate diet was provided to the larvae, those which had higher protein and lipid levels did accumulate energy reserves at a greater rate and had lower mortality rates. Therefore maximizing the energy reserves in the eggs of the giant scallop will lead to greater success in rearing the larvae.

4.2 ENERGY RESERVES

As in other bivalve larvae (Collyer, 1957; Millar and Scott, 1967; Bayne, 1972; Holland and Spencer, 1973; Holland and Hannant, 1974), carbohydrate is a minor reserve material in the eggs, larvae and spat of scallops. In the eggs of the giant scallop, the protein:lipid:carbohydrate ratio was constant (60:30:10) regardless of the conditioning

treatments of the adults (Table 5). When additional energy reserves were available to the adults these reserves were deposited in the eggs and partitioned equally between protein and lipid fractions. This is an indication of the importance of both protein and lipid in the successful development of scallop larvae.

Lipid has been shown to be the major source of energy for growth and metamorphosis in most bivalve larvae (see Holland, 1978). Since survival time is very important and there is an upper limit to the amount of reserves that the adults can deposit in the egg or that the larvae can accumulate during their pelagic life stage, it is important for them to use the most efficient energy reserve (Crisp, 1976). In the oyster, Ostrea edulis, Holland and Spencer (1973) reported that neutral lipid accounted for 41% of the total organic matter lost during starvation, whereas protein accounted for 34%. During development of the egg to the larva Bayne et al. (1975) observed a much greater decrease in lipid levels (from 160 μg/mg in the eggs to 17 μg/mg in the larvae) than in the other components. Protein levels decreased from an average of 350 μg/mg in the eggs to 86 μg/mg in the larvae. Although they found that for Mytilus edulis larvae, greater losses of protein occurred than in Ostrea edulis larvae, carbohydrate still played only a minor role.

In the larvae of the giant scallop both lipid and protein are important in providing energy for morphogenesis and growth. Protein is a much more important energy source in Placopecten magellanicus larvae than in oyster larvae, providing 50-60% of the total organic matter required for energy during development of the egg to the veliger and 40% of the total energy (Tables 7 and 8). Other marine larvae use protein as an energy store. Bartlett (1979) suggested that the Pacific oyster, Crassostrea gigas, used protein rather than neutral lipid as an energy store in the late stages of larval development.

There is evidence of a protein sparing effect in the giant scallop. Conversion efficiencies show that protein from the egg is conserved when the amount of protein is very low. Relatively greater levels of lipid are used for development in this instance. On average the relative amounts of lipid decreased from 30% in the eggs to 25% in the veligers whereas protein levels increased from 60 to 65% (Table 7). Therefore although protein is a very important energy source in scallop larvae, lipid appears to be the more important energy reserve.

In all of the conditioning experiments it was noticed that the total energy used to cover the costs of development from the egg to the larva increased as the total amount of energy reserves deposited in the egg increased (Figure 7).

This was mainly due to an increase in the loss of protein during early development. It is possible that this protein is either directly incorporated into the shell or serves to cover the costs of producing a better shell.

Very little is known about the energy required to secrete the shell. There are, however, definite advantages to producing a stronger shell. The shell offers the larvae some protection against mechanical forces and helps in locomotion. In an aquaculture situation where the larvae are, by necessity, handled several times a week this might be an important factor in survival.

During starvation of the larvae, protein again seems to be very important, contributing 60-70% of the total energy required for metabolism. This is probably due to the fact that protein levels are three to four times greater than lipid levels in the larvae.

During starvation, protein and lipid are both lost from bivalve larvae in proportions that depend upon the age of the larvae and the length of the starvation period. Over a two-day starvation period, Ostrea edulis larvae lost approximately equal amounts of protein and lipid, although more energy came from the lipid than from the protein fraction because of the greater caloric yield per unit weight of lipid (Millar and Scott, 1967; Gabbott and Holland, 1973; Helm et al. 1973). In some bivalve larvae

protein is spared during the initial stages of starvation (Helm et al., 1973). However, during longer periods of starvation protein becomes the major source of energy. During seven days of starvation the ratio of protein:lipid:carbohydrate losses for Mytilus edulis larvae was 1.0:0.24:0.11 (Bayne, 1976) and for Mya arenaria larvae was 1.0:0.30:0.04 (Gustafson, 1980). For Placopecten magellanicus larvae the ratios were observed to be 1.0:0.36:0.07. No protein sparing effect was noted even in the first week of starvation. If such an effect is present in P. magellanicus larvae it might only last a few days in which case my analysis would not have detected it.

In oyster and mussel larvae the amount of neutral lipid is predictive of larval success (Holland and Spencer, 1973; Bayne et al., 1975; Bayne et al., 1978; Holland, 1978). In Placopecten magellanicus the total energy content of the eggs is a more accurate predictor of the potential success of larval growth and survival since both protein and lipid are important energy sources.

When both protein and lipid levels in the eggs are low, the recovery rate of larvae, the accumulation of energy reserves and survival are decreased. However if either protein or lipid levels are high there does not appear to be any significant detrimental effect on larval growth and survival even though the other energy reserves may be

present in relatively low amounts. For example, the growth rate, survival rate and rate of accumulation of protein, lipid and carbohydrate of larvae from naturally conditioned females in Experiment 4 (Table 10) were not significantly different from larvae obtained from females fed algal supplemented diets although the level of lipid in the eggs was significantly lower (Figure 6).

The growth rates of larvae obtained from starved females did not differ from the growth rate of the other larvae in Experiment 4 (Table 10). Veliger larvae have been shown to increase shell length even during starvation. Crepidula fornicata larvae grew approximately 50 μm in shell length when subjected to high temperatures but decreased in total carbon content indicating a decline in body tissues (Lucas and Costlow, 1979). Gustafson (1980) observed that the antibiotic, chloramphenicol, inhibited body tissue growth in Mya arenaria larvae, but allowed for shell growth of 145 μm in 12 days. We observed growth of 15-20 μm in shell height in Placopecten magellanicus larvae while the protein, lipid and carbohydrate levels declined steadily. The shell length/body weight relationship is made more complex by the capacity of veligers to add new shell in spite of loss of body tissue. A measure of the change in body tissue is therefore a better predictor of growth and condition of the larvae.

During the pelagic stage, the scallop larvae accumulated protein very rapidly. Lipid levels, however, did not increase dramatically until the third or fourth week (Figure 9). This increase in lipid towards the end of larval life is an indication that lipid might be important in providing energy for metamorphosis in Placopecten magellanicus larvae. This would agree with data on Ostrea edulis (Holland and Spencer, 1973) where neutral lipid reserves in the larvae are increased from a low of 8.8% at release to a maximum of 23.2% in pediveligers. Holland and Spencer (1973) reported that half of these reserves were used to satisfy the metabolic requirements during metamorphosis and that there was no evidence of protein or carbohydrate being used for energy. However in Placopecten magellanicus we were unable to determine the main energy source during metamorphosis due to the failure of larvae to settle successfully.

4.3 LARVAL DIET

The larval diet is more important than the level of energy reserves in the eggs in determining the success of growth in Placopecten magellanicus larvae. The larvae obtained from eggs spawned by females in Conditioning Experiments 1 and 2, which had high protein and lipid levels, did not survive any longer than the larvae obtained from starved females or the starved larvae from Females 53, 54 and 55.

When the diet is inadequate for growth the larvae must utilize body tissue to cover metabolic costs. The increased energy in the larvae of females fed algal supplemented diets is not sufficient to increase survival rates.

The algal cultures fed to the larvae changed constantly in unpredictable ways. These changes may have been harmful to the larvae. For example, the moderate success obtained with feeding *Isochrysis galbana* to the larvae from Female 53 (Table 10) was totally absent when the same diet was fed to the larvae of Female 54 two months later. Also even though the larvae from Female 53 grew during the first three weeks they were fed *Isochrysis galbana* this alga was unable to support further growth. This indicates that some change, unfavorable to the larvae, occurred which transformed a potentially good food into an inadequate one.

Researchers have reported that feeding larvae on a mixed algal diet may enhance growth as compared to feeding them unialgal diets (Calabrese and Davis, 1958; Bayne, 1965; Walne and Spencer, 1968; Davis and Guillard, 1970; Pilkington and Fretter, 1970; Helm, 1977; Kempf and Willows, 1977; Chia and Koss, 1978). The greater success of multialgal diets might be due to minimizing the detrimental effects of any given phytoplankter. When more than one species of alga is present in the diet any minor toxic effects of one phytoplankter might be masked by the others.

Also any nutrient deficiencies in one algal species may be corrected by the others. Larvae in their natural environment will normally encounter a large variety of cells from which they can, presumably, obtain all necessary macro and micronutrients.

It is generally accepted that the nutritional value of various phytoplankters is not equal. In addition to the biochemical composition of the algal cells, other factors such as the digestibility of the cell walls, the assimilation efficiency of each component and the cell size must be taken into account when evaluating the suitability of an algal species for food.

Several attempts have been made to correlate the gross biochemical content (protein, lipid and carbohydrate) of the algae to their suitability as food (Parsons et al., 1961; Walne, 1970b; Epifanio, 1979).

Walne (1970b, 1974) showed that the differences in chemical composition of Dunaliella tertiolecta., Phaeodactylum tricornutum and Monochrysis lutheri varied more within an alga because of chemical and physical culture changes than between species.

Webb and Chu (1983) observed the nutritional value of an alga to be correlated to its protein content and not its lipid or carbohydrate levels. Carbohydrates, which have a high nutritional value for juvenile and adult oysters

(Haven, 1965; Dunathan et al., 1969; Castell and Trider, 1974; Flaak and Epifanio, 1978), do not appear to be as important as lipid in determining larval success (Millar and Scott, 1967; Holland and Spencer, 1973; Helm et al., 1973; Holland, 1978; Chu and Dupuy, 1980).

The diet of Isochrysis galbana, Chaetoceros calcitrans and Thalassiosira pseudonana provided for good larval growth. The main difference between the diet of I. galbana and the mixed diet was the high carbohydrate levels in Chaetoceros calcitrans. The success of this diet might be due to its high energy content.

Chaetoceros species have been underutilized in bivalve aquaculture (Enright, 1984). Walne (1970b) reported that C. calcitrans was a good algal species for larval oysters and Langdon and Waldock (1981) used it in their studies. Enright (1984) found C. calcitrans to be the best algal species, of those tested, for juvenile oysters.

As Chu et al. (1982) pointed out it is possible that once the balance of protein, lipid and carbohydrates required in a diet has been reached then other minor components and trace nutrients such as minerals and vitamins are the determining factors. The presence of Chaetoceros calcitrans might be responsible for the success of this diet because of certain micronutrients which might be absent in other species.

The growth rate of _Ostrea_ _edulis_ and _Crassostrea_ _virginica_ larvae was observed to be unrelated to the total amount of lipld in the diet (Waldock and Nascimento, 1979; Chu and Dupuy, 1980) but was related to the triacylglycerol composition of the lipids in the algae (Waldock and Nascimento, 1979).

Essential fatty acids have been related to the success of oyster larvae. Chu and Webb (1984) found an accumulation of $20:5\omega3$ and $22:6\omega3$ fatty acids in older oyster larvae. This is consistent with the idea that accumulation of these fatty acids is from the diet and that the primary energy reserve in bivalve larvae is lipid (Walne, 1970b; DeMoreno et al., 1976a; Sargent, 1976; Holland, 1978; Holland et al., 1983). Langdon and Waldock (1981) reported that the deficiency of $22:6\omega3$ in the diets was the limiting factor in the growth of _C. gigas_ larvae. Enright (1984) suggested that when optimal rations were presented to juveniles oysters, _Ostrea_ _edulis_, the best diets were those with high levels of $22:6\omega3$ fatty acids.

Older bivalve larvae have a limited ability to biosynthesize long chain $\omega3$ fatty acids _de_ _novo_ (DeMoreno et al., 1976b; Waldock and Holland, 1984). Chain elongation of $18:2\omega6$ or $18:3\omega3$ is, however, not sufficient to produce maximum growth. Either $22:6\omega3$ or $20:5\omega3$ or both of these fatty acids have been found to be essential for the growth

of oysters (Langdon and Waldock, 1981; Ackman, 1983; Enright, 1984). These fatty acids must come mainly from the diet. If 22:6ω3 and 20:5ω3 are both essential fatty acids for scallop larvae then a diet of C. calcitrans and I. galbana would afford a good source of these nutrients, the first being high in 20:5ω3 and the second containing large amounts of 22:6ω3 (Waldock and Nascimento, 1979). Algal species which lack these essential fatty acids might not provide an adequate diet for bivalve larvae (Ackman, 1983). Therefore when a limited number of algal species are used in hatcheries and nurseries severe fatty acid deficiencies are more likely to occur (Enright, 1984).

The importance of both protein and lipid as energy sources in Placopecten magellanicus larvae might explain the difficulty in finding a suitable diet. Placopecten magellanicus larvae have been observed, by me, to grow better on exponentially growing algal cultures which generally possess high protein levels (Fogg, 1959). However growth in the pediveliger stage and successful metamorphosis have proven unreliable at best. This could be due to an increased need for lipid at this time. Multialgal diets would be more likely to satisfy the energetic needs of the larvae.

It is important to optimize growth and reduce the time required for bivalve larvae to set. Thus it is essential to

determine the nutritional requirements of the larvae in order to provide them with the proper diet. This can be achieved by supplementing algal diets with carefully prepared microencapsulated diets (Jones et al., 1979). Further work should be done on the nutritional requirements of Placopecten magellanicus larvae in order to develop a good diet.

4.4 ENERGY REQUIREMENTS OF THE LARVAE

The net growth efficiency for 3-13 day old scallop larvae, P. magellanicus, was estimated at 34% (Table 12). This value is at the lower end of net growth efficiency values for bivalve larvae. Jespersen and Olsen (1982) calculated a net growth efficiency of 43-73% and a gross growth efficiency of 19-22% for Mytilus edulis larvae. Gabbott and Holland (1973) estimated that during resting metabolism the k2 values for Ostrea edulis larvae might be as high as 71-88%. They determined the net growth efficiency for swimming oyster larvae to be 78.6% on release and then 55.5% on day 10, using the same method as employed in this study. Bayne (1976) estimated a net growth efficiency of 65% for Mytilus edulis larvae. Walne (1965) estimated a net growth efficiency of 68-80% for Ostrea edulis larvae. Jespersen (1981) calculated a k2 of 60% for Mytilus edulis larvae. However the net growth efficiency of

Placopecten magellanicus is comparable to those obtained for gastropod larvae (Pechenik, 1980).

When calculating the k2 values for Placopecten magellanicus larvae, the energy required for swimming was estimated as twice the amount found for metabolic costs. However during the first two weeks veligers were generally observed to be quite active and swimming in the water column. In starving larvae the energy required for swimming would necessarily be obtained from reserves. Therefore the loss of reserves during starvation is a measure of metabolic and swimming costs. If this is true then the k2 value would be closer to 60%. This estimate, however, may be high since metabolic rate and activity decrease during starvation as indicated in Table 12a.

The dramatic decrease im metabolic costs after two weeks might indeed result from the decrease in swimming rate as well as a décrease in resting metabolism. Therefore the net growth efficiency of Placopecten magellanicus larvae probably lies between 34-60%. This is closer to the findings of other researchers for bivalve larvae.

Figure 9 shows the sigmoidal growth curves for larvae obtained from females in Conditioning Experiment 4. Loosanoff et al. (1951) and Sprung (1984a) reported evidence of sigmoidicity in growth curves where the growth rate is decreased by low temperatures or reduced ration. Since

Placopecten magellanicus larvae were reared at $11-12^0C$ the low net growth efficiency, as compared to other bivalve larvae, might be caused by lower culture temperatures.

It is also possible that although the ration fed did produce growth it was still not sufficient for maximum growth rates. If less than the optimum raton is available to the larvae then growth efficiency will be decreased.

Ostrea edulis and Mytilus edulis larvae exhibit greater larval growth rates than P. magellanicus. Using the conversion of 1 ml O_2 at NTP is 20.08 joules (Crisp, 1971) we can calculate the oxygen uptake for P. magellanicus as 0.07×10^{-6} ml O_2/hour/larva. This is ten times lower than the rate of oxygen consumption for Mytilus edulis and Ostrea edulis larvae. Part of the difference results from higher culture temperatures for oyster and mussel larvae. The higher metabolic rates will lead to greater growth efficiency as long as nutrients are not limiting. Therefore lower growth efficiencies might be expected with Placopecten magellanicus larvae.

Welch (1968) suggested an inverse correlation between absorption efficiency and net growth efficiency (k2). This relationship would prediot low absorption efficiencies for bivalve larvae which have high k2 values. This is supported by several studies. Walne (1965) found an absortion efficiency (AE) of 15-45% for Ostrea edulis, and Gabbott and

Holland (1973) determined an AE of 28-52% for oyster larvae and Helm (in Bayne, 1983) observed an AE of 29-46% for _Ostrea edulis_; Sprung (1984c) measured AE as 18-44% for _Mytilus edulis_ larvae, and Pechenik (1980) calculated AE of _Nassarius obsoletus_ to be 17-58%, of _Crepidula fornicata_ to be 69% and of _Brittium alternatum_ to be 35-45%.

Corner and Davies (1971) have pointed out that absorption efficiency is greatly influenced by the quality of the food. Different components of the food are not absorbed with the same efficiency. Sprung (1984d) found high absorption efficiencies for mussel larvae at low food concentrations. Low absorption efficiencies in bivalve larvae might be an artefact of high algal concentration fed to the larvae.

If the absorption efficiency for _P. magellanicus_ larvae is approximately 30% then the larvae would only have to feed at a rate of 45 cells of _Isochrysis galbana_ per hour. Riisgård _et al._ (1980) calculated ingestion rates of 30-80 cells/hour/larva for mussel larvae. According to Jespersen and Olsen (1982) mussel larvae 125 μm in length will ingest _Isochrysis galbana_ and _Monochrysis lutheri_ at a rate of 175 cells.hour^{-1}.larva^{-1} at a concentration of 40-60 cells/μl. Sprung (1984b) calculated the ingestion of _Isochrysis_ cells for mussel larvae, _Mytilus edulis_, of 141 μm in length, to be approximately 47 cells.h^{-1} at 12°C and an algal concentration of 40 cells.μl^{-1}.

Therefore it seems quite likely that Placopecten magellanicus larvae may feed at a rate of 45 cells.h^{-1} and thus obtain the required energy for growth and metabolism. Feeding the larvae a ration of 2.5×10^3 to 5×10^4 cells.larva^{-1} of Isochrysis galbana every two days would barely satisfy the energy requirements of 3 to 13 day old larvae of P. magellanicus. The energy required for growth and metabolism increases as the larvae grow. According to these calculations a diet of Isochrysis galbana would not provide sufficient energy for growth after the second or third week of pelagic life. This coincides with the first plateau observed in the growth of Placopecten magellanicus larvae. Placopecten magellanicus larvae often reach a size of 130 μm in height and then cease to grow (Dabinett, pers comm). The second plateau in larval life occurs during metamorphosis.

Occasionally some diets support larval growth during the initial three weeks of larval life but not thereafter. This might be due to insufficient energy if the ration is low. The ration should be increased with growth of the larvae. The mixed diet fed to the scallop larvae had a much higher energy content which would have satisfied the requirements for metabolic costs and growth even as larvae grew. Therefore to ensure good growth it is essential to determine the energy requirements of the larvae as they increase in

size and the amount of food which they can potentially ingest. In order to obtain maximum growth rates it is essential to provide the larvae with a ration which will allow for maximum ingestion rates. The ingested ration must cover the costs of metabolism, swimming and growth. Since absorption efficiency is higher at low food concentrations it is important not to overfeed the larvae. Especially since the nursery culture of juveniles is cost prohibitive.

The inability to obtain good larval growth and successfully induce settlement with any degree of predictability is a major obstacle to the commercial culture of the giant scallop. This study has shown the importance of intensely conditioning the females held in the laboratory. Also the total energy content of the eggs can serve as an indication of the potential success of any given batch of larvae. However the nutritional requirements of the pelagic larvae are still largely unknown. It is essential to setermine the macro and micronutrient requirements of Placopecten magellanicus in order to be able to rear the larvae with any degree of success.

4.5 ENERGY REQUIREMENTS OF JUVENILES

The major energy store in bivalve larvae is lipid. In the adults, including P. magellanicus carbohydrate is a more important source of energy (Giese, 1969; Thompson, 1977).

The giant scallop switches from lipid to carbohydrate as the main energy reserve between the ninth and twenty-third month. Holland and Hannant (1974) found that oyster spat changed from lipid to carbohydrate as the major energy reserve five months after settlement.

The different biochemical reserves of the larvae and the adults, may be related to their different life styles. In the free-swimming larvae the use of lipid as an energy reserve will give them added buoyancy. Also, since lipid is a highly compact and efficient energy reserve, having almost twice the energy value of carbohydrate per unit weight, it allows marine organisms to survive periods of starvation which may be imposed because of developmental changes during certain stages of their life histories (Holland, 1978). Since larvae are continuously swimming in a well-oxygenated environment the lipid reserves may be used by normal pathways of oxidative degradation. The adults on the other hand may have to withstand periods of anoxia and would therefore have to resort to anaerobic metabolism. Carbohydrates, however, can be readily handled via the glycolytic pathway (Holland and Hannant, 1974). Therefore it can be concluded that the different energy reserves in the adults and the larvae are due to the metabolic and physiological requirements imposed by their different life styles.

Filtration rates can be determined using a closed system or a flow through system. When using a closed system four basic assumptions are made:

1. the reduction in cell concentration is due to the filtration activity of the animals and are not to gravitational settling

2. the animal's pumping rate is constant

3. the particle retention is 100% or a known percentage

4. the suspension is homogeneous at all times (Coughlan, 1969).

With the proper controls it is possible to assure that these conditions are met. To ensure that the solution was homogeneous we measured the particle concentration of three different samples from each container during the initial experiments. There was no difference in concentration, thus indicating a homogeneous solution. _Isochrysis_ is within the size range retained maximally by mussel larvae and spat (Riisgard et al., 1980; Sprung, 1984b). The time period between samples was deemed short enough to ensure that the pumping rate of the animals was accurately measured.

Many bivalves display some ability to regulate feeding in response to short-term changes in food availability (Thompson and Bayne, 1972, 1974; Winter, 1973; Foster-Smith, 1975a,b; Widdows, 1978a; Griffiths, 1980b; Palmer and Williams, 1980; Navarro and Winter, 1982; Gerdes, 1983;

Seiderer et al., 1984). This appears to be the case for juvenile scallops at the higher concentrations of Isochrysis galbana tested ($1.5 \times 10^4 - 2.5 \times 10^4$ cells/ml). The juveniles adjusted their filtering rate to obtain a ration of 3-6% of their body weight per day (Table 13).

The maintenance ration, expressed as a percentage of tissue dry weight, was found to be approximately 5% at 12°C for small mussels of 3 mg tissue dry weight and 1.5% of tissue dry weight for 1 g mussels (Bayne, 1975). Therefore the ingested ration of 5-10% of their body weight per day for Placopecten magellanicus juveniles of 5-8 mg and 3-5% for scallops of 200-400 mg tissue dry weight would appear to be sufficient for metabolism and growth. The proportionately higher daily rations required by smaller individuals are an indication of their higher metabolic rates.

For a scallop with a tissue dry weight of 6 mg to ingest 10% of its body weight it would have to filter the water at 171.23 ml/h if the concentration of algae was 5×10^3 cells/ml, but only at 42.8 ml/h if the algal concentration was 2×10^4 cells/ml. Scallops of 40 mg and 400 mg tissue dry weight would have to filter an algal solution of 5×10^3 cells/ml at a rate of 1140 ml/hour and 13 litres/hour, respectively, to obtain 10% of their body weight per day but at 2×10^4 cells/ml they would only have to filter at a rate

of 285 ml/hour and 3 litres/hour, respectively. The maximum filtration rates observed for Placopecten magellanicus are sufficient to provide a ration of 10% of body weight per day at the highest algal concentration tested. It would seem that these scallops would require 2.0×10^4-2.5×10^4 cells/ml of Isochrysis galbana on a continuous basis. The scallops would thus be able to maintain a constant ration of approximately 5-10% of their body weight per day.

The filtering rate of scallops 20-40 mg dry tissue weight was not correlated to the concentration of Isochrysis galbana present. Winter (1978) pointed out that it is difficult to detect a significant effect of particle concentration on filtration rates in a static system. However our analysis was done on individual measurements, instead of mean filtration rates versus initial cell concentration, thus taking into account the changing cell concentrations. In this manner we were able to detect a significant relationship between filtration rate and algal concentration for the smallest sized scallops (5-8 mg) and the largest sized juveniles (200-400mg tissue dry weight). The determination of filtration rates was done on individual scallops for these two groups. The filtration rate of the group of juveniles weighing 20-40 mg tissue dry weight was, however, measured with groups of 10 to 15 scallops. The filtration rate of these juveniles was not related to algal

concentration. In this instance the filtration rate would be altered if not all the scallops were pumping. Differences in pumping rate could thus easily be masked. Although the scallops were observed to be open during the experimental periods this only indicates the possibility that they were filtering.

The filtration rate increased rapidly with the size of the scallops, especially, with the smaller juveniles as seen by the exponents of the regression equations in Figure 11. Riisgard et al. (1980) observed the same trend. The smaller mussels (up to 10 mg dry tissue weight) had a weight exponent of 1.03; whereas, measurements of Mytilus edulis ranging from 10-1000 mg in dry tissue weight had a weight exponent of 0.72 (Riisgard and Mohlengberg, 1979). Gerdes (1983) also found that within the weight range of 5-811 mg the weight specific filtration rate of small oysters, Crassostrea virginica, was approximately 3.3 times higher than that of the larger specimens.

The filtration rate of small juvenile scallops was very low at low algal concentrations. A decrease in the filtration rate at very low particle concentrations has been discussed controversially in the literature, since it has been first described by Adams and Steele (1966) for copepods. Filtration is an energy-consuming process. If filtering activity is only enhanced when a certain

concentration of food particles is present in the water, then this would be an energy-economizing behavior (Lam and Frost, 1976; Lehman, 1976). This could explain the low filtration rates for small scallops at 5×10^3-1×10^4 cells/ml of Isochrysis galbana.

Riisgard et al. (1980) observed filtration rates for Mytilus edulis spat weighing 5-10 mg tissue dry weight to be five times higher (100-200 ml.h^{-1}) than the highest filtration rates observed for Placopecten magellanicus juveniles of the same weight. The algal species fed to the mussel larvae was Dunaliella marina. This phytoplankter is a larger alga (5.6 μm) than Isochrysis galbana (3.5 μm), which was used in our experiments. Generally filtration rates for a species will decrease when fed a larger algal species. The filtration rates for Mytilus edulis juveniles 100-1000 mg tissue dry weight observed by Riisgard and Mohlenberg (1979) are only twice as high as the filtration rates observed for Placopecten magellanicus juveniles of the same size. Mytilus edulis, being an intertidal bivalve, might be expected to exhibit higher filtration rates than a sublittoral species to compensate for being unable to feed when exposed during low tides.

At 5×10^4-1×10^5 cells/ml of Isochrysis galbana, juvenile oysters, Crassostrea virginica, filter at rates (Gerdes, 1983) comparable to those of Placopecten magellanicus fed

2.5×10^4 cells/ml of <u>Isochrysis galbana</u> over the same size range.

Food uptake in dense algal concentrations is characterized by a constant ingestion rate over a wide range of food concentrations and a declining filtration rate with increasing food concentration (Sprung, 1984b). This constant ingestion is limited by the passage of food through the gut. The ingestion capacity depends upon the digestibility of the food- the rate at which the gut can be cleared (McMahon and Rigler, 1965). Food uptake in dilute food concentrations is characterized by a constant filtration rate and an increase in ingestion rate with increasing food concentration (Sprung, 1984b). For mussel larvae, <u>Mytilus edulis</u>, Sprung (1984b) found that the transition between dilute and dense cultures occurs between 5 and 10 cells.μl^{-1} of <u>Isochrysis galbana</u>. At higher concentrations ingestion rates can be accurately estimated but filtration rates will be underestimated.

When ingestion rates are below the level which results in intestinal fecal production, absorption efficiency, defined as the energy uptake from the food in the digestive system (Crisp, 1971), is at a maximum (see Newell, 1983). If the stomach capacity is ecxeeded these cells will not be ingested. Furthermore they would be unavailable for other larvae and to all purposes would appear to have been

ingested. Other particles could be ingested but only partially digested or totally undigested and excreted as pseudofaeces. It can therefore be concluded that the optimum ration- the ration at which growth is most efficient- will depend on the digestibility of the algae, the stomach capacity of the larvae and the concentration of food particles present.

Rearing spat in a nursery is an expensive endeavour (Persoone and Claus, 1980). The juveniles require large amounts of phytoplankton as feed every day. It is important not only to determine the optimum ration but also to determine the best method of delivering the food on a daily basis. The production of pseudofaeces assumes critical importance in an aquaculture system because frequently one of the largest operating costs is the algal food.

The juveniles held in the laboratory were considerably smaller than juveniles of the same age left in the natural environment. The reasons for this are unknown.

Epifanio and Ewart (1977) found that for continuously submerged oysters, C. virginica, there were periods of feeding activity and periods of quiescence. They concluded that when rearing bivalves it would be advantageous to add food discontinuously. This would entrain the feeding and digestive activity of the animals to the feeding regime and hence prevent excess food being lost during periods of

non-feeding. Langdon and Mckay (1974, 1976) found higher growth rates when the same total amount of algae was fed discontinuously to the oyster, C. gigas, compared with continuous feeding regimes. This was attributed to the fact that the continuous addition of algae would have resulted in a low food concentration that may not have fully stimulated the oysters' feeding activity, whilst the discontinuous feeding regime resulted in higher initial concentrations (Newell, 1983). If it is necessary to reduce the ration, one way would be to adopt a discontinuous feeding regime where the daily feeding periods are balanced between economic restraints and achievement of maximum growth (Newell, 1983). A more effective solution might be the maintenance of optimum ration levels during seasonal growth and a reduction in ration during the quiescent period.

Low levels of particulate inorganic matter (PIM) have a positive effect on feeding and growth of bivalves (Loosanoff, 1962; Winter, 1976; Kiorboe et al., 1981). Winter (1976) demonstrated that this enhanced growth was due to the stimulatory effect of low levels of PIM on filtration rate, and a decrease in pseudofaecal production, which resulted in an increased rate of ingestion. Experiments by Ali (1980) confirmed the stimulatory effects of PIM on the growth of the oyster C. virginica. Recent research indicates that both the mussel, Mytilus edulis, (Kiorboe et

al., 1980) and the oyster, <u>Crassostrea virginica</u>, (in Newell, 1983) preferentially ingest algae and reject PIM in the pseudofaeces. Possible explanations for the effects of PIM include:

1. addition of bacteria or organic matter which enhances food levels

2. provision of a large surface area for dissolved organic material (Murken, 1976)

3. addition of unknown growth factors (Winter, 1976)

4. improvement of digestion through mechanical action (Murken, 1976)

5. stimulatory effect on filtration rate (Winter, 1976).

Differences between the PIM concentration in the seawater at MSRL and the natural environment could explain the poor growth of juveniles in the laboratory. Algal concentrations far exceeding those found in the natural environment are required to produce similar growth in laboratory held animals. The slower growth rates of juvenile scallops in the laboratory need to be explored further.

4.6   CONCLUSIONS

1) Adult scallops, <u>Placopecten magellanicus</u>, can be conditioned in the laboratory to achieve protein and lipid levels as high or higher than those present in naturally conditioned animals.

2) In the eggs and the larvae of the giant scallop both protein and lipid are important sources of energy. The total energy content of the eggs is therefore a better predictor of the potential success of the larvae than the level of any individual biochemical component.

3) The higher energy content of the eggs from females fed algal diets resulted in greater larval recovery rates and a higher rates of accumulation of protein and lipid when an adequate diet was provided.

4) A mixed diet of I. galbana, C. calcitrans and T. pseudonana provided for better larval growth than the unialgal diet of Isochrysis galbana or Isochrysis sp. (Tahitian strain). This might be due to the higher caloric content of the diet.

5) In the larvae, protein is accumulated very rapidly and serves as a better indication of the condition of young larvae. Lipid levels tend to increase dramatically in the third or fourth week of pelagic life.

6) Three to thirteen day old larvae have a net growth efficiency between 33-60%. This is comparable to net growth efficiencies of other marine larvae.

7) The concentration at which juveniles produce pseudofaeces lies between 2.0-2.5x10^4 cells/ml of Isochrysis galbana. The filtration rates observed at these concentrations provided the smallest scallops (5-8 mg tissue

dry weight) with a ration of 10% of their body weight per day and the largest juveniles (200-400 mg tissues dry weight) with a ration of 5% per day. This ration should provide the juveniles with sufficient energy for metabolism and growth.

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TABLE A-1

Data obtained from eggs spawned by females of <u>Placopecten</u>
<u>magellanicus</u>, in Conditioning Experiment 1.

FEMALE	NUMBER EGGS SPAWNED	DRY WEIGHT* g per million eggs			EGG DIAMETER (µm)	
		MEAN	S.D.		MEAN	RANGE
1	2.5×10^6	0.078		(2)	69.05	59.68-73.83
2	4.6×10^6	0.086	0.004	(3)	67.22	54.23-73.36
3	14.9×10^6	0.079	0.006	(3)	68.86	58.97-76.52
4	2.1×10^6	0.091		(2)	66.65	58.97-72.41
5	20.7×10^6	0.079	0.009	(3)	67.78	55.90-73.36
6	3.1×10^6	0.083		(2)	69.91	58.97-76.52
7	5.6×10^6	0.085	0.005	(3)	67.22	58.97-73.36
8	25.4×10^6	0.077	0.004	(3)	66.08	50.54-75.64
9	10.7×10^6	0.061	0.011	(3)	65.50	54.23-73.36
10	30.5×10^6	0.088	0.008	(3)	66.65	49.82-75.20
11	15.0×10^6	0.078	0.011	(3)	66.65	53.35-74.29
12	2.3×10^6	0.083		(2)	67.22	56.70-72.41
13	9.4×10^6	0.060	0.007	(3)	66.65	55.08-72.89
14	24.1×10^6	0.107	0.011	(3)	66.65	48.47-75.20
15	15.3×10^6	0.072	0.004	(3)	66.08	53.35-72.41
16	8.9×10^6	0.065	0.012	(3)	68.32	55.08-74.29
17	10.4×10^6	0.084	0.009	(3)	67.78	55.90-74.29
18	15.6×10^6	0.070	0.009	(3)	68.32	55.90-73.83
19	16.1×10^6	0.073	0.002	(3)	67.78	56.70-73.83
20	1.3×10^6	0.070		(1)	70.43	64.92-74.29
21	28.2×10^6	0.077	0.010	(3)	68.32	55.08-76.08
22	8.0×10^6	0.054	0.009	(3)	66.08	56.70-71.93
23	32.5×10^6	0.069	0.007	(3)	67.22	55.08-73.83
24	1.2×10^6	0.070		(1)	67.22	58.97-72.89
25	1.6×10^6				68.86	60.39-74.29
26	15.7×10^6	0.094	0.011	(3)	65.50	55.08-73.83
27	1.5×10^6	0.117		(1)	67.22	58.97-71.93
28	29.1×10^6	0.070	0.006	(3)	67.78	55.08-74.75
29	3.3×10^6	0.084		(2)	68.86	61.75-72.90
30	11.7×10^6	0.068	0.002	(3)	68.86	60.66-72.89

* ( ) indicates the number of replicates

TABLE A-2

Data obtained from eggs spawned by females of <u>Placopecten</u> in Conditioning Experiment 2.

FEMALE	NUMBER EGGS SPAWNED	DRY WEIGHT* g per million eggs			EGG DIAMETER (μm)	
		MEAN	S.D.		MEAN	RANGE
31	16.0×10^6	0.112	0.017	(3)	68.32	58.97 — 71.93
32	18.8×10^6	0.083	0.011	(3)	67.78	55.08 — 73.36
33	12.0×10^6	0.091	0.004	(3)	67.78	55.90 — 73.83
35	9.2×10^6	0.074	0.013	(3)	68.32	55.08 — 75.20
36	1.0×10^6	0.091		(1)	67.78	55.90 — 75.20
37	3.5×10^6	0.106		(2)	68.32	54.23 — 74.29
39	56.8×10^6	0.094	0.010	(3)	69.91	56.70 — 74.75
40	3.2×10^6	0.088		(2)	68.32	58.97 — 73.86
43	3.1×10^6	0.088		(2)	68.86	59.68 — 72.41
44	18.2×10^6	0.114	0.009	(3)	68.32	59.68 — 73.83
45	3.1×10^6	0.093		(2)	68.86	60.39 — 73.36
46	13.9×10^6	0.090	0.021	(3)	67.22	56.70 — 73.36
47	6.6×10^6	0.094	0.016	(3)	67.78	58.97 — 73.36
48	9.2×10^6	0.101	0.014	(3)	67.22	58.97 — 71.93
49	14.5×10^6	0.108	0.011	(3)	65.50	55.08 — 71.42
50	17.6×10^6	0.107	0.009	(3)	67.22	55.08 — 74.29
51	6.9×10^6	0.107	0.011	(3)	69.91	55.90 — 74.29
52	12.7×10^6	0.108	0.008	(3)	72.89	63.05 — 81.39

* ( ) indicates number of replicates.

TABLE A-3

Data Obtained from eggs spawned by females of <u>Placopecten</u> <u>magellanicus</u> in Conditioning Experiment 3.

FEMALE	NUMBER EGGS SPAWNED	DRY WEIGHT* g per million Eggs		EGG DIAMETER (µm)	
		MEAN	S.D.	MEAN	RANGE
7	5.33x10^6	0.053	(2)	71.43	66.08-76.08
8	3.08x10^6	0.056	(2)	73.83	66.08-80.62
9	0.60x10^6			74.29	69.39-78.62

* ( ) indicates number of replicates.

TABLE A-4

Data obtained from eggs spawned by females of <u>Placopecten</u>
<u>magellanicus</u> in Conditioning Experiment 4.

FEMALE	NUMBER EGGS SPAWNED	DRY WEIGHT* g per million eggs			EGG DIAMETER (μm)	
		MEAN	S.D.		MEAN	RANGE
11	17.42×10^6	0.088	0.002	(3)	71.93	60.39-80.62
12	34.07×16^6	0.093	0.004	(3)	68.86	58.23-76.95
13	26.28×10^6	0.109	0.009	(3)	68.32	55.90-75.20
14	24.28×10^6	0.091	0.006	(3)	68.32	55.08-76.52
15	6.39×10^6	0.068		(2)	70.93	61.75-76.52
16	17.96×10^6	0.070	0.005	(3)	69.91	60.66-75.20
17	3.50×10^6	0.064		(2)	67.78	56.70-75.20
18	3.10×10^6	0.066		(2)	68.86	60.34-76.95
19	4.32×10^6	0.066		(2)	68.86	60.66-74.29
20	5.46×10^6	0.070	0.005	(3)	69.39	61.75-75.64
21	9.12×10^6	0.072	0.005	(3)	69.91	63.05-75.64
22	0.48×10^6				69.91	60.39-76.52
23	11.10×10^6	0.072	0.008	(3)	72.41	64.29-77.79
24	4.66×10^6	0.083	0.004	(3)	72.41	64.92-78.62
25	9.61×10^6	0.061	0.004	(3)	70.93	61.75-79.43
26	3.42×10^6	0.071		(1)	71.43	63.05-76.95
27	2.06×10^6	0.073		(1)	68.32	58.97-73.36
28	1.59×10^6	0.072		(1)	66.65	58.23-73.36
29	3.58×10^6	0.075		(2)	67.78	58.23-73.83
30	4.05×10^6	0.081	0.002	(3)	66.65	57.47-71.43
31	9.63×10^6	0.084	0.003	(3)	69.91	60.66-75.20
32	8.66×10^6	0.076	0.003	(3)	68.32	58.23-73.83

* ( ) indicates the number of replicates

TABLE A-5

Protein, lipid and carbohydrate content of eggs
obtained from females of <u>Placopecten</u> <u>magellanicus</u>
in Conditioning Experiment 4.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
			mg per million eggs	
1	ST	11.95	9.44	7.62
1	ST	12.42	11.13	3.28
1	ST	11.44	10.85	4.63
1	ST	9.58	9.57	7.26
2	A	22.94	13.17	6.56
2	A	23.56	14.64	4.87
2	A	24.23	15.26	4.84
2	A	21.85	14.78	6.58
3	SA	21.14	16.09	2.02
3	SA	22.01	13.79	1.94
3	SA	22.01	13.07	4.86
3	SA	20.14	13.82	2.11
4	S	17.94	13.47	2.08
4	S	19.04	11.71	2.14
4	S	19.03	10.89	1.89
4	S	20.23	11.03	1.94
5	A	21.11	15.23	3.85
5	A	21.26	18.67	3.64
5	A	21.25	15.63	3.11
5	A	21.89	19.09	3.91
6	ST	12.46	13.53	4.67
6	ST	12.82	11.56	4.69
6	ST	12.89	12.14	4.10
6	ST	10.15	13.02	4.45
7	ST	23.05	9.61	1.64
7	ST	23.19	9.57	1.46
7	ST	22.28	9.73	1.92
7	ST	22.25	9.41	1.32
8	SA	22.15	16.04	3.61
8	SA	22.28	13.48	3.09
8	SA	22.34	20.98	2.63
8	SA	22.22	17.53	2.76
9	S	24.04	9.17	1.38
9	S	24.28	9.12	1.64
9	S	22.07	11.04	1.89
9	S	26.65	8.16	1.71
10	A	24.56	15.06	2.87
10	A	24.34	13.34	2.76

TABLE A-5: CONTINUED.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
		mg per million eggs		
10	A	23.17	17.46	2.64
10	A	23.11	15.24	2.64
10	A	24.27	16.14	2.75
10	A	23.46	17.37	2.45
11	SA	20.54	15.41	0.71
11	SA	20.37	18.00	0.85
11	SA	25.09	20.39	0.87
11	SA	25.13	20.57	0.99
12	S	18.95	8.74	2.45
12	S	18.78	8.80	2.43
12	S	20.29	11.05	2.59
12	S	20.07	10.08	2.51
13	NC	24.92	19.67	0.89
13	NC	25.08	19.75	0.93
13	NC	23.58	14.90	1.42
13	NC	-----	14.92	----
14	A	30.78	19.00	2.92
14	A	30.74	19.05	2.90
14	A	31.28	20.71	3.01
14	A	29.75	20.27	2.96
15	ST	23.73	14.26	1.78
15	ST	18.29	14.19	1.92
15	ST	21.08	10.87	1.84
15	ST	20.94	11.32	1.84
16	ST	20.75	7.90	1.94
16	ST	21.14	7.67	2.08
16	ST	20.35	7.89	2.42
16	ST	20.15	7.25	2.72
16	ST	19.84	11.06	2.58
16	ST	18.77	10.03	2.63
17	SA	18.60	9.84	2.94
17	SA	18.92	9.32	3.08
17	SA	17.45	8.67	2.70
17	SA	17.39	8.13	2.63
17	SA	19.73	9.47	2.57
17	SA	17.90	9.13	2.53
18	NC	27.64	10.73	3.21
18	NC	28.04	10.79	3.14
18	NC	24.97	11.35	2.52
18	NC	28.07	10.00	2.65
18	NC	27.78	10.05	2.74
18	NC	24.89	11.87	2.65
19	A	25.45	14.67	2.96

TABLE A-5: CONTINUED.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
			mg per million eggs	
19	A	25.68	13.20	3.38
19	A	21.64	13.82	3.26
19	A	21.86	17.66	3.31
19	A	26.19	14.23	2.94
19	A	27.19	14.78	2.84
19	A	26.15	13.15	2.88
19	A	27.85	14.96	3.07
20	S	14.52	9.35	5.32
20	S	15.79	9.24	5.09
20	S	17.47	7.13	5.07
20	S	17.03	7.54	4.28
21	A	26.84	23.46	2.22
21	A	28.28	23.22	2.08
21	A	31.78	26.63	2.18
21	A	31.35	26.57	2.21
21	A	25.91	24.82	1.87
21	A	26.41	24.44	1.74
21	A	26.26	25.88	1.68
21	A	27.25	26.90	1.81
22	S	12.33	11.31	1.51
22	S	12.34	12.29	1.57
22	S	14.74	11.46	1.85
22	S	13.72	10.32	1.57
23	SA	28.22	12.82	1.02
23	SA	28.24	13.17	1.83
23	SA	22.24	14.28	1.51
23	SA	24.75	14.73	1.27
23	SA	23.10	13.57	1.87
23	SA	25.57	14.22	2.09
23	SA	25.78	13.07	2.17
23	SA	23.90	14.82	1.96
24	NC	30.34	8.54	1.66
24	NC	31.45	8.60	1.67
24	NC	27.20	11.83	2.35
24	NC	28.42	10.83	2.30
25	ST	10.87	11.57	4.90
25	ST	8.64	11.63	4.95
25	ST	10.10	10.32	3.99
25	ST	13.20	11.04	3.85
26	NC	24.12	14.19	2.55
26	NC	27.65	14.16	2.67
26	NC	23.07	15.01	2.83
26	NC	23.91	14.78	2.41

TABLE A-5: CONTINUED.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
			mg per million eggs	
26	NC	24.63	18.46	2.63
26	NC	23.87	14.58	2.14
26	NC	25.11	15.92	2.78
26	NC	24.55	17.81	2.43
27	S	20.32	12.07	2.01
27	S	19.56	11.78	2.09
27	S	21.04	10.15	2.14
27	S	15.57	9.90	1.99
28	NC	42.56	20.95	3.25
28	NC	42.35	20.64	3.30
28	NC	38.53	16.44	2.74
29	SA	24.93	18.15	3.56
29	SA	25.05	20.68	3.37
29	SA	22.18	13.14	2.46
29	SA	20.12	18.84	2.72
30	NC	21.11	16.92	3.58
30	NC	21.34	19.04	3.32
30	NC	21.55	17.46	2.67
30	NC	22.09	18.96	2.58

TABLE A-6

Protein, lipid and carbohydrate content of three day old
larvae obtained from females of <u>Placopecten magellanicus</u>
Conditioned in Experiment 1.

FEMALE	CONDITION	% RECOVERY	PROTEIN	LIPID	CARBOHYDRATE
				mg per million larvae	
1	ST	36.6	14.86	4.46	2.23
1	ST	36.6	14.88	4.74	2.15
1	ST	36.6	15.62	5.62	2.86
1	ST	36.6	14.94	5.08	2.55
2	A	70.7	18.86	6.97	3.72
2	A	70.7	18.46	6.84	3.82
2	A	70.7	17.93	6.43	3.38
2	A	70.7	18.04	7.06	3.82
3	SA	58.7	14.24	6.95	1.59
3	SA	58.7	14.66	7.95	1.63
3	SA	58.7	15.02	6.74	1.76
3	SA	58.7	15.01	7.04	1.47
4	S	42.5	12.32	6.13	1.68
4	S	42.5	12.85	6.22	1.67
4	S	42.5	13.43	6.04	1.25
4	S	42.5	13.66	5.95	1.46
5	A	47.9	16.92	8.14	3.46
5	A	47.9	15.44	7.46	3.29
5	A	47.9	17.62	8.25	2.93
5	A	47.9	16.84	7.94	3.14
6	ST	45.5	8.25	7.26	2.96
6	ST	45.5	9.34	6.35	2.84
6	ST	45.5	8.86	7.34	2.42
6	ST	45.5	10.00	7.46	3.05
7	ST	39.8	15.73	5.72	1.33
7	ST	39.8	15.63	5.61	1.31
7	ST	39.8	14.27	5.02	1.26
7	ST	39.8	15.08	4.97	1.30
8	SA	69.0	18.25	7.40	1.38
8	SA	69.0	17.46	6.95	1.23
8	SA	69.0	17.03	5.92	1.24
8	SA	69.0	16.87	6.14	1.20
9	S	84.8	17.16	7.17	2.25
9	S	84.8	16.87	6.84	2.13
9	S	84.8	17.95	6.45	2.02
9	S	84.8	18.24	6.53	2.27
10	A	94.0	18.25	8.41	2.24
10	A	94.0	17.65	7.36	2.30
10	A	94.0	17.42	7.05	1.95

TABLE A-6:CONTINUED.

FEMALE	CONDITION	% RECOVERY	PROTEIN	LIPID	CARBOHYDRATE
				mg per million larvae	
10	A	94.0	16.97	6.98	1.87
11	SA	73.6	13.65	7.43	0.57
11	SA	73.6	13.96	6.67	0.85
11	SA	73.6	12.35	6.95	0.63
11	SA	73.6	14.43	6.42	0.65
12	S	46.3	14.87	4.62	2.04
12	S	46.3	14.93	4.59	2.14
12	S	46.3	13.46	4.27	1.75
12	S	46.3	15.09	4.97	1.94
13	NC	55.0	24.04	9.27	1.57
13	NC	55.0	22.65	8.74	1.47
13	NC	55.0	21.46	8.43	1.49
13	NC	55.0	20.89	8.39	1.36
14	A	82.4	24.65	8.74	2.06
14	A	82.4	23.75	8.76	1.74
14	A	82.4	25.62	7.95	2.00
14	A	82.4	22.99	9.04	2.14
15	ST	32.6	15.64	6.43	1.48
15	ST	32.6	16.74	6.94	1.54
15	ST	32.6	14.39	5.87	1.64
15	ST	32.6	14.97	6.24	0.54
16	ST	32.6	14.64	4.12	1.53
16	ST	32.6	14.73	3.87	1.49
16	ST	32.6	14.84	3.94	1.40
16	ST	32.6	15.76	3.66	1.87
17	SA	63.8	12.64	4.73	2.25
17	SA	63.8	13.76	4.23	2.38
17	SA	63.8	13.24	4.33	2.15
17	SA	63.8	14.01	4.27	2.34
18	NC	63.4	20.74	5.76	2.08
18	NC	63.4	19.84	5.28	2.08
18	NC	63.4	21.47	4.96	1.94
18	NC	63.4	20.05	4.87	2.12
19	A	71.5	20.46	7.46	2.76
19	A	71.5	19.64	6.94	2.95
19	A	71.5	18.43	8.03	2.55
19	A	71.5	18.98	6.49	2.46
20	S	28.4	11.46	3.65	3.75
20	S	28.4	11.94	3.79	3.43
20	S	28.4	12.46	4.29	3.39
20	S	28.4	13.00	4.35	3.25
21	A	83.2	24.40	12.88	1.46
21	A	83.2	24.96	13.87	1.47

TABLE A-6: CONTINUED.

FEMALE	CONDITION	% RECOVERY	PROTEIN	LIPID	CARBOHYDRATE
21	A	83.2	24.87	12.65	1.54
21	A	83.2	23.00	12.04	1.58
22	S	31.1	16.44	5.94	2.07
22	S	31.1	15.94	6.24	2.00
22	S	31.1	16.79	6.74	1.95
22	S	31.1	17.00	6.85	1.87
23	SA	64.0	16.69	7.97	2.14
23	SA	64.0	15.43	7.05	1.84
23	SA	64.0	15.46	6.57	1.44
23	SA	64.0	16.87	6.65	1.42
24	NC	56.0	18.83	5.76	2.28
24	NC	56.0	17.92	4.95	1.87
24	NC	56.0	18.76	4.88	1.96
25	ST	37.2	7.46	3.81	3.05
25	ST	37.2	7.26	4.12	3.00
25	ST	37.2	8.95	3.75	2.93
25	ST	37.2	7.84	3.66	2.85
26	NC	70.1	12.94	6.87	2.25
26	NC	70.1	13.87	7.91	2.15
26	NC	70.1	14.62	8.34	2.06
26	NC	70.1	15.84	8.46	2.25
27	S	43.6	14.64	4.61	1.44
27	S	43.6	14.67	4.73	1.58
27	S	43.6	13.82	4.58	1.96
27	S	43.6	15.47	4.59	1.87
28	NC	72.5	30.15	9.38	2.88
28	NC	72.5	30.24	9.46	2.96
28	NC	72.5	27.46	9.56	2.44
28	NC	72.5	28.05	8.83	2.35
29	SA	68.9	18.46	8.46	2.39
29	SA	68.9	17.63	8.87	2.46
29	SA	68.9	15.48	8.76	2.57
29	SA	68.9	16.62	9.44	2.43
30	NC	59.3	17.46	8.46	1.87
30	NC	59.3	18.94	8.57	1.92
30	NC	59.3	16.48	7.63	1.44
30	NC	59.3	15.99	7.03	2.05

TABLE A-7

Protein, lipid, and carbohydrate content of eggs in
Placopecten magellanicus obtained from females in
Conditioning Experiment 2.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
			mg per million eggs	
31	ST	14.64	12.42	2.44
31	ST	14.83	11.28	2.49
31	ST	14.14	12.06	2.75
31	ST	12.81	11.68	2.81
32	S	18.67	12.55	3.25
32	S	18.89	12.03	3.04
32	S	19.63	13.56	2.84
32	S	19.09	13.53	2.88
33	A	30.15	13.46	2.63
33	A	30.85	13.56	2.59
33	A	31.28	14.24	2.90
33	A	32.43	14.28	3.00
35	S	22.46	12.07	2.09
35	S	22.64	12.14	2.29
35	S	23.12	11.14	2.34
35	S	22.76	13.04	2.29
36	ST	26.54	12.69	2.11
36	ST	24.79	10.85	2.24
36	ST	25.54	13.47	1.99
36	ST	24.92	10.16	2.08
37	A	27.43	12.38	3.10
37	A	25.47	12.35	3.19
37	A	26.05	11.59	3.27
37	A	26.15	11.53	3.43
39	ST	23.15	12.57	2.15
39	ST	22.49	10.04	2.27
39	ST	22.76	11.24	2.44
39	ST	22.92	11.76	2.15
40	SA	24.03	10.38	2.43
40	SA	24.78	10.57	2.35
40	SA	23.10	11.48	2.67
40	SA	24.57	11.53	2.43
43	ST	19.15	12.21	2.30
43	ST	19.64	15.26	2.22
43	ST	18.94	12.83	1.84
43	ST	18.67	13.46	1.70
44	A	33.87	14.73	1.75
44	A	35.43	16.67	1.65

TABLE A-7: CONTINUED.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
			mg per million eggs	
44	A	36.78	15.28	1.64
44	A	33.49	14.63	2.08
45	SA	26.83	14.03	3.08
45	SA	26.82	13.46	3.14
45	SA	27.15	13.25	3.63
45	SA	27.10	13.31	2.88
46	S	22.34	14.26	1.25
46	S	20.57	13.68	1.32
46	S	19.90	14.04	1.79
46	S	23.47	13.78	1.72
47	SA	27.30	13.05	2.69
47	SA	26.82	13.87	2.72
47	SA	27.14	14.82	2.34
47	SA	28.66	15.17	2.39
48	S	19.04	11.54	1.74
48	S	13.18	11.76	1.86
48	S	13.18	11.35	1.85
48	S	14.17	12.13	1.73
49	A	20.35	16.03	2.24
49	A	26.01	15.39	2.08
49	A	22.74	15.78	2.24
49	A	22.49	15.89	2.17
50	SA	28.57	20.48	2.33
50	SA	24.76	19.49	2.58
50	SA	22.38	20.02	2.71
50	SA	23.61	17.79	2.39
51	NC	28.35	13.14	3.25
51	NC	27.63	12.23	3.11
51	NC	30.72	19.01	2.93
51	NC	34.64	17.01	2.87
52	NC	28.79	12.48	5.38
52	NC	27.83	12.26	5.34
52	NC	26.01	14.74	6.03
52	NC	28.77	12.84	6.09

TABLE A-8

Protein, lipid and carbohydrate content of three day old
larvae of _Placopecten magellanicus_ obtained from
females in Conditioning Experiment 2.

FEMALE	CONDITION	% RECOVERY	PROTEIN	LIPID	CARBOHYDRATE
			mg per million larvae		
31	ST	61.8	13.91	7.24	1.10
31	ST	61.8	12.87	7.04	1.23
31	ST	61.8	9.74	5.95	1.26
31	ST	61.8	9.74	6.24	1.30
32	S	73.7	14.59	5.67	1.99
32	S	73.7	14.94	5.94	2.36
32	S	73.7	13.85	5.02	2.43
32	S	73.7	13.87	5.25	2.39
33	A	94.9	14.87	6.96	1.62
33	A	94.9	18.63	6.85	1.74
33	A	94.9	19.66	6.24	1.42
33	A	94.9	20.20	6.14	1.70
35	S	67.1	15.87	6.87	1.84
35	S	67.1	15.96	6.96	1.95
35	S	67.1	14.87	6.43	1.74
35	S	67.1	16.42	6.98	1.83
36	ST	57.1	13.84	7.35	1.58
36	ST	57.1	14.96	6.44	1.94
36	ST	57.1	15.23	6.56	1.67
36	ST	57.1	13.85	6.05	1.78
37	A	60.5	15.87	7.38	0.99
37	A	60.5	16.87	6.41	1.14
37	A	60.5	15.94	5.99	1.24
37	A	60.5	17.03	5.98	1.24
39	ST	69.0	12.94	6.23	1.30
39	ST	69.0	13.83	6.39	2.05
39	ST	69.0	14.20	6.94	2.05
39	ST	69.0	14.22	5.96	1.87
40	SA	97.2	13.93	7.17	1.80
40	SA	97.2	11.68	7.39	1.51
40	SA	97.2	14.47	6.91	1.68
40	SA	97.2	14.99	7.04	1.70
43	ST	58.0	19.42	11.95	1.18
43	ST	58.0	16.61	11.44	1.18
43	ST	58.0	22.28	12.05	1.01
43	ST	58.0	17.46	10.42	0.90
44	A	97.5	22.21	10.33	1.66
44	A	97.5	22.83	7.81	1.73

TABLE A-8: CONTINED.

FEMALE	CONDITION	% RECOVERY	PROTEIN	LIPID	CARBOHYDRATE
					, mg per million larvae
44	A	97.5	24.28	6.80	1.85
44	A	97.5	23.97	6.71	1.92
45	SA	78.6	14.36	8.18	5.33
45	SA	78.6	15.09	8.81	2.81
45	SA	78.6	16.07	7.05	1.94
45	SA	78.6	15.87	6.72	1.84
46	S	43.4	18.75	8.62	1.33
46	S	43.4	19.19	8.18	1.35
46	S	43.4	16.46	7.64	1.27
47	SA	58.9	13.96	6.38	0.95
47	SA	58.9	14.34	6.64	0.95
47	SA	58.9	12.35	5.94	0.48
47	SA	58.9	15.30	5.89	0.48
48	S	44.0	10.58	6.43	0.51
48	S	44.0	12.76	6.26	0.76
48	S	44.0	11.26	5.41	0.87
49	A	65.2	14.63	8.03	1.33
49	A	65.2	14.47	10.08	1.61
49	A	65.2	15.36	7.46	1.55
49	A	65.2	15.42	7.02	1.76
50	SA	72.9	13.73	10.28	0.82
50	SA	72.9	12.84	8.65	0.70
50	SA	72.9	17.85	11.44	0.95
50	SA	72.9	17.94	12.05	1.23
51	NC	86.3	13.44	4.26	2.84
51	NC	86.3	12.14	3.63	1.90
51	NC	86.3	15.44	5.44	2.45
51	NC	86.3	16.09	5.91	2.57
52	NC	46.7	24.43	6.74	1.65
52	NC	46.7	26.36	6.77	1.24
52	NC	46.7	24.03	7.57	1.19
52	NC	46.7	24.19	6.70	1.41

TABLE A-9

Protein, lipid and carbohydrate content of eggs (a) and three day old larvae (b) of females of <u>Placopecten magellanicus</u> from Conditioning Experiment 3.

FEMALE	CONDITION	% RECOVERY	PROTEIN	LIPID	CARBOHYDRATE
				mg per million	
7a	5% T-<u>Iso</u>		20.92	9.82	2.40
7a	5% T-<u>Iso</u>		21.86	10.19	2.53
7a	5% T-<u>Iso</u>		17.03	9.99	2.72
7a	5% T-<u>Iso</u>		16.86	9.12	2.62
8a	5% T-<u>Iso</u>		23.28	11.44	1.68
8a	5% T-<u>Iso</u>		24.45	11.18	1.64
8a	5% T-<u>Iso</u>		22.93	11.15	1.50
8a	5% T-<u>Iso</u>		24.25	11.14	1.72
9a	3% T-<u>Iso</u>	—	16.64	10.28	2.17
9a	3% T-<u>Iso</u>		14.79	12.13	2.14
9a	3% T-<u>Iso</u>		15.65	11.47	2.43
9a	3% T-<u>Iso</u>		16.09	10.43	2.09
7b	5% T-<u>Iso</u>	40.0	16.63	3.03	1.13
7b	5% T-<u>Iso</u>	40.0	15.76	3.41	1.09
7b	5% T-<u>Iso</u>	40.0	18.36	3.86	0.94
7b	5% T-<u>Iso</u>	40.0	16.35	3.46	0.99
8b	5% T-<u>Iso</u>	42.9	18.71	5.19	2.83
8b	5% T-<u>Iso</u>	42.9	15.43	4.72	1.85
8b	5% T-<u>Iso</u>	42.9	15.36	4.44	1.94
8b	5% T-<u>Iso</u>	42.9	16.87	5.06	1.42
9b	3% T-<u>Iso</u>	14.9	10.88	3.92	0.70
9b	3% T-<u>Iso</u>	14.9	10.73	3.64	0.75
9b	3% T-<u>Iso</u>	14.9	11.56	4.43	0.64
9b	3% T-<u>Iso</u>	14.9	10.34	3.87	0.62

TABLE A-10

Protein, lipid and carbohydrate content of eggs spawned
by females of Placopecten magellanicus in
Conditioning Experiment 4.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
			mg per million eggs	
11	A	33.45	16.14	3.66
11	A	31.03	15.56	3.45
11	A	30.48	13.74	3.75
11	A	30.73	15.68	3.46
12	M	30.79	13.97	2.54
12	M	31.52	13.87	2.67
12	M	30.43	12.81	2.65
12	M	31.30	13.52	2.59
13	M	31.10	12.03	2.61
13	M	30.18	10.80	2.68
13	M	31.36	10.90	2.45
13	M	31.41	10.54	2.57
14	ST	23.70	9.35	0.84
14	ST	23.99	8.98	0.86
14	ST	21.98	9.49	0.91
14	ST	23.17	9.09	0.98
15	A	29.62	19.38	1.70
15	A	29.53	19.11	1.61
15	A	32.36	19.10	1.46
15	A	31.06	18.46	1.52
16	A	31.51	14.02	2.20
16	A	31.72	13.19	2.27
16	A	31.29	12.30	2.45
16	A	30.52	14.24	2.57
17	ST	20.03	11.58	1.03
17	ST	19.87	11.02	1.03
17	ST	20.57	9.80	1.15
17	ST	21.00	9.55	1.18
18	M	24.62	14.56	1.32
18	M	27.02	15.03	1.36
18	M	25.47	14.16	1.36
18	M	27.38	15.07	1.57
19	NC	22.64	9.01	1.95
19	NC	24.97	8.59	1.97
19	NC	25.17	9.43	2.27
19	NC	24.78	9.43	2.21
20	NC	26.71	7.70	3.00
20	NC	28.09	6.96	2.97
20	NC	26.71	7.11	2.93

TABLE A-10: CONTINUED.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
			mg per million eggs	
20	NC	29.12	6.96	3.14
21	NC	31.26	12.49	2.46
21	NC	30.36	13.44	2.45
21	NC	28.85	12.83	2.43
21	NC	28.55	12.92	2.40
22	NC	23.51	8.71	2.20
22	NC	23.35	9.80	2.22
22	NC	22.71	10.01	2.17
22	NC	25.10	8.98	2.20
23	A	24.79	13.68	1.88
23	A	26.06	14.08	1.96
23	A	25.37	15.81	2.02
23	A	25.83	14.33	1.97
24	A	35.69	15.63	2.37
24	A	36.68	15.44	2.40
24	A	37.01	15.06	2.44
24	A	36.13	14.82	2.30
25	ST	24.04	10.91	1.71
25	ST	25.97	11.54	1.73
25	ST	24.38	10.62	1.88
25	ST	25.29	11.06	1.82
26	A	25.27	13.15	2.01
26	A	23.92	13.02	1.89
26	A	23.61	12.60	2.05
26	A	26.42	12.85	2.15
27	M	29.36	17.41	2.36
27	M	30.41	17.87	2.43
27	M	30.50	17.89	2.48
27	M	31.63	18.00	2.51
28	ST	20.06	9.41	2.48
28	ST	20.07	9.86	2.86
28	ST	20.14	9.24	3.00
28	ST	19.88	9.03	2.92
29	ST	18.62	8.36	2.95
29	ST	18.64	9.03	2.99
29	ST	18.78	7.95	2.76
29	ST	19.06	8.35	2.84
30	ST	17.62	8.09	1.71
30	ST	18.35	8.61	1.55

TABLE A-10: CONTINUED.

FEMALE	CONDITION	PROTEIN	LIPID	CARBOHYDRATE
			mg per million eggs.	
30	ST	18.56	7.83	1.31
30	ST	16.94	7.70	1.00
31	M	26.29	12.86	1.64
31	M	26.59	13.03	1.77
31	M	26.06	14.91	2.38
31	M	26.62	13.67	2.42
32	M	29.52	13.38	5.76
32	M	32.46	13.79	5.96
32	M	32.37	15.25	5.65
32	M	31.08	15.40	5.63

TABLE A-11

Protein, lipid and carbohyrate content of three day old
larvae obtained from females of <u>Placopecten magellanicus</u>
in conditioning experiment 4.

FEMALE	CONDITION	% RECOVERY	PROTEIN	LIPID	CARBOHYDRATE
				mg per million larvae	
11	A	15.7	18.29	7.91	0.90
11	A	15.7	18.41	6.56	0.98
11	A	15.7	19.67	6.98	1.04
11	A	15.7	20.34	7.32	1.10
12	M	13.4	18.92	6.24	0.82
12	M	13.4	10.24	7.35	0.90
12	M	13.4	17.31	6.85	0.93
12	M	13.4	19.04	7.04	0.98
13	M	9.8	18.46	5.22	1.32
13	M	9.8	17.88	5.84	1.44
13	M	9.8	19.06	6.04	1.04
13	M	9.8	18.72	5.77	1.83
14	ST	6.6	13.38	5.79	0.92
14	ST	6.6	15.37	5.43	0.98
14	ST	6.6	16.67	4.91	1.02
14	ST	6.6	16.03	5.82	1.14
15	A	23.8	16.22	5.44	0.69
15	A	23.8	15.44	6.36	0.80
15	A	23.8	16.97	6.88	0.73
15	A	23.8	16.70	5.84	0.83
16	A	24.6	14.41	5.82	0.99
16	A	24.6	14.96	5.30	1.05
16	A	24.6	13.87	4.87	1.02
16	A	24.6	14.59	4.96	0.95
17	ST	11.3	21.94	4.25	1.40
17	ST	11.3	20.88	4.67	0.98
17	ST	11.3	18.35	4.87	0.87
17	ST	11.3	16.39	5.21	0.93
18	M	21.6	18.53	6.00	0.62
18	M	21.6	16.47	6.74	0.74
18	M	21.6	17.87	5.32	0.82
18	M	21.6	16.38	5.46	0.75
19	NC	32.4	9.08	4.72	0.89
19	NC	32.4	11.25	5.03	1.07
19	NC	32.4	12.35	4.41	0.93
19	NC	32.4	12.75	3.89	0.93
20	NC	20.6	14.05	3.21	1.01
20	NC	20.6	15.07	3.46	1.07
20	NC	20.6	16.33	3.87	1.15
20	NC	20.6	15.87	3.15	1.27

FEMALE	CONDITION	% RECOVERY	PROTEIN	LIPID	CARBOHYDRATE
			mg per million larvae		
21	NC	41.2	9.27	6.07	0.80
21	NC	41.2	12.36	5.46	0.91
21	NC	41.2	14.36	5.87	1.06
21	NC	41.2	14.36	5.39	1.00
23	A	38.9	14.67	6.78	1.27
23	A	38.9	14.78	7.20	1.34
23	A	38.9	15.24	6.94	1.19
23	A	38.9	15.84	6.54	1.24
24	A	45.6	14.58	4.88	1.63
24	A	45.6	13.68	4.74	1.75
24	A	45.6	14.96	5.43	1.44
24	A	45.6	15.83	5.06	1.53
25	ST	5.4	12.65	4.12	1.10
25	ST	5.4	12.96	4.30	1.03
25	ST	5.4	11.46	3.54	0.96
25	ST	5.4	11.56	3.55	0.89
26	A	49.6	15.73	6.34	1.35
26	A	49.6	15.84	6.63	1.46
26	A	49.6	16.27	7.02	1.44
26	A	49.6	16.34	7.06	1.48
27	M	66.3	13.52	9.78	1.34
27	M	66.3	13.50	8.64	1.46
27	M	66.3	14.47	8.10	1.36
27	M	66.3	14.38	8.05	1.30
28	ST	12.4	14.08	4.18	0.89
28	ST	12.4	14.64	4.38	0.93
28	ST	12.4	13.69	3.99	1.03
28	ST	12.4	13.43	4.07	1.07
29	ST	8.6	18.74	3.81	1.55
29	ST	8.6	16.44	4.03	1.64
29	ST	8.6	15.78	4.08	1.84
29	ST	8.6	14.70	3.78	1.45
30	ST	9.2	24.83	3.45	1.38
30	ST	9.2	23.94	3.63	1.38
30	ST	9.2	13.66	4.05	1.05
30	ST	9.2	13.43	4.33	1.10
31	M	58.9	8.16	4.38	1.23
31	M	58.9	8.35	4.46	1.32
31	M	58.9	10.67	4.85	1.46
31	M	58.9	10.68	5.23	1.50
32	M	60.9	15.66	3.54	0.86
32	M	60.9	15.88	3.85	0.94
32	M	60.9	16.07	4.65	1.24
32	M	60.9	14.99	5.01	1.25

TABLE A-12

Filtration rates of groups of juveniles of <u>Placopecten</u>
<u>magellanicus</u> at various cell concentrations of <u>Isochrysis</u>
<u>galbana</u> (container volume= 2000 ml).

TEMP	INITIAL CELL CONCENTRATION		DRY WT*	DURATION	FILTRATION RATE	
	MEAN	S.D.			MEAN	S.D.
°C	cells/ml		(mg)	hours	ml/h/animal	
9.0	11002.6	324.6	36.50	8.0	116.6	32.7
9.0	10876.4	567.8	36.60	8.0	53.9	18.9
9.0	10563.2	259.5	39.13	8.0	120.2	13.9
9.0	10721.0	894.2	44.16	8.0	74.3	38.4
9.0	10643.2	342.8	14.78	8.0	8.8	4.6
9.0	10546.3	365.8	19.55	8.0	49.9	8.9
9.0	10835.1	26.9	19.94	8.0	44.8	7.7
9.0	10974.0	572.5	18.99	8.0	5.7	4.4
10.0	10231.7	329.9	36.52	9.5	130.6	38.8
10.0	11000.6	712.3	36.62	9.5	71.5	16.6
10.0	11125.0	915.4	39.13	9.5	164.6	30.8
10.0	10027.3	138.5	44.16	9.5	103.5	12.8
10.0	10624.6	784.6	14.78	9.5	31.4	1.4
10.0	11032.6	1145.3	19.55	9.5	88.9	8.2
10.0	10934.6	673.5	19.94	9.5	64.1	21.8
10.0	10734.9	238.1	18.99	9.5	12.0	10.6
9.0	12721.9	1349.6	35.38	9.0	124.6	28.9
9.0	10387.4	395.1	35.07	9.0	112.5	31.6
9.0	10826.7	39.7	37.26	9.0	84.6	12.0
9.0	10954.7	925.0	39.08	9.0	106.8	25.8
9.0	11547.0	259.8	17.88	9.0	52.8	30.1
9.0	10563.6	349.1	19.78	9.0	36.6	8.9
9.0	10627.9	953.4	16.44	9.0	64.9	7.3
9.0	11673.9	1369.6	18.77	9.0	80.4	9.1
8.0	10359.0	213.7	35.07	9.0	74.3	8.3
8.0	10763.9	927.4	35.38	9.0	143.7	49.8
8.0	10359.2	67.3	37.26	9.0	106.4	12.5
8.0	11306.9	982.3	39.02	9.0	83.8	14.9
8.0	10264.8	36.9	17.88	9.0	53.8	19.6
8.0	10097.3	643.3	19.72	9.0	49.6	22.8
8.0	10578.3	218.0	16.44	9.0	20.4	8.0
8.0	11025.8	879.4	18.77	9.0	26.3	14.6
9.0	5238.1	79.4	31.08	10.0	148.6	27.6
9.0	5340.7	129.4	35.17	10.0	119.9	20.1
9.0	5265.0	63.3	36.62	10.0	205.7	14.3
9.0	5523.9	82.6	42.91	10.0	55.1	8.7
9.0	5061.2	99.1	19.16	10.0	3.0	0.3

TABLE A-12: CONTINUED.

TEMP °C	INITIAL CELL CONCENTRATION cells/ml MEAN	S.D.	DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal MEAN	S.D.
9.0	5629.3	267.2	22.77	10.0	37.1	9.4
9.0	5529.5	357.3	18.24	10.0	55.6	9.0
9.0	5086.9	981.4	17.12	10.0	3.6	0.8
9.0	4874.3	209.1	31.08	7.5	276.0	32.6
9.0	4996.1	638.2	35.17	7.5	143.9	27.7
9.0	4638.5	498.6	36.12	7.5	200.4	43.9
9.0	4895.3	129.5	42.91	7.5	79.3	20.1
9.0	4904.9	398.4	19.16	7.5	21.0	2.0
9.0	4884.8	294.0	22.77	7.5	41.7	8.3
9.0	5019.3	106.9	18.24	7.5	66.6	8.0
9.0	4873.8	129.7	17.12	7.5	14.7	2.1
10.0	4719.2	598.0	42.66	7.0	212.6	39.4
10.0	4934.5	94.8	36.41	7.0	184.8	40.6
10.0	4893.2	490.6	38.79	7.0	136.5	21.9
10.0	5023.9	104.7	33.40	7.0	175.6	28.8
10.0	4970.1	249.5	17.37	7.0	42.9	19.6
10.0	4948.3	403.2	21.74	7.0	38.6	12.4
10.0	5106.7	293.4	20.06	7.0	19.4	8.5
10.0	5174.9	329.4	19.60	7.0	54.6	4.9
10.0	5209.6	269.4	42.66	10.0	303.8	50.9
10.0	5197.4	320.5	36.41	10.0	288.4	40.6
10.0	5301.1	560.6	38.79	10.0	226.4	36.9
10.0	5248.2	390.4	33.40	10.0	184.7	39.4
10.0	5164.9	264.1	17.37	10.0	53.3	8.9
10.0	5238.7	305.2	21.74	10.0	40.5	2.9
10.0	5199.3	286.7	20.06	10.0	69.0	14.2
10.0	5236.8	395.3	19.60	10.0	58.2	10.4
10.0	15993.5	934.6	37.69	9.0	272.6	31.1
10.0	16001.2	1035.0	42.41	9.0	208.9	39.6
10.0	15438.5	527.3	38.13	9.0	258.9	68.9
10.0	16230.8	1098.4	33.69	9.0	115.6	20.7
10.0	15433.0	345.7	18.77	9.0	88.4	12.9
10.0	15835.9	739.5	19.21	9.0	92.4	20.6
10.0	16215.4	834.6	19.72	9.0	15.6	2.8
10.0	15552.8	793.2	16.21	9.0	114.9	24.8
9.0	15932.0	438.2	37.69	9.5	306.9	41.4
9.0	16024.6	739.4	42.41	9.5	246.8	36.7
9.0	15290.4	376.7	38.13	9.5	272.9	29.6
9.0	15349.3	532.8	33.69	9.5	188.5	63.8
9.0	14983.7	765.3	18.77	9.5	106.4	21.0
9.0	15185.9	386.5	19.21	9.5	90.8	12.5

TABLE A-12: CONTINUED.

TEMP $°C$	INITIAL CELL CONCENTRATION cells/ml		DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal	
	MEAN	S.D.			MEAN	S.D.
9.0	14873.2	297.4	19.72	9.5	133.8	7.9
9.0	15398.5	667.0	16.21	9.5	143.5	18.9
9.0	15344.7	592.6	29.09	9.0	386.9	41.6
9.0	15648.9	309.4	27.14	9.0	208.1	64.8
9.0	16103.7	1103.8	38.79	9.0	272.3	59.1
9.0	15834.9	821.5	34.17	9.0	300.4	39.1
9.0	14993.6	246.2	19.72	9.0	81.7	24.1
9.0	15395.1	654.9	23.64	9.0	78.8	29.3
9.0	15249.4	378.2	20.35	9.0	101.4	31.0
9.0	16023.9	891.3	21.81	9.0	23.9	8.6
10.0	15209.3	104.6	29.09	8.5	346.3	72.1
10.0	15934.8	870.3	27.14	8.5	258.4	63.6
10.0	16032.7	994.0	38.79	8.5	268.8	56.7
10.0	16103.6	885.5	34.18	8.5	196.5	28.0
10.0	15349.5	409.6	19.72	8.5	84.3	16.9
10.0	15634.2	193.2	23.64	8.5	101.2	20.9
10.0	24985.9	652.0	20.35	8.5	74.4	18.8
10.0	15673.7	723.9	21.81	8.5	150.5	59.4
10.0	25402.1	1004.3	31.98	8.5	214.9	26.7
10.0	24965.4	104.8	30.63	8.5	144.7	14.3
10.0	25934.9	566.3	38.24	8.5	238.5	22.6
10.0	25749.0	399.5	35.68	8.5	65.3	10.5
10.0	24864.3	229.2	19.21	8.5	44.4	7.8
10.0	25003.4	34.6	21.13	8.5	47.8	9.9
10.0	25443.9	409.4	18.29	8.5	51.7	8.1
10.0	25736.3	632.7	16.26	8.5	6.5	5.6
9.0	24568.1	660.2	31.98	9.0	196.1	42.7
9.0	25390.2	227.6	30.63	9.0	97.8	15.4
9.0	25087.3	115.8	38.24	9.0	205.8	44.2
9.0	25534.8	745.9	35.68	9.0	86.2	5.8
9.0	24886.0	492.5	19.21	9.0	5.9	2.4
9.0	25340.7	287.4	21.13	9.0	94.9	21.8
9.0	25984.1	1104.7	18.29	9.0	94.5	36.7
9.0	25379.3	340.6	16.26	9.0	5.6	4.5
10.0	25901.7	766.0	39.47	8.5	247.3	21.7
10.0	24885.5	330.4	44.93	8.5	184.4	17.3
10.0	25003.5	100.8	43.28	8.5	136.5	40.4

TABLE A-12: CONTINUED.

TEMP °C	INITIAL CELL CONCENTRATION cells/ml		DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal	
	MEAN	S.D.			MEAN	S.D.
10.0	25443.8	298.6	35.68	8.5	137.1	11.8
10.0	25632.0	568.3	19.05	8.5	21.5	4.1
10.0	24560.2	743.9	21.74	8.5	83.3	8.1
10.0	26109.4	997.1	18.72	8.5	24.1	3.5
10.0	25395.7	395.3	20.94	8.5	7.5	3.9
10.0	25009.3	230.5	39.47	8.0	236.4	29.6
10.0	25492.6	802.5	44.93	8.0	178.6	29.4
10.0	26038.9	208.4	43.28	8.0	159.4	31.6
10.0	25600.2	304.6	35.68	8.0	189.4	38.8
10.0	25304.6	209.8	19.05	8.0	29.4	7.2
10.0	25093.5	459.2	21.74	8.0	38.4	9.6
10.0	25993.5	309.8	18.72	8.0	41.4	12.9
10.0	24985.0	201.1	20.94	8.0	7.6	3.7

* Dry weight estimated by regression equation.
ln DRY WEIGHT = -1.16 + 0.143 (HEIGHT) + 0.144 (LENGTH)
R^2 = 88.1%

Filtration rates of juveniles of <u>Placopecten magellanicus</u>
at various cell concentrations of <u>Ischrysis galbana</u>
(container volume= 25 ml).

TEMP °C	INITIAL CELL CONCENTRATION cells/ml		DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal	
	MEAN	S.D.			MEAN	S.D.
12.0	6397.0		8.9	3.0	1.8	0.5
12.0	5771.3		6.7	3.0	2.8	1.6
12.0	5783.3		5.1	3.0	1.4	0.7
12.0	5686.7		8.4	3.0	1.5	1.5
12.0	5683.3		5.4	3.0	5.0	3.6
12.0	5935.3		6.3	3.0	3.6	2.9
12.0	5020.7		7.5	3.0	3.6	2.3
12.0	5636.0		6.3	3.0	6.4	3.6
12.0	6256.7		8.9	3.0	4.5	2.2
12.0	6496.0		6.7	3.0	1.9	1.6
12.0	6028.0		5.1	3.0	0.7	0.7
12.0	6171.0		8.4	3.0	2.4	1.0
12.0	6164.0		5.4	3.0	2.6	0.6
12.0	6110.7		6.3	3.0	2.8	1.6
12.0	6060.7		7.5	3.0	2.5	1.0
12.0	6092.7		6.3	3.0	4.4	1.2
12.0	6533.5	70.0	7.1	6.0	3.1	1.7
12.0	7064.0	48.1	6.7	6.0	13.3	6.3
12.0	6485.4	278.0	5.6	6.0	2.6	1.1
12.0	5805.0	279.6	6.8	6.0	2.2	0.8
12.0	5067.3	82.7	6.6	6.0	3.9	1.6
12.0	5013.0	92.9	8.4	6.0	3.2	1.7
12.0	5363.5	423.6	5.8	6.0	8.4	6.3
12.0	6500.0	93.3	6.2	6.0	2.7	1.4
10.5	6045.0	73.9	7.4	6.0	3.7	1.5
10.5	5930.7	214.9	8.7	6.0	2.7	2.7
10.5	6162.7	116.9	6.6	6.0	4.9	2.7
10.5	5768.0	79.2	4.7	6.0	3.0	1.4
10.5	6091.7	116.5	6.5	6.0	7.6	4.2
10.5	6615.3	17.0	7.6	6.0	2.3	1.4
10.5	6221.7	512.4	6.4	6.0	2.0	0.7
10.5	6193.0	245.6	5.9	6.0	2.4	1.2
10.5	5209.7	154.2	5.4	6.0	2.2	0.6
10.5	5931.7	93.8	5.0	6.0	3.7	2.6
10.5	6460.0	77.4	7.4	6.0	5.5	3.5
10.5	5894.3	52.3	5.5	6.0	3.4	1.6

TABLE A-13: CONTINUED.

TEMP °C	INITIAL CELL CONCENTRATION cells/ml MEAN	S.D.	DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal MEAN	S.D.
10.5	5968.4	403.9	7.4	6.0	2.9	2.9
10.5	5740.0	411.9	6.4	6.0	4.2	3.0
10.5	5623.7	240.9	6.5	6.0	3.8	1.9
10.0	5637.2	178.6	6.5	6.0	16.2	10.0
10.0	5916.9	472.6	5.7	6.0	10.8	5.9
10.0	12137.7	24.9	5.8	6.0	6.9	5.8
10.0	10830.7	106.6	6.2	6.0	25.5	10.6
10.0	11473.5	1007.6	7.1	6.0	17.6	14.9
10.0	9636.7	128.7	5.7	6.0	20.8	12.7
10.0	11008.0	558.7	3.1	6.0	20.1	15.6
10.0	11117.0	594.4	6.3	6.0	13.4	9.5
10.0	10104.0	375.3	6.1	6.0	14.4	10.3
10.0	10154.0	154.7	8.4	6.0	12.7	12.7
12.0	11872.0	150.7	5.8	6.0	24.9	9.7
12.0	11653.0	88.3	7.4	6.0	15.9	14.6
12.0	10558.7	213.4	5.9	6.0	23.4	11.6
12.0	10764.3	186.0	6.6	6.0	9.7	5.6
12.0	11863.0	791.2	5.8	6.0	19.1	17.4
12.0	10800.0	226.1	5.9	6.0	11.6	8.3
12.0	11537.3	453.2	4.4	6.0	16.6	7.3
12.0	10755.4	366.8	5.8	6.0	29.1	13.9
12.0	11653.3	246.1	5.4	6.0	13.8	6.7
12.0	10558.9	362.6	6.2	6.0	18.4	5.2
12.0	11082.4	88.9	6.2	6.0	16.6	8.9
12.0	10088.2	72.6	5.8	6.0	26.6	17.4
12.0	10696.5	184.8	5.0	6.0	3.5	1.8
12.0	10600.7	172.2	8.9	6.0	19.3	5.7
12.0	11749.8	344.0	6.7	6.0	26.8	15.4
12.0	10154.0	586.5	6.0	6.0	4.6	2.8
12.5	12120.4	120.8	5.3	6.0	18.6	9.9
12.5	12155.8	300.6	4.9	6.0	16.2	9.2
12.5	11863.3	751.4	7.0	6.0	7.3	5.8
12.5	10314.0	806.5		5.4	6.0	
12.5	10906.6	86.2	7.3	6.0	20.9	15.9
12.5	10764.8	442.9	6.2	6.0	10.5	7.5
12.5	12186.8	156.7	6.6	6.0	9.2	8.6
12.5	11030.2	59.3	5.7	6.0	12.8	4.1
12.5	16974.7	238.1	9.1	6.0	30.8	8.3
12.5	15491.3	786.7	6.3	6.0	6.0	1.4

TABLE A-13: CONTINUED.

TEMP °C	INITIAL CELL CONCENTRATION cells/ml		DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal	
	MEAN	S.D.			MEAN	S.D.
12.5	15717.5	862.2	8.0	6.0	12.1	8.9
12.5	14656.7	429.3	6.5	6.0	67.5	28.1
12.5	15991.8	672.5	6.5	6.0	9.1	5.8
12.5	15337.9	528.2	4.8	6.0	17.5	9.5
12.5	15932.6	469.6	6.4	6.0	59.4	33.2
12.5	16325.3	602.4	9.1	6.0	46.7	19.2
12.5	15994.2	86.1	6.6	6.0	14.6	2.4
12.5	15829.1	954.8	5.0	6.0	42.8	46.8
14.5	15829.6	954.2	6.6	6.0	44.6	30.9
14.5	15300.8	765.8	6.3	6.0	32.2	6.8
14.5	16024.9	594.1	7.0	6.0	9.4	3.3
14.5	15461.2	639.8	9.4	6.0	13.4	9.6
14.5	16099.1	728.2	6.1	6.0	19.3	9.1
14.5	15563.0	228.9	5.3	6.0	40.8	25.2
14.5	14934.6	244.4	6.5	6.0	49.5	28.2
14.5	16133.4	815.5	6.7	6.0	59.9	13.3
14.5	15392.6	749.5	6.3	6.0	14.6	8.3
14.5	15736.3	826.5	6.1	6.0	2.8	1.2
13.5	15196.3	104.6	6.1	6.0	26.3	12.7
13.5	15104.2	749.8	8.5	6.0	35.2	17.1
13.5	15749.0	374.2	6.2	6.0	12.8	8.3
13.5	15375.9	454.1	7.2	6.0	68.4	36.6
13.5	15467.7	367.9	6.2	6.0	46.4	21.7
13.5	15154.9	857.5	5.8	6.0	27.5	14.3
13.5	15875.1	607.2	6.9	6.0	17.6	7.3
13.5	16026.3	960.2	6.8	6.0	28.4	16.7
13.5	16960.2	862.7	7.2	6.0	20.5	22.5
13.5	16645.5	999.0	7.0	6.0	36.4	17.7
13.5	20790.1	662.2	6.1	6.0	22.3	9.5
13.5	19662.4	810.4	4.7	6.0	33.0	17.5
13.5	20626.5	696.0	6.4	6.0	26.1	5.6
13.5	20540.3	486.2	7.4	6.0	52.8	11.7
13.5	21323.2	276.6	6.6	6.0	61.0	15.8
13.5	20738.7	202.9	6.5	6.0	23.6	17.9
13.5	20918.8	953.7	8.1	6.0	37.8	26.5
13.5	20855.9	1664.5	6.8	6.0	58.1	37.1

TABLE A-13: CONTINUED.

TEMP	INITIAL CELL CONCENTRATION		DRY WT*	DURATION	FILTRATION RATE	
°C	cells/ml		(mg)	hours	ml/h/animal	
	MEAN	S.D.			MEAN	S.D.
13.5	21158.7	1223.4	8.4	6.0	13.8	2.6
13.5	20767.6	540.3	5.5	6.0	19.0	8.9
14.5	21002.5	898.5	6.0	6.0	31.1	13.4
14.5	21130.2	972.2	6.6	6.0	21.3	10.4
14.5	20743.9	1323.3	5.9	6.0	34.4	24.0
14.5	21000.5	334.2	6.1	6.0	12.0	7.7
14.5	20748.8	881.7	7.7	6.0	7.8	2.2
14.5	20672.0	836.9	6.9	6.0	14.7	6.1
14.5	20767.4	918.6	6.3	6.0	24.2	1.3
14.5	20714.6	855.1	7.1	6.0	3.9	2.1
14.5	20720.7	1158.1	6.6	6.0	9.3	7.0
14.5	20583.3	767.4	5.9	6.0	10.3	9.3

* Dry weight estimated by regression equation.
ln DRY WEIGHT = -1.16 + 0.143 (HEIGHT) + 0.144 (LENTH)
R^2 = 88.1%

Filtration rates of juveniles of <u>Placopecten magellanicus</u> at various cell concentrations of <u>Isochrysis galbana</u> (container volume= 2000 ml).

TEMP °C	INITIAL CELL CONCENTRATION cells/ml		DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal	
	MEAN	S.D.			MEAN	S.D.
10.5	5689.4	540.7	779.1	2.5	1182.6	533.8
10.5	5532.3	329.8	639.7	6.0	1638.8	801.7
10.5	5877.5	697.7	579.6	6.0	2050.2	1148.7
10.5	5774.2	525.8	740.6	6.0	759.9	333.2
10.5	5533.3	284.0	250.4	6.0	408.5	417.7
10.5	5523.7	904.4	231.9	6.0	268.1	321.3
10.5	5272.7	14.0	276.4	6.0	612.2	450.8
10.5	5729.0	541.7	135.5	6.0	367.7	278.0
10.0	5482.0	268.4	642.5	9.5	1032.3	1082.0
10.0	5743.8	372.4	693.7	9.5	812.2	576.9
10.0	6037.8	450.4	575.6	9.5	535.1	316.2
10.0	5724.3	625.3	588.6	9.5	996.8	502.7
10.0	5408.7		264.4	9.5	208.2	121.9
10.0	5564.0	172.7	190.3	9.5	290.3	153.8
10.0	5872.9	381.8	285.4	9.5	615.4	527.7
10.0	6126.2	636.4	288.6	9.5	478.8	416.3
12.0	6259.0	491.8	635.4	7.0	2078.7	1240.4
12.0	6053.7	331.0	224.3	7.0	534.9	382.8
12.0	6144.8	393.3	701.4	7.0	2604.3	1449.9
12.0	5798.8	108.9	315.0	7.0	872.5	795.8
12.0	6543.5	808.0	582.1	7.0	1284.4	820.7
12.0	6714.9	771.8	264.4	7.0	983.6	580.8
12.0	7178.9	1166.4	315.0	7.0	1229.4	877.8
8.0	6332.0	334.8	845.2	7.5	704.9	1132.6
8.0	5120.8	316.2	701.4	7.5	326.9	278.4
8.0	6339.2	317.8	635.4	7.5	550.0	408.4
8.0	5667.9	608.1	835.9	7.5	1388.8	1151.9
8.0	6338.2	312.9	452.4	7.5	240.4	126.3
8.0	6495.9	281.2	371.3	7.5	296.7	174.4
8.0	6410.4	75.7	412.8	7.5	305.4	350.7
8.0	6208.7	208.7	412.8	7.5	687.7	350.7
10.0	12621.2	1307.2	493.9	8.5	2388.5	833.4
10.0	11488.7	1101.6	693.7	10.0	1605.2	896.3
10.0	10972.5	572.0	409.8	9.5	955.3	378.3
10.0	12020.8	1116.7	635.4	10.0	2672.0	1392.3
10.0	11135.7	1161.7	242.2	10.0	261.0	284.9

TABLE A 14: CONTINUED

TEMP	INITIAL CELL CONCENTRATION cells/ml		DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal	
°C	MEAN	S.D.			MEAN	S.D.
10.0	11473.2	1343.3	244.9	10.0	1562.6	1016.3
10.0	10924.9	815.6	190.3	10.0	321.2	187.7
10.0	11854.7	1816.2	264.4	10.0	390.2	216.4
10.0	10379.3	473.3	447.4	8.5	816.9	309.9
10.0	11380.2	1130.5	447.4	8.5	675.9	455.3
10.0	10559.3	356.9	412.2	8.5	602.7	382.7
10.0	10905.0	822.5	322.1	8.5	1141.4	752.6
10.0	10658		190.3	8.5	242.9	213.7
10.0	10646.0	34.7	188.2	8.5	343.3	299.3
10.0	10313.4	290.4	224.3	8.5	501.8	307.3
10.0	9714.2	176.9	242.2	8.5	708.3	323.2
10.0	10247.7	25.9	156.2	9.0	170.1	118.4
10.0	10721.9	825.9	452.4	9.0	1492.1	663.9
10.0	10015.9	778.9	488.4	9.0	2803.9	555.9
10.0	11853.5	2632.9	409.8	9.0	791.4	508.6
10.0	10556.4	222.7	533.2	9.0	501.4	253.9
10.0	10837.4	441.0	499.9	9.0	1730.4	935.3
10.0	10558.7	152.5	156.2	9.0	442.2	223.7
10.0	10336.0	323.0	188.2	9.0	144.9	96.5
10.0	10066.0	121.0	190.3	9.0	158.9	85.0
10.0	10330.2	276.7	172.4	9.0	334.4	164.1
11.0	14610.9	954.4	267.3	9.0	594.7	354.5
11.0	13781.4	915.4	409.8	9.0	353.4	360.8
11.0	14691.0	928.9	379.6	9.0	720.8	521.2
11.0	15895.4	1558.1	313.9	9.0	2075.3	790.3
11.0	14322.6	824.6	317.4	9.0	147.7	118.3
11.0	15213.0	996.1	409.8	9.0	4164.0	462.1
11.0	15924.2	2596.8	371.3	9.0	662.4	337.2
11.0	14978.0	1430.1	317.4	9.0	742.4	371.9
11.0	14678.4	1183.6	264.4	9.0	554.7	379.7
11.0	14264.7	674.7	205.5	9.0	860.7	624.8
10	18110.7	328.7	488.4	4.5	2405.0	1027.5
10	16716.5	1301.2	447.4	9.5	2456.9	668.9
10	17302.0	1497.1	317.4	9.5	2133.6	1390.6
10	16073.7	1497.0	447.4	9.5	646.1	512.0
10	16073.7	952.9	379.6	9.5	1278.0	741.9
10	15078.7	480.0	347.7	9.5	282.3	209.3
10	15348.7	2197.9	447.4	9.5	1508.2	1159.7
10	15176.0	809.7	347.7	9.5	219.1	161.3
10	15595.4	2192.3	409.8	9.5	1600.4	971.3
10	14052.7	616.7	317.4	9.5	58.4	41.9

TABLE A-14: CONTINUED.

TEMP °C	INITIAL CELL CONCENTRATION cells/ml		DRY WT* (mg)	DURATION hours	FILTRATION RATE ml/h/animal	
	MEAN	S.D.			MEAN	S.D.
9.0	15736.0	504.0	414.4	9.5	271.6	206.6
9.0	16991.0	1315.0	447.4	9.5	150.1	141.7
9.0	15678.0	9.8	375.4	9.5	185.5	102.8
9.0	15302.0	214.7	488.4	9.5	261.8	188.3
9.0	15628.4	293.7	408.3	9.5	197.3	147.1
9.0	16209.0	891.7	347.7	9.5	299.5	189.9
9.0	15200.0	168.2	264.4	9.5	115.4	81.8
9.0	15199.7	287.1	318.5	9.5	187.8	206.6
9.0	15166.5	142.2	264.4	9.5	132.6	181.0
9.0	14835.7	346.5	244.9	9.5	246.5	154.3
10.0	21833.5	1062.0	379.6	9.5	1625.8	1070.6
10.0	22809.0	766.1	244.9	9.0	1625.8	1070.6
10.0	23741.7	2652.9	447.4	9.0	1160.5	473.9
10.0	22010.7		457.5	9.0	168.5	204.0
10.0	22612.7	274.4	288.6	9.0	284.8	147.9
10.0	21240.7	1024.9	347.7	9.0	501.5	263.2
10.0	22260.7		242.2	9.0	272.7	264.7
10.0	22365.2	1366.1	313.9	9.0	1004.6	472.9
10.0	21464.4	480.3	156.7	9.0	263.7	164.3
10.0	21565.3		188.2	9.0	91.9	60.7
10.5	21557.7	1212.3	493.9	10.0	534.8	362.4
10.5	21815.3	905.3	269.4	10.0	667.4	590.2
10.5	21630.4	648.4	318.5	10.0	1324.8	956.2
10.5	22388.9	1781.9	318.5	10.0	1156.8	706.6
10.5	22715.0	1367.1	244.9	10.0	988.7	782.1
10.5	23618.0	504.4	375.4	10.0	472.2	388.1
10.5	23231.0	1290.5	375.4	10.0	525.4	496.4
10.5	20708.0		221.8	10.0	128.3	101.7
10.5	21364.0	467.1	205.5	10.0	146.6	87.3
10.5	23302.9	2008.9	244.9	10.0	535.6	327.1
10.5	28140.0		264.4	7.0	157.6	78.8
10.5	26390.5	1887.3	375.4	7.0	754.2	730.7
10.5	28064.7		244.9	7.0	255.8	10.6
10.5	27709.3		347.7	7.0	260.4	310.5

TABLE A-14: CONTINUED.

TEMP	INITIAL CELL CONCENTRATION		DRY WT*	DURATION	FILTRATION RATE	
°C	cells/ml		(mg)	hours	ml/h/animal	
	MEAN	S.D.			MEAN	S.D.
10.5	27837.9	174.2	347.7	7.0	415.2	137.1
10.5	27641.4	403.5	343.9	7.0	857.5	675.3
10.5	28099.3		188.2	7.0	90.5	72.1
10.5	27873.3		318.6	7.0	411.1	328.8
10.5	27906.0		188.2	7.0	45.2	48.8
10.5	30643.3		157.9	7.0	900.9	566.4
10.5	27344.4	2059.9	452.4	10.5	700.1	575.5
10.5	25757.6	1059.2	582.1	10.5	510.8	248.3
10.5	26725.2	2440.7	588.6	10.5	1475.4	1200.9
10.5	26845.5	1658.8	533.2	10.5	700.1	575.5
10.5	27136.0	231.9	533.2	10.5	242.9	201.5
10.5	26763.8	162.8	447.4	10.5	470.7	266.7
10.5	26524.7	2831.9	375.4	10.5	1810.0	822.8
10.5	26532.7		267.3	10.5	150.9	111.6
10.5	25990.4	979.1	291.8	10.5	256.5	247.3
10.5	27017.4	1650.3	294.0	10.5	2492.8	1717.2

* Dry weight estimated from regression equation
ln DRY WEIGHT = 2.53 + 0.0766 (HEIGHT) + 0.0111 (LENGTH)
R^2 = 97.2%

TABLE A-15

Protein, lipid and carbohydrate content of Placopecten
magellanicus juveniles obtained from spat collectors in
Little Bay, Newfoundland, on August 31, 1983.

HEIGHT	LENGTH	ESTIMATED DRY WT*	PROTEIN	LIPID	CARBOHYDRATE
mm	mm	mg		mg per juvenile	
15.65	14.25	22.87	13.34	1.21	3.65
			13.18	1.19	3.40
16.80	15.55	32.51	6.30	1.02	2.55
			6.64	1.10	2.45
15.40	13.65	20.24	9.24	0.90	2.00
			9.42	0.85	2.0G
13.30	12.15	12.08	7.70	0.73	2.30
			7.94	0.80	2.30
11.15	10.50	7.00	6.02	0.51	1.33
			5.91	0.54	1.35
12.10	10.90	8.50	5.76	0.35	1.32
			5.78	0.35	1.22
11.20	10.30	6.85	4.72	0.54	1.13
			4.96	0.54	1.08
10.50	9.50	5.53	3.46	0.26	0.78
			3.20	0.29	0.95
18.40	17.20	51.83	15.84	2.23	4.57
			16.22	2.19	4.99
18.40	16.10	44.24	14.62	1.52	2.85
			14.26	1.61	1.35
15.60	14.50	23.54	5.44	0.74	1.37
			5.76	0.70	1.30
9.90	9.00	4.72	3.36	0.26	0.95
			3.43	0.27	1.19
11.00	10.15	6.52	2.54	0.17	1.01
			2.66	0.17	1.01
11.50	11.00	7.91	4.66	0.37	1.36
			4.68	0.35	1.13
12.50	11.25	9.46	3.20	0.27	1.13
			3.14	0.26	1.12
10.60	9.50	5.61	2.06	0.22	1.09
			2.19	0.23	1.00
11.00	10.40	6.76	3.20	0.33	1.01
			3.23	0.29	1.11
10.80	9.90	6.11	3.97	0.39	1.09
			4.14	0.38	0.99

TABLE A-15: CONTINUED.

HEIGHT	LENGTH	ESTIMATED DRY WT*	PROTEIN	LIPID	CARBOHYDRATE
mm	mm	mg		mg per juvenile	
11.45	10.40	7.21	4.40	0.32	1.49
			4.44	0.31	1.39
12.15	10.60	8.20	3.58	0.39	1.18
			3.60	0.40	1.48
11.70	10.85	7.97	5.95	0.43	1.75
			4.72	0.43	1.86
10.10	9.50	5.22	1.78	0.18	0.86
			1.88	0.18	0.86
9.75	9.10	4.69	2.28	0.22	0.95
			2.34	0.23	0.86
10.10	9.70	5.37	2.84	0.19	0.86
			2.75	0.20	0.98
10.05	9.20	4.96	4.44	0.21	0.74
			4.49	0.21	0.84
15.45	14.00	21.44	6.86	0.71	2.15
			6.72	0.66	2.24
9.7	8.9	4.52	1.70	0.14	0.94
			1.60	0.14	0.73
10.00	9.10	4.81	2.66	0.20	1.05
			2.66	0.20	1.12
8.50	7.80	3.25	1.44	0.16	0.37
			1.88	0.16	0.26
9.20	8.40	3.92	4.39	0.25	1.13
			4.56	0.26	0.82
8.00	7.25	2.80	1.35	0.16	0.56
			1.33	0.16	0.56
8.20	7.50	3.98	1.92	0.15	0.47
			1.82	0.14	0.37
8.40	7.90	3.25	2.59	0.15	0.63
			2.68	0.16	0.70
8.00	7.10	2.74	7.35	0.34	2.24
			7.68	0.34	2.20
16.50	15.70	31.83	13.74	0.86	3.25
			12.48	0.81	3.35
16.85	15.45	32.28	9.46	0.76	2.86
			9.76	0.77	2.98
18.00	16.55	44.58	11.98	1.01	3.86
			12.32	1.01	4.45
16.10	14.80	26.40	9.02	0.78	2.51
			9.26	0.84	2.50
17.05	15.35	32.74	10.12	0.80	3.37
			10.42	0.89	3.33
15.45	14.30	22.39	6.32	0.47	2.48
			6.62	0.49	2.49

TABLE A-15: CONTINUED.

HEIGHT mm	LENGTH mm	ESTIMATED DRY WT* mg	PROTEIN	LIPID	CARBOHYDRATE
			mg per juvenile		
16.25	14.50	25.84	15.94	0.70	2.36
			15.56	0.70	2.25
13.20	12.20	11.99	4.40	0.35	1.24
			5.52	0.35	1.36
13.20	12.60	12.71	8.88	0.44	1.63
			9.08	0.44	1.76
15.43	14.00	21.38	6.96	0.49	1.99
			7.12	0.49	1.89
10.20	9.50	5.29	3.00	0.14	0.81
			3.28	0.14	0.85
14.20	12.85	15.20	8.20	0.19	2.01
			8.20	0.22	1.90
9.90	9.20	4.86	2.60	0.14	0.62
			2.72	0.15	0.73
14.35	13.40	16.80	5.40	0.36	2.48
			5.80	0.39	2.38
16.85	15.50	32.51	7.96	0.76	2.87
			7.44	0.75	2.87
10.40	9.65	5.57	5.34	0.24	1.24
			5.15	0.25	1.25
13.60	12.40	13.07	4.52	0.55	1.87
			4.76	0.54	1.77
8.30	7.80	3.20	1.22	0.11	0.63
8.50	7.80	3.20	1.32	0.10	0.73
8.55	7.70	3.26	1.58	0.09	0.49
8.60	7.80	3.26	1.55	0.11	0.59
10.05	9.10	4.89	2.62	0.13	0.62
			2.75	0.13	0.52
18.45	17.20	52.20	11.97	1.10	3.86
			12.27	1.09	5.87
12.50	11.00	9.13	2.92	0.25	1.25
			3.01	0.22	1.27
13.60	12.60	13.45	4.05	0.31	1.51
			4.20	0.32	1.39
9.40	8.70	4.21	0.87	0.10	0.38
			0.99	0.10	0.49
13.20	12.20	11.99	2.83	0.21	1.03
			2.96	0.21	0.63

* ln DRY WEIGHT = -1.16 + 0.143 (HEIGHT) + 0.144 (LENGTH)
R^2 = 88.1%

TABLE A-16

Protein, lipid and carbohydrate levels in groups of two or
three juveniles of <u>Placopecten magellanicus</u> obtained
from spat collectors in Little Bay Newfoundland on July 5,
1984.

HEIGHT	LENGTH	ESTIMATED Dry wt*	PROTEIN	LIPID	CARBOHYDRATE
mm	mm	mg		µg per spat	
5.50	5.40	1.36	672.49	123.34	50.15
5.40	5.25	1.32	648.93	136.10	51.78
5.70	5.50	1.41			
4.30	4.20	1.02	72.20	60.01	21.10
4.50	4.40	1.07	74.57	56.00	20.89
4.15	3.85	0.95	54.59	41.78	29.97
4.00	3.95	0.96	57.36	41.78	30.58
3.85	3.80	0.92			
6.10	5.70	1.51	797.87	147.36	51.60
5.90	5.85	1.51	876.76	146.01	52.99
3.60	3.60	0.88	127.13	61.82	18.16
3.80	3.70	0.91	142.68	54.23	19.60
3.90	3.80	0.93			
7.30	7.00	2.04	521.79	200.58	69.25
			472.02	187.58	75.61
4.00	4.00	0.97	158.24	72.71	23.94
4.20	3.70	0.94	142.68	82.46	27.69
6.75	6.50	1.80	922.72	221.64	87.71
			874.50	215.57	79.16
3.90	4.00	0.96	144.36	58.67	27.64
4.00	4.10	0.98	168.36	59.11	26.89
4.00	4.00	0.97	168.36	73.46	28.92
4.30	4.10	1.01	160.12	72.04	29.22

* ln DRY WEIGHT = $-0.998 + 0.0878$ (HEIGHT) $+ 0.153$ (LENGTH)
$R^2 = 65.7\%$

Dry tissue weight and protein, lipid and carbohydrate
composition of algal species used for larval and adult
diets.

SPECIES	DRY WEIGHT mg per million cells ($\times 10^{-5}$)		PROTEIN	LIPID	CARBOHYDRATE
	MEAN	S.D.	mg per million cells		
I. galbana	2.42	0.29	8.31	4.65	1.31
			8.24	4.82	1.91
			8.01	4.74	1.10
			8.13	4.69	1.16
			12.52	3.16	0.48
			12.24	3.09	0.52
			12.08	2.97	0.47
			12.42	2.84	0.50
			9.09	5.89	1.23
			8.64	5.97	1.16
			8.76	5.95	1.58
			9.20	6.17	1.56
			10.90	6.97	1.53
			12.28	6.78	1.58
			12.18	6.73	1.70
			10.90	6.59	1.62
			12.89	6.24	1.29
			12.89	5.94	1.28
			12.59	6.16	1.35
			12.82	6.02	1.36
T-Iso	3.08	0.59	10.36	7.56	6.10
			10.43	7.33	6.08
			10.33	7.33	5.97
			10.59	7.10	6.02
			14.68	10.42	9.07
			16.74	10.63	8.35
			14.76	11.36	8.97
			16.28	11.20	8.68
			14.84	7.65	6.07
			14.49	7.48	5.91
			14.56	7.62	5.83
			14.56	7.65	5.84

TABLE A-17: CONTINUED.

| SPECIES | DRY WEIGHT mg per million cells ($x 10^{-5}$) | | PROTEIN | LIPID | CARBOHYDRATE |
	MEAN	S.D.	mg per million cells		
T. pseudonna			13.78	8.13	13.04
			14.54	7.69	13.28
			13.97	7.69	12.82
			14.16	8.46	12.91
			13.41	9.46	12.46
			12.02	8.72	13.73
			8.71	7.36	10.41
			9.24	8.41	11.52
			10.38	7.38	10.56
			9.46	9.57	15.38
			8.73	6.24	12.41
			9.90	8.48	14.36
C. calcitrns			12.67	8.39	40.15
			12.96	9.19	39.53
			14.04	8.39	38.02
			14.13	8.79	38.95
			51.38	18.42	66.17
			47.12	17.73	67.36
			48.83	17.33	64.40
			47.12	18.02	64.45
			58.73	19.02	53.70
			50.46	19.86	50.73
			60.03	22.41	56.38
			55.40	20.73	44.39
T. suecica	16.71	2.50	29.86	14.17	9.09
			29.86	13.89	8.96
			28.96	13.26	10.35
			29.14	14.43	9.85
			38.25	6.14	14.63
			37.68	6.57	16.42
			38.81	6.83	15.06
			39.99	6.84	16.31

TABLE A-18

Experiment 1 (Eggs): Two level nested ANOVAS testing for differences among conditions and females.

	Source variation	DF	MS	F	Sig F	R^2
PROTEIN	Among groups	4	544.74	6.35	0.0011	.881
	Among subgroups	112	2.59	33.11	0.0000	.504
	Within subgroups	25	85.73			
LIPID	Among groups	4	314.62	5.85	0.0018	.483
	Among subgroups	112	2.36	22.79	0.0000	.834
	Within subgroups	25	53.79			
CARB	Among groups	4	6.28	0.92	0.4694	.128
	Among subgroups	112	0.27	22.50	0.0000	.834
	Within subgroups	25	5.76			
TE	Among groups	4	1346157.4	12.33	0.0000	.664
	Among subgroups	112	29547.46	3.69	0.0000	.447
	Within subgroups	25	108956.16			

Experiment 1 (Larvae):

	Source variation	DF	MS	F	Sig F	R^2
PROTEIN	Among groups	4	266.12	5.97	0.0016	.488
	Among subgroups	90	10.26	61.41	0.0000	.945
	Within subgroups	25	44.61			
LIPID	Among groups	4	44.46	4.24	0.0093	.404
	Among subgroups	90	0.22	47.81	0.0000	.930
	Within subgroups	25	10.48			
CARB	Among groups	4	2.24	1.24	0.3177	.166
	Among subgroups	90	0.04	50.56	0.0000	.934
	Within subgroups	25	1.80			
TE	Among groups	4	413162.86	7.45	0.0004	.544
	Among subgroups	90	1060.11	52.32	0.0000	.936
	Within subgroups	25	55470.43			

Among groups (conditions)
Among subgroups (females)
Within groups (error)

Experiment 2 (Eggs): Two level nested ANOVAS testing for differences among conditions and females.

	Source variation	DF	MS	F	Sig F	R^2
PROTEIN	Among groups	4	288.18	4.59	0.0157	.586
	Among subgroups	54	2.33	26.94	0.0000	.866
	Within subgroups	13	62.75			
LIPID	Among groups	4	17.93	1.07	0.4119	.247
	Among subgroups	54	1.25	13.43	0.0000	.764
	Within subgroups	13	16.81			
CARB	Among groups	4	7.69	3.76	0.0303	.537
	Among subgroups	54	0.04	47.94	0.0000	.920
	Within subgroups	13	2.04			
TE	Among groups	4	341531.06	5.68	0.0072	.636
	Among subgroups	54	4137.99	14.53	0.0000	.778
	Within subgroups	13	60120.52			

Experiment 2 (Larvae).

		DF	MS	F	Sig F	R^2
PROTEIN	Among groups	4	60.99	1.35	0.3452	.293
	Among subgroups	53	2.17	20.89	0.0000	.837
	Within subgroups	13	45.23			
LIPID	Among groups	4	7.83	0.67	0.6231	.171
	Among subgroups	53	0.68	17.25	0.0000	.809
	Within subgroups	13	11.65			
CARB	Among groups	4	0.28	0.163	0.9533	.048
	Among subgroups	53	0.19	9.12	0.0000	.691
	Within subgroups	13	1.71			
TE	Among groups	4	20392.38	0.30	0.8713	.085
	Among subgroups	53	2927.85	23.05	0.0000	.850
	Within subgroups	13	67481.32			

Among groups (conditions)
Among subgroups (females)
Within subgroups (error)

Experiment 3 (eggs): Two level nested ANOVAS
testing for differences among conditions and
females.

	Source variation	DF	MS	F	Sig F	R^2
PROTEIN	Among groups	1	85.28	2.05	0.3830	.672
	Among subgroups	9	2.63	15.81	0.0032	.637
	Within subgroups	1	41.59			
LIPID	Among groups	1	0.88	0.21	0.7267	.173
	Among subgroups	9	0.34	12.46	0.0064	.581
	Within subgroups	1	4.19			
CARB	Among groups	1	0.03	0.17	0.9167	.017
	Among subgroups	9	0.02	102.81	0.0000	.920
	Within subgroups	1	1.74			
TE	Among groups	1	31454.76	0.71	0.5537	.416
	Among subgroups	9	1971.23	22.39	0.0011	.713
	Within subgroups	1	44137.89			

Experiment 3 (Larvae).

		DF	MS	F	Sig F	R^2
PROTEIN	Among groups	1	89.90	1349.6	0.0173	.999
	Among subgroups	9	1.33	0.05	0.8278	.006
	Within subgroups	1	0.07			
LIPID	Among groups	1	0.88	0.02	0.9063	.021
	Among subgroups	9	0.11	35.09	0.0002	.796
	Within subgroups	1	3.99			
CARB	Among groups	1	1.90	1.01	0.4895	.502
	Among subgroups	9	0.12	15.70	0.0033	.636
	Within subgroups	1	1.89			
TE	Among groups	1	66824.98	7.18	0.2274	.878
	Among subgroups	9	1646.89	5.66	0.0413	.386
	Within subgroups	1	9319.3			

Among groups (conditions)
Among subgroups (females)
Within subgroups (error)

Experiment 4 (eggs):Two level nested ANOVAS
testing for differences among conditions and
females.

Source variation	DF	MS	F	Sig F	R²
PROTEIN Among groups	3	418.54	10.33	0.0004	.633
Among subgroups	66	0.87	46.38	0.0000	.927
Within subgroups	18	40.53			
LIPID Amomg groups	3	192.78	12.24	0.0001	.671
Among subgroups	66	0.38	41.08	0.0000	.918
Within subgroups	18	15.76			
CARB Among groups	3	4.03	1.01	0.4123	.144
Among subgroups	66	0.02	172.57	0.0000	.979
Within subgroups	18	3.99			
TE Among groups	3	1054892.7	16.34	0.0000	.732
Among subgroups	66	1211.70	53.12	0.0000	.935
Within subgroups	18				

Experiment 4 (larvae).

Source variation	DF	MS	F	Sig F	R²
PROTEIN Among groups	3	28.00	1.02	0.4063	.153
Among subgroups	63	4.03	6.79	0.0000	.647
Within subgroups	17	27.32			
LIPID Among groups	3	20.05	4.01	0.0249	.415
Among subgroups	63	0.21	24.05	0.0000	.866
Within subgroups	17	4.99			
CARB Among groups	3	0.08	0.08	0.8387	.047
Among subgroups	63	0.19	15.25	0.0000	.805
Within subgroups	17	0.28			
TE Among groups	3	59299.99	2.31	0.1126	.290
Among subgroups	63	2185.85	11.73	0.0000	.760
Within subgroups	17	25642.63			

Among groups (conditions)
Among subgroups (females)
within subgroups (error)

Oneway analysis of variance testing for differences in
larval recovery rate, and the number, dry weight and size of
eggs spawned by females of _Placopecten magellanicus_ in the
various conditioning regimes.

RECOVERY RATE

	Source	DF	MS	F	Sig F
Experiment 1	Between groups	4	1216.81	7.145	0.006
	Within groups	25	170.30		
Experiment 2	Between groups	4	374.56	1.40	0.289
	Within groups	13	268.35		
Experiment 4	Between groups	3	995.5	3.48	0.038
	Within groups	18	5153.90		

NUMBER OF EGGS SPAWNED

	Source	DF	MS	F	Sig F
Experiment 1	Between groups	4	0.293E+15	4.49	0.0072
	Within groups	25	0.653E+14		
Experiment 2	Between groups	4	0.729E+14	0.403	0.8032
	Within groups	13	0.181E+15		
Experiment 4	Between groups	3	0.756E+14	0.927	0.4480
	Within groups	13	0.816E+14		

DRY WEIGHT OF EGGS

	Source	DF	MS	F	Sig F
Experiment 1	Between groups	4	0.02	0.935	0.4603
	Within groups	25	0.02		
Experiment 2	Between groups	4	2.332	0.112	
	Within groups	13	0.00		
Experiment 4	Between groups	3	0.02	0.805	0.5081
	Within groups	18	0.02		

TABLE A-22: CONTINUED.

EGG SIZE

	Source	DF	MS	F	Sig F
Experiment 1	Between groups	4	0.99	0.609	0.6598
	Within groups	25	1.62		
Experiment 2	Between groups	4	6.15	5.449	0.0084
	Within groups	13	1.13		
Experiment 4	Between groups	3	13.48	12.247	0.0001
	Within groups	18	1.10		

TABLE A-23

Regression analysis of the effect of dry tissue weight and algal concentration on the filtration and ingestion rates of juveniles of <u>Placopecten magellanicus</u>.

FILTRATION RATE VS ALGAL CONCENTRATION
$F = a \: conc^b$

WEIGHT	SOURCE	D.F.	MS	F
5-8 mg $a=2.2 \times 10^{-6}$ $b=1.66$	Regression Residual	1 121	76.21 0.49	154.59
20-40mg $a=33.82$ $b=0.089$	Regression Residual	1 1016	0.348 1.107	0.32
200-600mg $a=9.78 \times 10^{5}$ $b=-0.856$	Regression Residual	1 1617	2487.668 2.269	1096.37

INGESTION RATE VS ALGAL CONCENTRATION
$IR = a \: conc^b$

WEIGHT	SOURCE	D.F.	MS	F
5-8 mg $a=2.3 \times 10^{-6}$ $b=2.66$	Regression Residual	1 121	196.062 0.493	397.69
20-40 mg $a=33.78$ $b=1.09$	Regression Residual	1 1016	52.231 1.107	47.18
200-600 mg $a=2.21 \times 10^{3}$ $b=0.818$	Regression Residual	1 1617	2870.72 1.83	1569.95

TABLE A-23: CONTINUED.

FILTRATION RATE VS TISSUE DRY WEIGHT
$F = a W^b$

CONCENTRATION	SOURCE	D.F.	MS	F
5x10³ cells/ml a=0.505 b=1.245	Regression Residual	1 102	502.620 0.838	599.36
10x10³ cells/ml a=2.513 b=0.972	Regression Residual	1 87	222.413 0.336	660.89
15x10³ cells/ml a=8.182 b=0.730	Regression Residual	1 90	132.434 0.807	164.18
20x10³ cells/ml a=4.383 b=0.831	Regression Residual	1 38	101.441 0.598	169.48
25x10³ cells/ml a=3.436 b=0.855	Regression Residual	1 50	62.078 0.938	66.21

INGESTION RATE VS TISSUE DRY WEIGHT
$IR = a W^b$

CONCENTRATION	SOURCE	D.F.	MS	F
5x10³ cells/ml a=3.75x10³ b=1.19	Regression Residual	1 102	475.524 0.888	535.68
10x10³ cells/ml a=2973x10⁴ cells/ b=0.943	Regression Residual	1 87	214.409 0.404	531.37
15x10³ cells/ml a=1.206x10⁵ b=0.728	Regression Residual	1 90	129.724 0.833	155.78
20x10³ cells/ml a=8.80x10⁴ b=0.854	Regression Residual	1 38	103.324 0.722	143.11
25x10³ cells/ml a=9.87x10⁴ b=0.837	Regression Residual	1 50	59.695 0.813	73.39

TABLE A-24

Regression analysis of the combined effect of algal
concentration and tissue dry weight on the ingestion
rate of juveniles of Placopecten magellanicus.

$$IR = 0.066 \ (W)^{0.939} \ (conc)^{1.388}$$

Source of variation	D.F.	MS	F	R^2
Regression	2	637.789	7.334	0.797
Residual	373	0.869		