

THE EFFECTS OF UNIALGAL AND MIXED DIETS ON  
GROWTH OF HATCHERY - PRODUCED SPAT OF THE  
SEA SCALLOP, Placopecten magellanicus (GMELIN)

CENTRE FOR NEWFOUNDLAND STUDIES

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CAROLYN A. GILLIS







The Effects of Unialgal and Mixed Diets on Growth  
of Hatchery - Produced Spat of the Sea Scallop,  
Placopecten magellanicus (Gmelin)

by

© Carolyn A. Gillis, B.Sc.

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## ABSTRACT

Sea scallop (Placopecten magellanicus) juveniles have been reared in a hatchery at the Ocean Sciences Centre, Memorial University of Newfoundland, for several years and transplanted to scallop farms located in three Newfoundland bays. This study was undertaken to investigate the effect of diet on growth of spat to help optimize hatchery production. Four experiments were performed to determine which available algal diet(s) resulted in greatest growth (in terms of shell height, dry weight and organic weight) of juvenile P. magellanicus ranging in size from 1 - 4 mm shell height. It was found that of the diets tested, a ternary mixed diet consisting of the three species Isochrysis galbana, Isochrysis aff. galbana (T-Iso) and Chaetoceros spp. (muelleri or calcitrans), fed in a 1:1:1 ratio at a final concentration of 50 cells/ $\mu$ l, resulted in the greatest growth for juveniles sized 2 - 4 mm. A unialgal Isochrysis galbana diet supported as much growth as the ternary mixed diet in scallops sized 1 - 2 mm. The unialgal Chaetoceros diets did not support any appreciable growth. ALGAL 161, a commercially available, spray dried, heterotrophically grown Tetraselmis suecica product, was found to be a poor food for P. magellanicus juveniles. Gross growth efficiencies were determined for scallops for the different diets tested. No

significant differences in growth efficiencies among the diets were found.

A short-term experiment was done to study the effect of ration on filtration and ingestion rates for three size classes of juvenile Placopecten magellanicus (2-4 mm, 4-6mm, >6mm). It was found that as ration increased, filtration rates decreased and ingestion rates increased. Values for filtration and ingestion rates (F) were related to growth rate (W) using the general allometric equation  $F = aW^b$ . The physiological rates were found to be increasing faster than the growth rates of these young juvenile scallops with values of the exponent (b) ranging from 1.47 - 2.24.



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## CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
CONTENTS.....	v
LIST OF TABLES.....	vii
LIST OF FIGURES.....	ix
LIST OF APPENDICES.....	xii
<b>I INTRODUCTION.....</b>	<b>1</b>
I.1. GENERAL.....	1
I.2. PARTICLE SELECTION AND PARTICLE RETENTION EFFICIENCY.....	3
I.3. EFFECT OF MIXED AND UNIALGAL DIETS.....	5
I.4. BIOCHEMICAL CONSIDERATIONS.....	9
I.5. ENERGY REQUIREMENTS OF LARVAE AND JUVENILES... 15	
I.5.i. Filtration Rate.....	15
I.5.ii. Ingestion.....	18
I.5.iii. Efficiencies.....	18
I.6. OBJECTIVES .....	20
<b>II MATERIALS AND METHODS .....</b>	<b>22</b>
II.1. SPAWNING ADULTS AND REARING LARVAE.....	22
II.2. ALGAL CULTURE.....	24
II.3. ALGAL CULTURES USED IN GROWTH EXPERIMENTS... 26	
II.4. ALGAL DRY WEIGHT AND ORGANIC WEIGHT DETERMINATIONS.....	27
II.5. EFFECT OF DIET ON GROWTH.....	27
II.5.i. Experiments 1 and 2.....	28
II.5.ii. Experiment 3.....	29
II.5.iii. Experiment 4.....	30
II.6. GROWTH DETERMINATIONS.....	31
II.6.i. Shell Height.....	31
II.6.ii. Dry Weight.....	31
II.6.iii. Organic Weight.....	32
II.7. DETERMINATION OF INGESTION RATES.....	32
II.8. DETERMINATION OF GROSS GROWTH EFFICIENCY.... 32	
II.9. EFFECT OF RATION ON FILTRATION AND INGESTION RATES.....	33
II.10. DETERMINATION OF FILTRATION AND INGESTION RATES.....	34
II.11. STATISTICAL METHOD.....	36

<b>III</b>	<b>RESULTS</b> .....	38
III.1.	ALGAL SPECIES' CHARACTERISTICS.....	38
III.2.	GROWTH - EXPERIMENT 1.....	40
III.2.i.	Shell Height.....	40
III.2.ii.	Dry Weight.....	42
III.2.iii.	Organic Weight.....	42
III.3.	GROWTH - EXPERIMENT 2.....	45
III.3.i.	Shell Height.....	45
III.3.ii.	Dry Weight.....	46
III.3.iii.	Organic Weight.....	46
III.4.	GROWTH - EXPERIMENT 3.....	49
III.4.i.	Shell Height.....	51
III.4.ii.	Dry Weight.....	53
III.4.iii.	Organic Weight.....	55
III.5.	GROWTH - EXPERIMENT 4.....	55
III.5.i.	Shell Height.....	57
III.5.ii.	Dry Weight.....	59
III.5.iii.	Organic Weight.....	59
III.6.	GROSS GROWTH EFFICIENCIES.....	60
III.7.	EFFECT OF RATION ON FILTRATION AND INGESTION RATES.....	65
<b>IV</b>	<b>DISCUSSION</b> .....	80
IV.1.	EFFECT OF DIET.....	80
IV.2.	GROSS GROWTH EFFICIENCIES.....	87
IV.3.	EFFECT OF RATION ON FILTRATION AND INGESTION RATES.....	89
IV.4.	SUMMARY.....	92
IV.5.	CONCLUSIONS.....	95
	<b>REFERENCES</b> .....	96
	<b>APPENDIX</b> .....	112

LIST OF TABLES

TABLE 1:	Summary of experimental factors and algal combinations used in growth experiments.	28
TABLE 2:	General characteristics of the algal species used in Experiments 1 - 4.	38
TABLE 3:	Literature values for algal cell characteristics.	39
TABLE 4:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in shell heights of spat (2 - 4 mm) reared on various diets (Experiment 1).	41
TABLE 5:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in dry weights of spat (2 - 4 mm) reared on various diets (Experiment 1).	43
TABLE 6:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in organic weights of spat (2 - 4 mm) reared on various diets (Experiment 1).	44
TABLE 7:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in shell heights of spat (2 - 4 mm) reared on various diets (Experiment 2).	47
TABLE 8:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in dry weights of spat (2 - 4 mm) reared on various diets (Experiment 2).	48
TABLE 9:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in organic weights of spat (2 - 4 mm) reared on various diets (Experiment 2).	50
TABLE 10:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in shell heights of spat (1 - 2 mm) reared on various diets (Experiment 3).	52

TABLE 11:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in dry weights of spat (1 - 2 mm) reared on various diets (Experiment 3).	54
TABLE 12:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in organic weights of spat (1 - 2 mm) reared on various diets (Experiment 3).	56
TABLE 13:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in shell heights of spat (2 - 4 mm) reared on various diets (Experiment 4).	58
TABLE 14:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in dry weights of spat (2 - 4 mm) reared on various diets (Experiment 4).	61
TABLE 15:	<u>Placopecten magellanicus</u> . Summary of results of Duncan's means test for differences in organic weights of spat (2 - 4 mm) reared on various diets (Experiment 4).	62
TABLE 16:	Literature values for gross growth efficiencies of laboratory-reared juvenile bivalve molluscs fed algal diets.	64
TABLE 17:	Mean filtration and ingestion rates for three size classes of scallops (2-4, 4-6 and 6+ mm) at three food rations (20, 40 and 80 cells/ $\mu$ l).	71
TABLE 18:	Literature values for filtration rates of various bivalve species.	72
TABLE 19:	Specific filtration and ingestion rates for three size classes of scallops (2-4, 4-6 and 6+ mm) at three food rations (20, 40 and 80 cells/ $\mu$ l).	74

LIST OF FIGURES

FIGURE 1:	<u>Placopecten magellanicus</u> . Mean shell heights ( $\pm$ sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 1).	41
FIGURE 2:	<u>Placopecten magellanicus</u> . Mean dry weights ( $\pm$ sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 1).	43
FIGURE 3:	<u>Placopecten magellanicus</u> . Mean organic weights ( $\pm$ sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 1).	44
FIGURE 4:	<u>Placopecten magellanicus</u> . Mean shell heights ( $\pm$ sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 2).	47
FIGURE 5:	<u>Placopecten magellanicus</u> . Mean dry weights ( $\pm$ sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 2).	48
FIGURE 6:	<u>Placopecten magellanicus</u> . Mean organic weights ( $\pm$ sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 2).	50
FIGURE 7:	<u>Placopecten magellanicus</u> . Mean shell heights ( $\pm$ sd) for spat (1 - 2 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 3).	52
FIGURE 8:	<u>Placopecten magellanicus</u> . Mean dry weights ( $\pm$ sd) for spat (1 - 2 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 3).	54
FIGURE 9:	<u>Placopecten magellanicus</u> . Mean organic weights ( $\pm$ sd) for spat (1 - 2 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 3).	56

- FIGURE 10: Placopecten magellanicus. Mean shell heights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 4). 58
- FIGURE 11: Placopecten magellanicus. Mean dry weights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 4). 61
- FIGURE 12: Placopecten magellanicus. Mean organic weights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l (Experiment 4). 62
- FIGURE 13: Placopecten magellanicus. Gross growth efficiencies (K1) of spat (2 - 4 mm) reared on various diets batch fed at 50 cells/ $\mu$ l (Experiment 1). 66
- FIGURE 14: Placopecten magellanicus. Gross growth efficiencies of spat (2 - 4 mm) reared on various diets batch fed at 50 cells/ $\mu$ l (Experiment 2). 67
- FIGURE 15: Placopecten magellanicus. Gross growth efficiencies of spat (1 - 2 mm) reared on various diets batch fed at 50 cells/ $\mu$ l (Experiment 3). 68
- FIGURE 16: Placopecten magellanicus. Gross growth efficiencies of spat (2 - 4 mm) reared on various diets batch fed at 50 cells/ $\mu$ l (Experiment 4). 69
- FIGURE 17: Placopecten magellanicus. Specific filtration rate for spat of various size classes when fed varying rations of a mixed algal diet. 75
- FIGURE 18: Placopecten magellanicus. Specific ingestion rate for spat of various size classes when fed varying rations of a mixed algal diet. 76
- FIGURE 19: Placopecten magellanicus. Filtration rate per animal as a function of tissue organic weight at varying food rations. 78

FIGURE 20: Placopecten magellanicus. Ingestion rate per animal as a function of tissue organic weight at varying food rations.

79



LIST OF APPENDICES

TABLE A-1:	Results for growth parameters of juvenile <u>Placopecten magellanicus</u> in Experiment 1.	112
TABLE A-2:	Ingestion values for <u>Placopecten magellanicus</u> juveniles during the course of Experiment 1.	115
TABLE A-3:	Results for growth parameters of juvenile <u>Placopecten magellanicus</u> in Experiment 2.	116
TABLE A-4:	Ingestion values for <u>Placopecten magellanicus</u> juveniles during the course of Experiment 2.	120
TABLE A-5:	Results for growth parameters of juvenile <u>Placopecten magellanicus</u> in Experiment 3.	121
TABLE A-6:	Ingestion values for <u>Placopecten magellanicus</u> juveniles during the course of Experiment 3.	125
TABLE A-7:	Results for growth parameters of juvenile <u>Placopecten magellanicus</u> in Experiment 4.	128
TABLE A-8:	Ingestion values for <u>Placopecten magellanicus</u> juveniles during the course of Experiment 4.	132
TABLE A-9:	Summary of conditions yielding highest growth rates of <u>P. magellanicus</u> spat from experiments 1-4.	135
FIGURE A-1:	<u>Placopecten magellanicus</u> . Filtration rate per animal for spat of varying size classes when fed varying rations of a mixed algal diet.	136
FIGURE A-2:	<u>Placopecten magellanicus</u> . Ingestion rate per animal for spat of various size classes when fed varying rations of a mixed algal diet.	137

TABLE A-10: Inorganic dry weights and organic dry weights of scallops for each treatment studied. 138

## I INTRODUCTION

### I.1. General

The sea scallop, Placopecten magellanicus, is an important native commercial mollusc (class Bivalvia, family Pectinidae), found in the northwest Atlantic from the north shore of the Gulf of St. Lawrence to Cape Hatteras, North Carolina. Most scallop landings are obtained from the commercial fisheries on Georges Bank off the east coast of North America. The past ten years have shown annual catches from all sources ranging from about 10,000 mt to 40,000 mt, with landed prices ranging from a high of C\$17/kg in the early 1980s to the present value of C\$8 - 10/kg (Couturier, 1990).

In recent years there has been considerable interest in the aquaculture of this species. There are two possible sources of seed (spat or juvenile scallops). The first involves collecting spat on artificial substrates deployed in suitable sites in the natural environment, and then using these spat to enhance natural populations or for aquaculture purposes. The second involves production of hatchery-grown spat, an approach that is presently being studied as a possible alternative to naturally-produced spat.

Successful rearing of P. magellanicus larvae through metamorphosis in the laboratory has been limited (Culliney, 1974; Fournier and Marsot, 1986). Research at the Ocean Sciences Centre in St. John's, Newfoundland, on hatchery

methods for the sea scallop commenced in the late seventies and by 1987 had advanced to produce spat of adequate size to transport and suspend in several aquaculture sites.

Artificial spat production consists of 2 phases: hatchery and nursery. The hatchery phase involves rearing the larvae to post-larvae (up to 1 mm). In the nursery phase, spat are reared until transplantation to the natural environment (2 - 10 mm). The nursery phase is important in that it minimises losses due to handling, predation, temperature shocks and shortage of food (DePauw, 1981). For both the hatchery and nursery phases, it is important to know which algal diets support growth of larvae, survival through metamorphosis and provide optimum growth of the post-larvae and juveniles.

The successful rearing of bivalve larvae (Loosanoff and Davis, 1963; Walne, 1964) has led to studies on their nutritional requirements (Ukeles, 1970) and aspects of their biochemistry (Millar and Scott, 1967; Holland, 1978; Whyte et al., 1987; Gabbott, 1976). These studies investigated factors influencing larval success and viability through metamorphosis and settlement. Much of the research available concerns oyster, clam and mussel larvae. Some information concerning larvae of P. magellanicus is reported in Culliney (1974) and Manning (1986). Naidu et al. (1989) present an overview of culture methods for P. magellanicus

and, Dabinett (1989) has reported successes in hatchery production and grow-out in Newfoundland. Other research on juvenile P. magellanicus has focused on effect of ration (Hollett and Dabinett, 1989), effect of current speed and food concentration using a microparticulate diet (Kean-Howie et al., 1989) and the effects of unialgal and mixed diets on growth (Gillis and Dabinett, 1989). However, as the scallop aquaculture industry grows, there is a need for further research on requirements for larval and juvenile scallops.

#### **I.2. PARTICLE SELECTION AND PARTICLE RETENTION EFFICIENCY**

Scallops have been described as non-siphonate, ciliary suspension feeders (Bricelj and Shumway, 1991) and have been termed suspensivores rather than filter feeders (Benninger and Le Pennec, 1991). Suspended particles are captured on the gills by a combination of direct ciliary action, mucus secretion and entrapment on the gill filaments. Due to the dimensions of the eu-latero-frontal cirri on the gill filaments, it is believed that different species of bivalves can retain particles of different sizes with differing efficiencies. Bricelj and Shumway (1991) point out how most post-settlement stages of suspension feeding bivalves can retain particles above 3 - 4  $\mu\text{m}$  with 100% efficiency, with retention efficiency decreasing with decreasing particle

size. However, the limit for effective retention of particles in members of the Pectinidae is about 5 - 7  $\mu\text{m}$ . Winter (1978) found that in four species of bivalves (Chlamys islandica, Mytilus edulis, Crassostrea virginica, Cardium edule), particles were effectively retained down to about 7  $\mu\text{m}$  in diameter. Mytilus edulis could retain particles down to about 2  $\mu\text{m}$  in diameter and particles below 1  $\mu\text{m}$  were retained to a very limited degree by all species. Recent studies with P. magellanicus have reported 100% retention of particles down to 3  $\mu\text{m}$  (Cranford and Grant 1990, D.Deibel and R.Thompson, Ocean Sciences Centre, Memorial University, pers. com.).

Haven and Morales-Alamo (1970) found that the American oyster, Crassostrea virginica (2 years old) exhibited a sharp increase in the percentage of particles removed as particle size increased from 1  $\mu\text{m}$  to between 2 and 4  $\mu\text{m}$ , with a consistent inflection point at a particle size of 2 - 3  $\mu\text{m}$ , indicating that the distance between adjacent latero-frontal cilia determines the particle sizes retained, and the absence of a mucous sheet. Percentage removed levelled off at larger sizes with no increase in efficiency.

Riisgard et al. (1980) found that juvenile M. edulis (1 - 4 mm long) completely withheld particles above 4  $\mu\text{m}$ , with retention efficiency gradually decreasing below 4  $\mu\text{m}$  to 20% for 1  $\mu\text{m}$  particles. Wilson (1980) found that while grazing

rates increase with the size of Ostrea edulis spat, particle-size preference remains constant.

Vahl (1973) has shown that the Iceland scallop (Chlamys islandica), 45.5 - 51.2 mm shell height, effectively retained particles down to 7  $\mu$ m diameter with few particles 1.2  $\mu$ m in diameter being retained. This is consistent on observations on the queen scallop, Chlamys (Aequipecten) opercularis (Vahl, 1972). Sprung (1984) suggested a maximum retention efficiency for M. edulis larvae of 3.5  $\mu$ m diameter, the maximum ingestible particle size being 9  $\mu$ m diameter.

### I.3. EFFECT OF MIXED AND UNIALGAL DIETS

For hatchery and nursery purposes, it is important to know the algal diet supporting growth and survival of larvae through metamorphosis and resulting in optimum growth of the juveniles. There are several features of the diet which must be taken into account when determining which algal species will provide the best food such as: the cost of production of algae; the ease with which the algae can be cultured in a laboratory; the optimum food ration (algal cell density) for growth. These features may vary according to the bivalve species under consideration.

Some algae support better growth of bivalves than

others. Walne (1970) studied the food value of 19 genera of algae, presented as unialgal diets to juvenile bivalves. Some algal species resulted in greater growth depending on the species of bivalve used. Walne (1970) considered that differences in the rigidity of the cell wall or in chemical composition cannot explain all the results obtained, and he suggested that differing food values may lie in protein digestibility as a whole or in some other aspect of protein chemistry.

As well as studying the food values of unialgal diets for bivalves, some researchers have also considered mixed algal diets. Walne (1974) described how diets consisting of more than one species tend to result in better growth. Epifanio (1979) studied the effects of 15 diets composed of various mixtures of 4 species of algae on growth of juvenile Crassostrea virginica and Mercenaria mercenaria. There was little correlation between gross chemical composition and nutritional value of the diets. 'Non-additive' growth was defined as growth different from that predicted by summing the growth responses of the oysters fed the individual components of the diets. The term 'synergistic' was applied to mixed diets which yield greater growth than that produced by either of the dietary components alone. Observed synergism was suggested to be due to micronutrients or fatty acid composition of the algae.



Romberger and Epifanio (1981) studied the effects of three species of algae on assimilation efficiency and growth in juvenile oysters, Crassostrea virginica, fed single or mixed diets. In general, it was found that ingestion of any two-species mixture was greater than for any unialgal diet, with a mixed diet consisting of Thalassiosira pseudonana and Isochrysis galbana resulting in synergistic growth responses which could not be expressed in terms of assimilation or ingestion. It was suggested that this was probably due to the availability and balance of fatty acids or micronutrients such as vitamins or minerals.

Enright et al. (1986) studied the food value of 16 phytoplankton diets on growth of juvenile Ostrea edulis compared to a reference diet of Isochrysis aff. galbana (T-Iso). The highest growth rates were observed using a mixed algal diet containing five species. In general, the best diets had high levels of the fatty acids, 22:6n3 and 20:5n3 and high carbohydrate levels, while protein levels appeared to be less critical. Discrepancies from literature values were attributed to such differences as the species used, the strains and physiological state of the algal cells (which is influenced by culture conditions), and the ration used.

Davis and Guillard (1958) also determined the value of ten genera of microorganisms as food for the larvae of oysters (Crassostrea virginica) and clams (Mercenaria

mercenaria). Again, a mixed diet provided better growth than unialgal diets. The four best foods for oyster larvae were all naked flagellates, while clam larvae were able to utilize several forms with cell walls. Toxicity of algal metabolites was thought to affect the value of an alga as food, and a metabolite that inhibits growth in one species of bivalve may promote growth in another.

Helm (1977) also found that a mixed diet of Isochrysis galbana and Tetraselmis suecica resulted in improved growth in Ostrea edulis larvae and provided greater yields of spat. He concluded that the mixed diet provides a better balance, such that any deficiencies in one species are compensated for by the other species in the mixture, and suggested that the triglyceride fraction of the algae may be responsible for differences in the food value for oyster larvae.

Cary et al. (1981) found that a mixed diet of Isochrysis, Monochrysis and Rhodomonas showed a negative synergistic effect on growth in larvae of the purple-hinge rock scallop Hinnites multirugosus, possibly due to ciliate contamination or buildup of toxic metabolites. It was also shown that some algae (Isochrysis galbana) resulted in better growth when harvested in the stationary phase, during which the cells contained the lowest carbohydrate and highest lipid level, while others (Monochrysis lutheri) supported scallop growth better when harvested in the

exponential phase, during which high levels of protein and lipid were produced. Isochrysis aff. galbana (T-Iso) produced similar growth when harvested in either the exponential or the stationary phase.

The effect of yeast-algal mixed diets as foods for bivalves has also been examined. Epifanio (1979) compared four diets of varying proportions of the yeast Candida utilis and the diatom Thalassiosira pseudonana on four species of bivalve molluscs. It was found that Candida utilis was suitable as a replacement for up to 50% of algae in diets of 3 of the 4 species tested. Mean growth was not closely related to gross chemical composition of the diets or amino acid composition. Diets higher in lipid generally yielded greater growth. This is probably due to lipid quality rather than lipid quantity.

#### **I.4. BIOCHEMICAL CONSIDERATIONS**

Many researchers have studied and compared algal diets in an attempt to determine the optimal diet for bivalves. It is important to consider why such diets result in increased growth. Although the presence of a rigid cell wall may have some effect, this alone cannot account for many of the results obtained and many researchers have looked further to assess the importance of the biochemical

composition of the algae on its food value.

In many marine invertebrate larvae, lipid is the major dietary reserve material (Holland 1978). The different stages of the bivalves's life are marked by varying biochemical compositions. Holland (1978) measured lipid reserves and energy metabolism of benthic marine invertebrates. Marine invertebrate eggs are comprised primarily of protein, followed by lipid and then carbohydrate. Results from Pecten maximus indicate that lipid is the main reserve during the larval stage up to and including metamorphosis (Holland, 1978). It appears that the larvae utilise mainly lipid stores whereas the adults store glycogen as an energy reserve.

Holland and Hannant (1974) studied the biochemical changes which occur in the spat of oysters, Ostrea edulis, following metamorphosis. For a few months after settlement, the neutral lipid content was greater than the glycogen content. Following this time, glycogen reserves were accumulated faster than neutral lipid so that 3 to 5 month-old spat showed greater amounts of glycogen than neutral lipid. Holland (1978) associated this increase in glycogen with an increase in the number of Leydig cells, connective tissue cells scattered throughout the adult oyster and responsible for glycogen storage. Furthermore, glycogen levels fluctuated with the reproductive cycle, showing an

increase in levels during the spring and early summer prior to gonad ripening, and a decrease during the summer spawning season with a subsequent increase in the autumn.

Holland (1978) suggested that the possible reasons why larvae store mainly lipid whereas adults store glycogen concerns the fact that lipids can only be utilized aerobically, whereas carbohydrates can be used either aerobically or anaerobically. Glycogen storage may enable the adults to survive anaerobic conditions, such as when they are exposed at low tide. Another important difference may lie in the lifestyles, since the larvae are planktonic and the adults sessile. In the free-swimming larval stage the stored lipid will also contribute more to the buoyancy of the animal.

Whyte et al. (1987) identified lipid and protein as major components and carbohydrate (glucan) a minor component in the eggs of the scallop Patinopecten yessoensis, with total energy levels twice those observed in the larvae of other scallops and oysters. The stored glucan, probably glycogen, was depleted during early larval development. During development, scallop larvae utilised reserves of lipid and protein for approximately 20 days, after which lipid was accumulated as the larvae approached metamorphosis, a process requiring significant energy reserves.

Whyte et al. (1990) correlated the nutritional condition of rock scallop (Crassadoma gigantea) larvae with dietary carbohydrate, lipid, and protein. The greatest energy reserves were observed in larvae fed a diet with the highest level of carbohydrates, rather than lipid or protein. Furthermore, it was shown that while the levels of 20:5n3 or 22:6n3 fatty acids do not effect the nutritional condition of the larvae, a certain threshold level of essential fatty acids must be met in order to maintain nutritional levels. Apparently, requirements for fatty acids are more easily satisfied than for other micronutrients.

Langton and Waldock (1981), studying Crassostrea gigas spat, found that either 20:5n3 or 22:6n3 could fulfill the higher n3 polyunsaturated fatty acid requirements for the spat which are unable to produce long-chain fatty acids of the n3 family. A dietary deficiency of these fatty acids was found to limit growth.

Enright et al. (1986) studied the growth of juvenile Ostrea edulis fed Chaetoceros gracilis of varying chemical composition. This was done by varying the nutrient conditions of the culture. Cells cultured in complete f/2 nutrient medium (control) and a silicate-limited medium had a similar protein content, whereas cells from a nitrogen-limited medium contained approximately 60% less protein.

All three cultures had a similar amino-acid composition. The lipid content of silicate-limited cells was more than twice that of the control or nitrogen-limited cells. At the lowest ration levels, the silicate-limited diet produced the highest growth rate. This was attributed to the fact that since none of the diets probably had sufficient caloric content for maximum growth, the increased amount of fatty acids could be used to supply energy so the proteins could be used for growth. At the highest feeding ration, the control culture resulted in the best growth. The control had 3 times the 22:6n3 content of the silicate-limited diet, which may be important once the caloric requirements are met. The high carbohydrate content of the nitrogen-limited diet seems to be of little use if the protein content is limiting.

Holland (1978) pointed out that both n3 and n6 polyunsaturated fatty acids are important for membrane fluidity and essential membrane functions. The double bonds formed in the n3 fatty acids may be responsible for retaining membrane fluidity at low and relatively constant temperatures, such as are found in the marine environment. Other advantages of lipid as an energy source include the fact that it is an efficient fuel, especially for organisms that may undergo periods of food shortages or critical developmental changes in their life histories. Lipids are

also important for buoyancy, maintenance of body structure and thermal insulation in various marine organisms.

Whyte (1987) compared the chemical composition in both the stationary and the exponential phases of growth, of the following species of algae: (1) Thalassiosira pseudonana, (2) Chaetoceros calcitrans, (3) Chaetoceros sp., (4) Isochrysis galbana, (5) Isochrysis aff. galbana (T-Iso) and (6) Tetraselmis suecica. It was found that both species of Isochrysis at both stages of growth had the highest lipid levels. The diatoms examined had higher levels of mono-oligosaccharides and total carbohydrates than did phytoflagellates. With the exception of Thalassiosira, the phase of growth had little effect on the polysaccharide content. The phytoflagellates had higher total nitrogen concentrations than the diatoms, with a greater concentration observed during the stationary phase. The concentration of free amino acids, peptides and amines was similar for most species. The algae studied were ranked as follows in terms of energy from the constituents: (1) Isochrysis aff. galbana (T-Iso), (2) Isochrysis galbana, (3) Chaetoceros calcitrans, (4) T. suecica, (5) T. pseudonana and (6) Chaetoceros sp.



## I.5. ENERGY REQUIREMENTS OF LARVAE AND JUVENILES

### I.5.i. Filtration Rate:

Two approaches to the study of the passage of water through bivalves are: (1) direct methods, which separate and measure the exhalent flow, and give a "pumping rate"; and (2) indirect methods, which measure "filtration or clearance rates" (the volume of water cleared of suspended particles per unit time), using the rate of removal of suspended material from a known volume of water to estimate the rate of flow through the mantle cavity (Owen, 1974). Owen (1974) also gives four assumptions of indirect methods when determining filtering rates:

- (1) the reduction in the concentration of particles is due to filtration;
- (2) the animal's pumping rate is constant;
- (3) particle retention is 100% efficient;  
alternatively, a known constant percentage is retained; and
- (4) the test suspension is at all times homogenous.

An inverse relationship between filtration rate and algal concentration (below threshold levels) has been reported for pectinids (Palmer and Williams, 1980; Malouf and Bricelj, 1989). Generally, above a threshold concentration of about 60 cells/ $\mu$ l (2 mg dry weight/l), the ingestion rate becomes independent of concentration (Palmer

and Williams, 1980) until very high concentrations cause the filtering mechanism to block.

In general, filtration rates decrease with increasing cell concentrations (Winter, 1978; Owen, 1974; Schulte, 1975; Foster-Smith, 1975). In terms of hatchery-produced bivalves, it is important to determine the optimum food concentration which is characterized by low filtration rates, no production of pseudofaeces (particles filtered, bound in mucus and eliminated before ingestion) and complete ingestion of food particles. This concentration has been termed "pseudofaeces-free cell density" (Winter, 1978) or "critical cell density" (Schulte, 1975). If there is no production of pseudofaeces, the amount of food ingested is equal to the amount of food filtered.

As algal concentration increases from minimum levels, the bivalve filters more algae per hour, although the water volume passing through the gills decreases (Schulte, 1975). Sprung (1984) found that with mussel larvae in dense food concentrations, there was a constant ingestion rate over a wide range of food concentrations and a declining rate with increasing food concentrations. In dilute food concentrations, there was a constant filtration rate and an increase of ingestion rate with increasing food concentration. Schulte (1975) reported that filtration rates at different concentrations change considerably in

short periods of time and, generally, filtration rates decrease with time at all concentrations.

Winter (1973) also found varying filtration rates in Mytilus edulis under constant experimental conditions and algal concentrations. As well, the results showed that filtration rates and amount of algae filtered increased with increasing body size, while filtration rate per mg dry weight of tissue decreased with increasing body size. It was shown that mussels of the same body size filtered approximately the same amount of algae at high or low concentrations, with lower concentrations being counterbalanced by higher filtration rates.

There is an allometric relationship between clearance rate and body size:  $CR = aW^b$ , where CR = clearance rate (l/h) and W = tissue dry weight (g). Values for the parameters (a) and (b) are given in Bricelj and Shumway (1991) for a range of pectinids; the weight exponent b is variable, ranging from 0.58 to 0.94, with a mean of 0.7, which is within the range reported for other bivalves.

It has been observed that different bivalve species may have different filtration rates over a period of time. For example, Palmer (1980) found that filtration activity remained relatively constant over a period of 24 to 33 h for Argopecten irradians concentricus, although Crassostrea virginica exhibited high and low periods of filtration

activity. It was suggested that this serves to regulate ingestion rate.

**I.5.ii. Ingestion:**

Bayne (1983) reported that ingestion rate varied directly with particle concentration until a threshold concentration was reached above which a further increase in concentration did not result in an increase in ingestion rate. This is referred to as the "functional response" to food. The rate at which cells are ingested is a function of clearance rate, particle concentration, particle size and the rate at which the gut is emptied.

**I.5.iii. Efficiencies:**

For aquaculture purposes, it is not only important that growth be maximized, but also that the optimum growth can be achieved with the least possible amount of food. Growth can be described in terms of energy or caloric content, wet weight or dry weight. Crisp (1971) suggests that growth efficiency ratios should be based on energy content rather than wet or dry weight, but if only wet or dry weight is known, the term "conversion rate" should be used.

The literature (Sprung, 1984; Jorgensen, 1976; Bayne and Newell, 1983; Warren and Davis, 1967; Crisp, 1971; Morton, 1983) describes different types of efficiencies

which describe energy budgets within an organism.

Assimilation efficiency (AE) refers to the proportion of ingested food (I) used for growth (G) and respiration (R) (assimilated), and is given by:

$$AE = [(R + G) / I] \times 100$$

Gross growth efficiency (K1) refers to the proportion of ingested food that is converted to growth, and is given by:

$$K1 = [G / I] \times 100$$

Net growth efficiency (K2) refers to the proportion of assimilated food which is converted into growth, and is given by:

$$K2 = [G / (G + R)] \times 100$$

Since the amount of food assimilated is always less than the amount of food ingested, K2 is always greater than K1.

Growth efficiencies have been determined in terms of dry weight, nitrogen, phosphorus, or carbon content (Corner and Davis, 1971).

The term "maintenance ration" has been used to describe the rate of food consumption which allows maintenance activities to be performed with no increase in biomass (Crisp, 1971). Another term that has been widely used to describe energetics in molluscs is "scope for growth", which is defined as "the difference between the energy of the food an animal consumes and all other energy utilizations and losses" (Warren and Davis, 1967). The scope for growth and

growth efficiencies may vary with the species, size and age of the bivalve and with ration level, temperature and salinity (Bayne, 1983; Bayne and Newell, 1983).

#### I. 6. OBJECTIVES

Experience at the pilot hatchery at the OSC/MSRL based on twelve successive spawnings (Dabinett, pers. comm.) has shown that survival and growth of larvae through settlement to post larvae of 1 mm in shell height (i.e. the hatchery phase) is predictable and of uniform rate. In contrast, nursery growth of post-larvae from 1 mm shell height onwards has been unpredictable. Growth rates have fluctuated indicating that conditions such as diet, ration, and physical conditions such as temperature and water flow were not ideal.

This study was undertaken to investigate the use of unialgal, binary and ternary algal mixtures of available algal species cultured to support scallop (*P. magellanicus*) growth with nursery-sized animals (>1 mm shell height). These cultures were *Isochrysis galbana*, *Isochrysis* (Tahitian strain) T-Iso, *Chaetoceros muelleri* and *Chaetoceros calcitrans*. Furthermore, a commercially available spray-dried heterotrophically grown culture of *Tetraselmis suecica* known as ALGAL 161 was tested for efficacy as a diet supplement for juvenile scallops in a hatchery-nursery

situation.

The hypothesis tested was that, based on studies previously cited in this chapter, growth of juvenile scallops on the various algal diets would differ significantly with the prediction that the ranked order would be ternary, binary and unialgal diets respectively.

Conversion efficiency or gross growth efficiency is a useful index which has been used in the culture of many species enabling hatchery managers to optimize growth and minimize food costs. A further aim of this study was to investigate the usefulness of conversion efficiency in a hatchery culturing scallops a potential tool to describe the effectiveness of hatchery practice.

The effect of food ration on filtration and ingestion rates for three size classes of juvenile P. magellanicus spat was investigated to complement the diet studies. Information was thereby provided for hatchery management on both the diet and ration to help optimise the growth of spat in nursery culture.

## II MATERIALS AND METHODS

## II.1. SPAWNING ADULTS AND REARING LARVAE, POST-LARVAE AND SPAT

Scallops for use as broodstock were collected by SCUBA divers from natural populations in Newfoundland in late May, three months prior to natural spawning. Males and females were put into separate tanks in ambient seawater and fed cultured algae, at approximately 2% dry weight of the scallop dry weight per day, for 6 weeks prior to spawning. At the time of spawning, a male and female with ripe gonads were chosen. The shells of the chosen scallops were cleaned, measured and labelled.

The male and female were then placed in a single plastic tank (34 x 28 x 16cm) containing 10 l seawater filtered to 1  $\mu$ m and sanitised with UV at 15°C. Water was vigorously recirculated over the animals with a pump until spawning commenced. When the male spawned, the water became frothy with bubbles. When the female spawned, the water became pink due to the color of the eggs. Once either of the adults commenced spawning, the pump was turned off and the adults were put into clean filtered seawater in separate containers to complete the spawning process.

If the female had not spawned by this point, she was induced to spawn by reimmersion in the original filtered seawater to which sperm had been added for a short period of time. Alternatively, the pump was used again to stimulate spawning.



Once the male and female completed spawning in the separate containers, the adults were removed from the egg or sperm suspensions. A count of the egg suspension was done using a Coulter Counter. The required number of eggs was removed and re-suspended in a volume of filtered seawater. Sperm was added to this egg suspension to obtain a ratio of 2 - 10 sperm per egg based on observations using dark field microscopy. A higher ratio is not recommended since this may cause polyspermy, which may result in lysis of the eggs or abnormal larvae.

The newly fertilized eggs were held in pyrex dishes (38 x 26 x 5 cm) until they reached the D-veliger stage 3 days later. Filtered seawater was put into each dish, together with the antibiotic neomycin at a concentration of 0.025 g/l as a precautionary measure to control bacterial growth. Approximately 70,000 fertilised eggs were added per dish (100 eggs/cm<sup>2</sup> area) in a final volume of 750 ml. The dishes were covered and maintained at 15°C.

The larvae were left for 3 days until they reached the D-veliger stage. They were then removed from the dishes by filtration using a submerged 50 µm mesh screen. The larvae were then put in a 1000 l tank (1 m<sup>3</sup>) at a density of approximately 1/ml and fed a diet of Isochrysis galbana, Isochrysis aff. galbana (T-Iso), Chaetoceros muelleri and Thalassiosira pseudonana, maintained at a concentration of

12 cells/ $\mu$ l in a ratio of 2:1 phytoflagellates to diatoms. Water in the tanks was changed either twice or three times weekly and replaced with fresh filtered seawater. After 10 days, the larvae were counted using a Coulter counter. This procedure was repeated for approximately 35 - 40 days while gradually increasing the food supply to 30 cells/ $\mu$ l, by which time the larvae began to show adhesive tendencies.

Corrugated plastic sheets were hung in the tanks and left for 4 - 6 weeks with fresh filtered seawater and food added daily by displacement. During this time the larvae underwent metamorphosis to post-larvae, set or spat.

After 4 - 6 weeks, the spat were gently brushed off the plastic sheets and placed on either 300 $\mu$ m or 500  $\mu$ m mesh screens in a 1000 l tank containing filtered seawater. The water in the tanks was changed by displacement daily and the tanks were cleaned approximately twice a month. The ration of algae fed was increased as required by the spat maintaining a food concentration of 30 cells/ $\mu$ l.

Spat were size graded using mesh screens to obtain sizes appropriate for the feeding and growth experiments.

## II.2. ALGAL CULTURE

A culture collection of various algal species was maintained at a constant temperature of 15°C. These unialgal cultures (although not axenic) were maintained in

20 ml culture tubes with F/2 media (Guillard, 1975), and transferred every month.

Algal cultures for feeding broodstock and juveniles were grown in 250 l fibreglass cylinders on F/2 growth medium under continuous light from coolwhite and daylight fluorescent tubes at 20°C. The volume harvested every 2 - 3 days was replaced with fresh filtered seawater and F/2 medium. Cultures were counted daily using a model Z<sub>f</sub> Coulter Counter with a 100 µm aperture orifice tube, and the volume of culture required for feeding was calculated.

Cultures for feeding larvae were grown in 2 or 4 l Erlenmeyer flasks on F/2 medium. These cultures were harvested every 2 or 3 days and fresh filtered seawater and medium were added. Algal cell counts were made and the appropriate ration was fed to the larvae.

Cultures for feeding juveniles in experimental work were grown in 10 l glass battery jars inoculated with 2 l of algae collected from 4 l Erlenmeyer flasks. Cultures were harvested every 1 - 2 days and fresh filtered seawater and medium were added. Algal cell counts were performed daily using a Coulter Counter to determine the appropriate culture volume for the ration required.

All seawater used for the culture of algae was passed through a series of four filters: a prefilter designed to remove sediment and large particles, followed by a series of

three Gelman cartridge filters of pore sizes 10  $\mu\text{m}$ , 3  $\mu\text{m}$  and 1  $\mu\text{m}$ . The filtered seawater was then passed through a Trojan Technologies Inc. UV 405 water sterilizer. When the temperature of the ambient seawater fell below 8°C, the filtered seawater was heated to 10 - 11°C. Air was bubbled through the filtered seawater during heating to ensure proper mixing and avoid supersaturation.

### II.3. ALGAL CULTURES USED IN GROWTH EXPERIMENTS

Four species of algae were used in either unialgal or mixed algal diets. These species were chosen because they proved to be successful in rearing P. magellanicus larvae through metamorphosis. The four species employed included two phytoflagellates, Isochrysis galbana and Isochrysis aff. galbana (T-Iso), both high-temperature tolerant species, from the Class Prymnesiophyceae, and two diatoms, Chaetoceros calcitrans and Chaetoceros muelleri, both from the Class Bacillariophyceae. In addition, a dried algal food, known as "ALGAL 161" (Cell Systems, Cambridge, England) was used in Expt. 4. ALGAL 161 consists of spray dried cells of heterotrophically grown Tetraselmis suecica (Class Prasinophyceae) (1 gram of ALGAL 161 is equivalent to  $5 \times 10^9$  cells). The necessary mass of ALGAL 161 to give the required number of cells was added to 100 - 200 ml filtered seawater and mixed gently.

#### II.4. ALGAL DRY WEIGHT AND ORGANIC WEIGHT DETERMINATIONS

Five to ten counts were performed on three separate aliquots of each algal culture to determine cell concentration. Measured volumes of the algal culture, in the range of 10 - 300 ml, depending on cell concentration, were filtered through pre-weighed, ashed Whatman GF/C filters using gentle vacuum. The cells in the filtrate were also counted, and the total number of cells caught on the filter was determined. The cells on the filter were rinsed with 5 - 10 ml 3% ammonium formate (isotonic with seawater) and the filter papers were placed in aluminum weighing pans and dried to a constant weight at 90°C for 24 hours.

Once algal dry weights were determined, the filters and aluminum weighing pans were placed in a muffle furnace at 450°C for 5 hours to completely oxidise and remove any organic material. The resulting inorganic weight was subtracted from the dry weight to give the organic weight of the algae.

#### II.5. THE EFFECT OF DIET ON GROWTH:

Table 1 gives a summary of the experimental conditions and algal species used in each of the four experiments to determine the effect of diet on growth of juvenile P. macellanicus.

Table 1. Summary of experimental factors and algal combinations used in growth experiments.

	Experiment			
	1	2	3	4
Scallop shell height(mm)	2 - 4	2 - 4	1 - 2	2 - 4
Stocking density (#/l)	10	10	100	16
Food ration (cells/ $\mu$ l)	50	50	50	50
Age of scallops (months)	7	11	3	9
Temperature ( $^{\circ}$ C)	10	10	7	10
Algal diet (1)	Iso	Iso	Iso	161
(2)	Ch	Ch	Ch	C.cal
(3)	T-Iso	T-Iso	T-Iso	161; Iso
(4)	Iso;Ch	Iso;Ch	Iso;Ch	Iso;C.cal
(5)	Ch;T-Iso	Ch;T-Iso	Ch;T-Iso	T-Iso;C.cal
(6)	Iso;Ch; T-Iso	Iso;Ch; T-Iso	Iso;Ch; T-Iso	Iso;Ch; T-Iso
(7)	-	Iso;T-Iso	Iso;T-Iso	Iso;C.cal; T-Iso
(8)	-	-	starved control	starved control

Iso = Isochrysis galbana  
 Ch = Chaetoceros muelleri  
 T-Iso = Isochrysis galbana (T-Iso)  
 161 = ALGAL 161  
 C.cal = Chaetoceros calcitrans

#### II.5.i. Experiments 1 and 2:

Scallops aged 7 months (Experiment 1) and 11 months (Experiment 2) were size graded using mesh screens to give a

size class of 2 - 4 mm shell height. Scallops (200) were put on each of 3 screens suspended in tanks containing 60 litres of filtered seawater standing in a wet bench to maintain temperature at 10°C. Water was circulated over the screens using air lifts to create a downwelling circulation of water over the screens. Water was changed 3 times per week by displacement.

Each tank was batch-fed algae daily a ration of 50 cells/ $\mu$ l with the mixed diets consisting of equal portions of each constituent species based on cell number. The water was mixed thoroughly to resuspend any settled algal cells prior to sampling for cell counts because no control tanks were available. Cell counts were made daily using a Coulter Counter and growth (shell height, dry weight and organic weight) was monitored every two weeks by subsampling 30 scallops per screen.

#### II.5.ii. Experiment 3:

Scallops aged 3 months were size graded using 1 mm and 2 mm mesh screens. Thirty-two experimental tanks were set up in four water baths at  $7^{\circ}\pm 1^{\circ}$ C. Batches of 1000 juveniles were placed on screens suspended in the tanks in 10 l filtered seawater, so that the density of animals was 1 scallop per 10 ml filtered seawater. Three replicates were set up for each treatment, together with controls with

no animals to determine the settling out of algal cells in the calculation of filtration rates. Water was circulated, and food kept in suspension, by gentle bubbling and an airlift downweller. Fresh filtered seawater was added 3 times a week by displacement at a flow rate of approximately 8 l/min. for 5 minutes.

The ration of the appropriate diet was batch-fed daily at 50 cells/ $\mu$ l with mixed diets made up from equal ratios of each component species based on cell number.

Cell counts were done daily using a Coulter Counter and growth (shell height, dry weight and organic weight) was monitored every two weeks by subsampling 50 scallops per tank.

#### II.5.iii. Experiment 4:

Juveniles aged 9 months were size graded using 2 mm and 4 mm mesh screens resulting in selection of scallops 2 - 4 mm in length. Thirty-two tanks were set up in three water baths maintained at a temperature of  $10^{\circ}\pm 1^{\circ}\text{C}$ , with three replicates of each treatment, plus controls with no animals. 160 scallops were placed on screens suspended in the tanks containing 10 l filtered seawater. The density of animals was 1 scallop per 63 ml filtered seawater. Conditions were similar to experiment 3, except that C. calcitrans and Algal



161 were tested as dietary components. Growth was monitored by subsampling 20 scallops per tank every seven days.

## **II.6. GROWTH DETERMINATIONS:**

### **II.6.i. Shell Height:**

The required number of scallops were removed from the experimental rearing tank. Scallops sized 2 - 4 mm were placed in a petri dish and an image was recorded on a photocopier. The scallop images on the photocopy were then imported into Jandel Scientific's Sigma Scan program (Version 3.90) for measurement of shell height. Scallops 1 - 2 mm in size were videotaped on a JVC ER videotape using a Wild M420 Microscope and Cohu Model 4815 camera connected to a JVC model H4-D4400 video cassette recorder. Scallops were sized using image analysis (JAVA R program, Version 1.20, Jandel Scientific).

### **II.6.ii. Dry Weight:**

The whole scallops used for shell height determinations were filtered onto a pre-ashed, pre-weighed Whatman GF/C filter using a minimum volume of filtered seawater. The scallops were then rinsed with 5 - 10 ml isotonic ammonium formate dried at 90°C (24 hours) and weighed.

**II.6.iii. Organic Weight:**

Aluminum weighing pans containing filter papers and scallops were placed in a muffle furnace at 450°C for 5 hours to remove any organic components. The organic weight was determined by subtracting the remaining inorganic component from the dry weight.

**II.7. DETERMINATION OF INGESTION RATES**

Since the ration used (50 cells/ $\mu$ l) was assumed to be below the critical cell concentration (Hollelt and Dabinett, 1989), that is, the concentration above which pseudofaeces is produced, the number of cells removed from suspension was considered to be ingested by the animals. Therefore, the ingestion rate (cells/h) was calculated by determining the number of cells removed from suspension over a period of time.

The dry weight or organic weight of cells ingested can then be determined by multiplying the number of cells ingested by the dry weight or organic weight per cell of the appropriate algae.

**II.8. DETERMINATION OF GROSS GROWTH EFFICIENCY**

Gross growth efficiency (K1) is growth per unit of ingested ration. Because respiration rates were not

determined, assimilated portion of food could not be determined and net growth efficiencies (K2) were not calculated. Because the algal ration used was below the level necessary for production of pseudofaeces, the food removed from the suspension was equal to the food ingested by the animals.

Growth was measured as an increase in organic weight per animal during a specified period of time. The number of cells ingested by the animals was determined using the Coulter Counter. The organic weight of cells ingested was calculated by multiplying this number by the organic weights of the algal cells as shown in Table 2.

#### II.9. EFFECT OF RATION ON FILTRATION AND INGESTION RATES

An experiment was done to determine the effects of ration on filtration rates for three size classes of juvenile P. magellanicus aged 5 months. The three size classes studied were 2 - 4 mm shell height, 4 - 6 mm shell height and >6 mm shell height. The required sizes were collected by gently sieving scallops through appropriately sized submerged screens as in previous experiments. The three food rations studied were 20 cells/ $\mu$ l, 40 cells/ $\mu$ l and 80 cells/ $\mu$ l.

Thirty tanks were set up in four water baths. Each tank contained 10 l filtered seawater. Water was circulated

and food kept in suspension by gentle bubbling and a downweller. Temperature in each tank was maintained at 7°C - 7.5°C. There were three replicates of each of the following treatments:

- (1) 72 scallops (2 - 4 mm) at a ration of 20 cells/ $\mu$ l
- (2) 144 scallops (2 - 4 mm) at a ration of 40 cells/ $\mu$ l
- (3) 288 scallops (2 - 4 mm) at a ration of 80 cells/ $\mu$ l
- (4) 20 scallops (4 - 6 mm) at a ration of 20 cells/ $\mu$ l
- (5) 40 scallops (4 - 6 mm) at a ration of 40 cells/ $\mu$ l
- (6) 80 scallops (4 - 6 mm) at a ration of 80 cells/ $\mu$ l
- (7) 12 scallops (>6 mm) at a ration of 20 cells/ $\mu$ l
- (8) 24 scallops (>6 mm) at a ration of 40 cells/ $\mu$ l
- (9) 48 scallops (>6 mm) at a ration of 80 cells/ $\mu$ l

and controls at each cell density with no animals.

The number of scallops was increased with increasing ration in order for any decrease in ration to be observed in the half-hour measuring time. The experiment lasted 30 hours. Counts were done every 30 minutes and algae were added to maintain the appropriate level using a 1:1:1 mixture of *I. galbana*, *C. muelleri* and *I. galbana* (T-Iso) based on cell number. Another count was then done 30 minutes later and the number of cells removed from suspension was used to determine ingestion rate. Cell counts were done using a model Z<sub>c</sub> Coulter Counter with a 100  $\mu$ m aperture orifice tube.

For animals 2 - 4 mm shell height, organic weights were determined using the whole animal. For those animals sized 4 - 6 mm and >6 mm shell height, organic weights were determined separately for tissue and shells.

## II.10. DETERMINATION OF FILTRATION AND INGESTION RATES

The cells removed from suspension during a 30-minute sampling period were considered to be ingested by the scallops, as there was no production of pseudofaeces and settling was negligible. Filtration rate was determined using the following formula (Coughlan, 1969):

$$m = M / (n \cdot t) \log_e \text{Conc}_0 / \text{Conc}_t$$

where  $m$  = filtering rate of animal  
 $M$  = volume of suspension  
 $n$  = number of animals  
 $t$  = period of time

$\text{Conc}_0$  and  $\text{Conc}_t$  = concentrations initially and after time  $t$  in suspension

Filtration rates were determined for each 30-minute sampling period and an average filtration rate was calculated for the entire experimental period for each experimental tank (ml/scallop/hr). Dividing these filtration values by the organic weight of animals in the tank (mg/scallop) resulted in filtration rates per unit weight of animal (ml/mg/hr). Corresponding ingestion rates were determined by multiplying these filtration rate values by the concentration of food in the tank (cells/scallop/hr) and specific ingestion rates (cells/mg/hr) were calculated.

## II.11. STATISTICAL METHODS

All statistical analyses were performed using SAS (Statistical Analysis System), developed by SAS Institute Inc., Cary, N.C., USA.

The growth data for the juveniles was subjected to a two-way analysis of variance with interaction using the categorical variables of diet and time. A probability of greater than 0.05 for the W test for normality (Shapiro-Wilks test) was accepted. Residual plots were examined for independence and constant variance.

In those cases where variance was not constant, a log transformation of the data was performed before analysis of variance. In certain cases, where a log transformation did not result in constant variance, outliers were removed or a weighted analysis of variance was performed. Of the twelve analyses performed, only two did not result in a p value greater than 0.05 in the W test after such manipulations. In these cases, examination of the normality plots indicate that they are close to normal and are symmetrical.

Time 0 points were not included in the analysis since only one average initial sample was taken for all the experimental groups.

Duncan's means tests were used to test for differences between diets at individual sampling times.

Two non-parametric tests, Friedman's method for randomized blocks and the Kruskal-Wallis test, were used to test for significance in the gross growth efficiency data.

For the study of the physiological rates of filtration and ingestion, log physiological rate was plotted against log organic weight and the plot inspected for linearity. A linear regression was performed to determine the parameters for the allometric equation relating physiological function (F) to size (organic weight) namely:  $F = aW^b$ . The values for the constant 'a' and weight exponent 'b' were determined from the linear regression of the log transformed variables namely:  $\log F = \log a + b \log W$  (Sokal and Rohlf, 1969).

## III RESULTS

## III.1. CHARACTERISTICS OF ALGAL SPECIES

The cell characteristics of the algal species used in this study are given in Table 2.

Table 2. General characteristics of the algal species used in Experiments 1 - 4.

Species	Dry weight (pg/cell) Mean $\pm$ sd	Organic weight (pg/cell) Mean $\pm$ sd	Mean cell diameter ( $\mu$ )
<u>Isochrysis</u> <u>galbana</u>	21.8 $\pm$ 1.0 (n = 10)	19.8 $\pm$ 0.9	3.8
<u>Chaetoceros</u> <u>muelleri</u>	26.4 $\pm$ 2.4 (n = 7)	16.1 $\pm$ 1.6	3.5
<u>I. aff.</u> <u>galbana</u> T-Iso	30.5 $\pm$ 2.1 (n = 6)	26.1 $\pm$ 1.0	5.2
<u>Chaetoceros</u> <u>calcitrans</u>	14.4 $\pm$ 1.8 (n = 8)	10.5 $\pm$ 1.0	4.2

The weights and sizes found in this study are in general agreement with those reported in the literature, which for comparison are presented in Table 3.



Table 3. Literature values for algal cell characteristics.

Species	Dry Wt. (pg/cell)	Org Wt. (pg/cell)	Size ( $\mu$ )	Source
<u>Isochrysis galbana</u>	23.5			Epifanio and Ewart (1977)
<u>Isochrysis</u> aff. <u>galbana</u> (T-Iso)	20.1			Urban <u>et.al.</u> (1983)
<u>Isochrysis galbana</u>	16.1			Romberger and Epifanio (1981)
<u>Isochrysis galbana</u>	24.2			Manning (1986)
<u>Isochrysis</u> aff. <u>galbana</u> (T-Iso)	30.8			Manning (1986)
<u>Chaetoceros calcitrans</u>		7		Laing and Millican (1986)
<u>Isochrysis</u> aff. <u>galbana</u> (T-Iso)		20		Laing and Millican (1986)
<u>Isochrysis galbana</u>			5 x 2.5	Cary <u>et.al.</u> (1981)
<u>Isochrysis</u> aff. <u>galbana</u> (T-Iso)			5 x 2.5	Cary <u>et.al.</u> (1981)

The algal species used were in the size range of 3 - 5  $\mu$ m diameter. Isochrysis aff. galbana (T-Iso) had the highest dry and organic weights and the greatest cell diameter. The Chaetoceros calcitrans cells, although not the smallest, had the least dry and organic weights. The other two species studied, Chaetoceros muelleri and Isochrysis galbana fall between these two extremes. In terms of organic weight supplied to the juveniles, the diets were ranked as follows

(in descending order): (1) T-Iso; (2) Isochrysis galbana/T-Iso; (3) Chaetoceros muelleri/ T-Iso; (4) Isochrysis galbana/Chaetoceros muelleri/T-Iso; (5) Isochrysis galbana; (6) Isochrysis galbana/Chaetoceros muelleri; and (7) Chaetoceros muelleri.

### III.2. GROWTH: EXPERIMENT 1

In Experiment 1, growth, as measured in terms of shell height, dry weight and organic weight, was determined for 3 replicates of each of the following diets: (1) Isochrysis galbana; (2) Chaetoceros muelleri; (3) Isochrysis galbana (T-Iso); (4) Isochrysis galbana and Chaetoceros muelleri; (5) T-Iso and Chaetoceros muelleri; and (6) Isochrysis galbana, Chaetoceros muelleri and T-Iso.

#### III.2.i. Shell Height:

The relationship between shell height, diet and time is shown in Figure 1. An analysis of variance was done on the log transformed shell height data. Shell height varied significantly with the diet\*time interaction ( $F=11.03$ ;  $df=20$ ;  $p=0.0001$ ), suggesting that neither main effect can be studied individually.

The results of the Duncan's means test indicate that there was no significant difference in shell height in any of the diets before Day 14. By Day 42, the ternary diet had

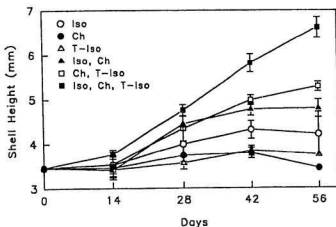


Figure 1. *Placopecten magellanicus*. Mean shell heights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=*Isochrysis galbana*; Ch=*Chaetoceros muelleri*; T-Iso= *Isochrysis galbana* (T-Iso)). (Experiment 1)

Table 4. *Placopecten magellanicus*. Summary of results of Duncan's means test for differences in shell heights of spat (2 - 4 mm) reared on various diets (Experiment 1).

		Time (days)			
		14	28	42	56
	A(6)	A(6)	A(6)	A(6)	A(6)
	A(5)	A(5)	A(5)	B(5)	B(5)
	A(1)	AB(4)	B(4)	B(4)	C(4)
	A(2)	BC(1)	C(1)	C(1)	D(1)
	A(3)	CD(3)	D(3)	D(3)	E(3)
	A(4)	D(2)	D(2)	D(2)	E(2)

- (1) *Isochrysis galbana*
- (2) *Chaetoceros muelleri*
- (3) *Isochrysis galbana* (T-Iso)
- (4) *Isochrysis galbana*; *Chaetoceros muelleri*
- (5) *Isochrysis galbana* (T-Iso); *Chaetoceros muelleri*
- (6) *Isochrysis galbana*; *Chaetoceros muelleri*; *Isochrysis galbana* (T-Iso)

A - E Diets in each column with the same letter do not differ significantly

produced significantly more growth than the other diets, followed by the two binary diets, although these were not significantly different from each other. By Day 56, the three mixed diets are significantly different from each other and the unialgal diets, with the ternary diet resulting in the greatest growth, followed by the T-Iso/Chaetoceros muelleri diet and the Isochrysis galbana/Chaetoceros muelleri diet, respectively.

#### III.2.ii. Dry Weight:

An ANOVA was performed on the log transformed data for dry weight, diet and time, as was done for shell height. Similar results were obtained ( $F=13.09$ ;  $df=15$ ;  $p=0.0001$ ). Figure 2 depicts dry weight versus time for the different diets studied. Duncan's means test shows that by Day 42, the ternary diet had produced significantly greater growth than any of the other diets and by Day 56, each of the diets are significantly different from each other, with the ternary diet producing the greatest growth and the unialgal Chaetoceros diet producing the least growth.

#### III.2.iii. Organic Weight:

Changes in organic weight over time on each diet are shown in Figure 3. An ANOVA of the log transformed data was performed. Again, organic weight varied significantly with the diet\*time interaction ( $F=13.89$ ;  $df=15$ ;  $p=0.0001$ ). The

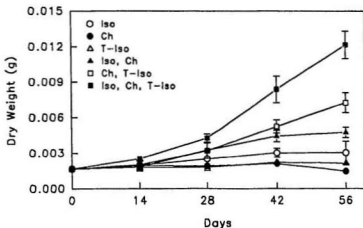


Figure 2. Placopecten magellanicus. Mean dry weights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; Ch=Chaetoceros muelleri; T-Iso= Isochrysis galbana (T-Iso)). (Experiment 1)

Table 5. Placopecten magellanicus. Summary of results of Duncan's means test for differences in dry weights of spat (2 - 4 mm) reared on various diets (Experiment 1).

	Time (days)			
	14	28	42	56
	A(6)	A(6)	A(6)	A(6)
	AB(5)	AB(4)	B(5)	B(5)
	AB(1)	AB(5)	B(4)	C(4)
	AB(2)	BC(1)	C(1)	D(1)
	B(4)	CD(2)	D(3)	E(3)
	B(3)	D(3)	D(2)	F(2)

- (1) Isochrysis galbana
- (2) Chaetoceros muelleri
- (3) Isochrysis galbana (T-Iso)
- (4) Isochrysis galbana; Chaetoceros muelleri
- (5) Isochrysis galbana (T-Iso); Chaetoceros muelleri
- (6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)

A - F Diets in each column with the same letter do not differ significantly

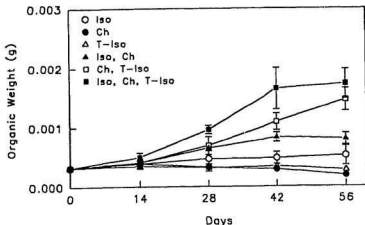


Figure 3. Placopecten magellanicus. Mean organic weights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; Ch=Chaetoceros muelleri; T-Iso= Isochrysis galbana (T-Iso)). (Experiment 1)

Table 6. Placopecten magellanicus. Summary of results of Duncan's means test for differences in organic weights of spat (2 - 4 mm) reared on various diets (Experiment 1).

	Time (days)			
	14	28	42	56
	A(6)	A(6)	A(6)	A(6)
	AB(4)	B(5)	B(5)	A(5)
	AB(5)	B(4)	B(4)	B(4)
	AB(2)	C(1)	C(1)	C(1)
	AB(1)	D(2)	D(3)	D(3)
	B(3)	D(3)	D(2)	E(2)

- (1) Isochrysis galbana
- (2) Chaetoceros muelleri
- (3) Isochrysis galbana (T-Iso)
- (4) Isochrysis galbana; Chaetoceros muelleri
- (5) Isochrysis galbana (T-Iso); Chaetoceros muelleri
- (6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)

A - E Diets in each column with the same letter do not differ significantly

results of Duncan's means test show that by Day 28, the ternary diet had produced significantly greater growth than any of the other diets studied, followed by the two binary diets which were not significantly different from each other. This pattern also held true on Day 42; by Day 56, the ternary diet and the binary diet of T-Iso/Chaetoceros muelleri both showed the greatest growth, although they were not significantly different from each other. The unialgal Chaetoceros diet supported the least growth.

### III.3 GROWTH: EXPERIMENT 2

The second experiment was basically a repeat of the first with an extra diet combination, and using scallops four months older. The following diets were compared: (1) Isochrysis galbana; (2) Chaetoceros muelleri; (3) Isochrysis aff. galbana (T-Iso); (4) Isochrysis galbana and Chaetoceros muelleri; (5) Isochrysis galbana and T-Iso; (6) Chaetoceros muelleri and T-Iso; and (7) Isochrysis galbana, Chaetoceros muelleri and T-Iso.

#### III.3.1. Shell Height:

A plot of shell height against time for the different diets studied is shown in Figure 4. A weighted analysis of variance was performed on the shell heights, with shell height varying significantly with the diet\*time interaction

( $F=4.48$ ;  $df=18$ ;  $p=0.0001$ ). The results of Duncan's means test indicate that on Day 14, there was no significant difference between any of the diets. By Day 42, the ternary diet had resulted in significantly more growth than any of the other diets, which were not significantly different from each other. On Day 56, both the ternary and binary diet of Isochrysis galbana and T-Iso showed the most growth, although they were not significantly different from each other.

#### III.3.ii. Dry Weight:

The relationship between dry weight, diet and time is shown in Figure 5. An analysis of variance was done on the dry weights after two outliers had been removed. As observed with shell height, dry weight varied significantly with the diet\*time interaction ( $F=10.80$ ;  $df=18$ ;  $p=0.0001$ ).

The results of Duncan's means test indicate that at Day 14, there was no significant difference between any of the diets. By Day 42, the ternary diet had produced significantly greater growth than any of the other diets.

#### III.3.iii. Organic Weight:

Growth in terms of organic weight versus time for the different diets is given in Figure 6. An analysis of variance was done on the log transformed organic weight data. Although the  $p$  value for the  $W$  test for normality was



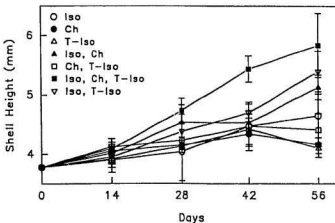


Figure 4. *Placopecten magellanicus*. Mean shell heights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=*Isochrysis galbana*; Ch=*Chaetoceros muelleri*; T-Iso= *Isochrysis galbana* (T-Iso)). (Experiment 2)

Table 7. *Placopecten magellanicus*. Summary of results of Duncan's means test for differences in shell heights of spat (2 - 4 mm) reared on various diets (Experiment 2).

		Time (days)			
		14	28	42	56
	A(5)	A(5)	A(6)	A(6)	A(6)
	A(6)	A(6)	AB(4)	B(7)	AB(7)
	A(2)	A(2)	BC(7)	B(4)	BC(4)
	A(4)	A(4)	BC(5)	B(5)	CD(1)
	A(7)	A(7)	C(2)	B(1)	D(5)
	A(3)	A(3)	C(3)	B(3)	D(2)
	A(1)	A(1)	C(1)	B(2)	D(3)

- (1) *Isochrysis galbana*
- (2) *Chaetoceros muelleri*
- (3) *Isochrysis galbana* (T-Iso)
- (4) *Isochrysis galbana*; *Chaetoceros muelleri*
- (5) *Isochrysis galbana* (T-Iso); *Chaetoceros muelleri*
- (6) *Isochrysis galbana*; *Chaetoceros muelleri*; *Isochrysis galbana* (T-Iso)
- (7) *Isochrysis galbana*; *Isochrysis galbana* (T-Iso)

A - D Diets in each column with the same letter do not differ significantly

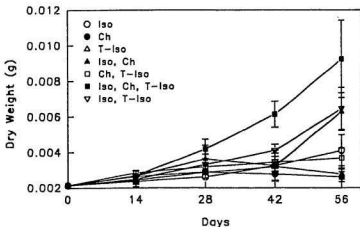


Figure 5. Placopecten magellanicus. Mean dry weights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; Ch=Chaetoceros muelleri; T-Iso= Isochrysis galbana (T-Iso)). (Experiment 2)

Table 8. Placopecten magellanicus. Summary of results of Duncan's means test for differences in dry weights of spat (2 - 4 mm) reared on various diets (Experiment 2).

Time (days)			
14	28	42	56
A(5)	A(6)	A(6)	A(6)
A(6)	AB(4)	B(7)	B(7)
A(4)	ABC(7)	BC(5)	B(4)
A(2)	BC(5)	BC(4)	C(1)
A(7)	BC(2)	BC(3)	CD(5)
A(3)	BC(3)	BC(1)	DE(3)
A(1)	C(1)	C(2)	E(2)

- (1) Isochrysis galbana
- (2) Chaetoceros muelleri
- (3) Isochrysis galbana (T-Iso)
- (4) Isochrysis galbana; Chaetoceros muelleri
- (5) Isochrysis galbana (T-Iso); Chaetoceros muelleri
- (6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (7) Isochrysis galbana; Isochrysis galbana (T-Iso)

A - D Diets in each column with the same letter do not differ significantly

not greater than 0.05, examination of the normality plot shows it to be symmetrical and close to normal. The diet\*time interaction had a significant effect on organic weight ( $F=2.30$ ;  $df=21$ ;  $p=0.0141$ ).

The results of Duncan's means test indicate that on Day 14, there was no significant difference between any of the diets. However, on Day 56, the ternary diet showed significantly more growth than any of the other diets. Furthermore on Day 56, the two unialgal diets of Chaetoceros muelleri and T-Iso showed the least growth, although they were not significantly different from each other.

#### III.4. GROWTH: EXPERIMENT 3

This experiment studied the effect of different diets on growth of juvenile scallops 1 - 2 mm shell height using an experimental design which permitted better replication than was used in experiments 1 and 2. The following diets were examined: (1) Isochrysis galbana; (2) Chaetoceros muelleri; (3) Isochrysis aff. galbana (T-Iso); (4) Isochrysis galbana and Chaetoceros muelleri; (5) Isochrysis galbana and T-Iso; (6) Chaetoceros muelleri and T-Iso; and (7) Isochrysis galbana, Chaetoceros muelleri and T-Iso. These were compared to growth of scallops suspended in ambient seawater with no additional food added.

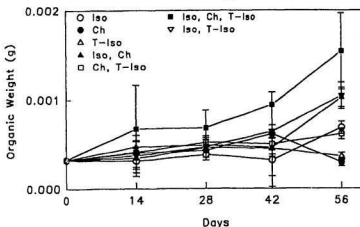


Figure 6. Placopecten magellanicus. Mean organic weights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; Ch=Chaetoceros muelleri; T-Iso= Isochrysis galbana (T-Iso)). (Experiment 2)

Table 9. Placopecten magellanicus. Summary of results of Duncan's means test for differences in organic weights of spat (2 - 4 mm) reared on various diets (Experiment 2).

Time (days)			
14	28	42	56
A(6)	A(6)	A(6)	A(6)
A(4)	AB(5)	AB(7)	B(7)
A(2)	AB(4)	AB(1)	B(4)
A(5)	B(7)	AB(2)	C(1)
A(7)	B(3)	B(5)	C(5)
A(1)	B(2)	B(3)	D(3)
A(3)	B(1)	B(4)	D(2)

- (1) Isochrysis galbana
- (2) Chaetoceros muelleri
- (3) Isochrysis galbana (T-Iso)
- (4) Isochrysis galbana; Chaetoceros muelleri
- (5) Isochrysis galbana (T-Iso); Chaetoceros muelleri
- (6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (7) Isochrysis galbana; Isochrysis galbana (T-Iso)

A - D Diets in each column with the same letter do not differ significantly

#### III.4.1. Shell Height:

Growth in terms of shell height versus time for Experiment 3 is shown in Figure 7. Although the p value for the W test for normality was not greater than 0.05, examination of the normality plot shows it to be symmetrical and close to normal. The diet\*time interaction had a significant effect on shell height ( $F=5.72$ ;  $df=21$ ;  $p=0.0001$ ).

The results of Duncan's means test indicate that by day 42, the following four diets had produced the greatest growth, although they were not significantly different from each other: (1) Isochrysis galbana/T-Iso; (2) Isochrysis galbana/Chaetoceros muelleri/T-Iso; (3) Isochrysis galbana; and (4) Isochrysis galbana/Chaetoceros muelleri. The two diets of Chaetoceros muelleri/T-Iso and T-Iso produced less growth than the aforementioned, although not significantly different from each other. The least growth was observed in the scallops fed Chaetoceros muelleri alone and in those grown with no food supplement, although, again, these two were not significantly different from each other. The same trends were observed on Day 56, except that the unialgal Chaetoceros muelleri diet produced significantly greater growth than the seawater alone, although less than all the other diets examined.

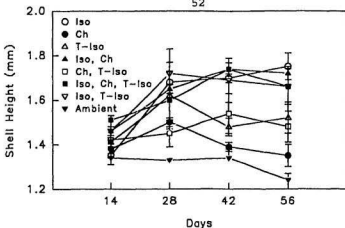


Figure 7. Placopecten magellanicus. Mean shell heights ( $\pm$  sd) for spat (1 - 2 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; Ch=Chaetoceros muelleri; T-Iso= Isochrysis galbana (T-Iso)). (Experiment 3)

Table 10. Placopecten magellanicus. Summary of results of Duncan's means test for differences in shell heights of spat (1 - 2 mm) reared on various diets (Experiment 3).

		Time (days)			
		14	28	42	56
	A(6)	A(7)	A(4)	A(1)	
	AB(7)	A(1)	A(6)	A(4)	
	AB(4)	A(4)	A(1)	A(7)	
	BC(5)	AB(3)	A(7)	A(6)	
	BC(3)	AB(6)	B(5)	B(3)	
	BC(2)	BC(2)	B(3)	B(5)	
	C(1)	C(5)	C(2)	C(2)	
	C(8)	D(8)	C(8)	D(8)	

- (1) Isochrysis galbana
- (2) Chaetoceros muelleri
- (3) Isochrysis galbana (T-Iso)
- (4) Isochrysis galbana; Chaetoceros muelleri
- (5) Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (7) Isochrysis galbana; Isochrysis galbana (T-Iso)
- (8) Ambient

A - D Diets in each column with the same letter do not differ significantly

#### III.4.ii. Dry Weight:

Changes in dry weight versus time are shown in Figure 8. An analysis of variance was done on the log transformed data after one outlier had been removed. Dry weight varied significantly with the diet\*time interaction ( $F=8.34$ ;  $df=21$ ;  $p=0.0001$ ), indicating that neither main effect could be studied individually.

The results of Duncan's means test showed that by Day 29, the ternary diet had produced significantly greater growth than any of the other diets. At this time, the Chaetoceros muelleri unialgal diet had produced significantly less growth than any of the other diets and was not significantly different than growth produced in scallops which were not given any additional food. However, on Day 43, the following four diets had produced significantly greater growth than the other diets, although they were not significantly different from each other: (1) Isochrysis galbana, Chaetoceros muelleri and T-Iso; (2) Isochrysis galbana and T-Iso; (3) Isochrysis galbana and Chaetoceros muelleri; and (4) Isochrysis galbana. Of the diets tested, the Chaetoceros muelleri diet produced significantly less growth, although it resulted in greater growth than the ambient filtered seawater. These same trends were observed on Day 60.

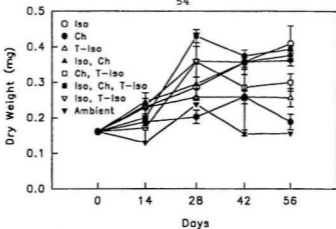


Figure 8. Placopecten magellanicus. Mean dry weights ( $\pm$  sd) for spat (1 - 2 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; Ch=Chaetoceros muelleri; T-Iso= Isochrysis galbana (T-Iso)). (Experiment 3)

Table 11. Placopecten magellanicus. Summary of results of Duncan's means test for differences in dry weights of spat (1 - 2 mm) reared on various diets (Experiment 3).

Time (days)			
14	28	42	56
A(4)	A(6)	A(6)	A(1)
AB(7)	B(5)	A(7)	A(6)
AB(1)	B(7)	A(4)	A(4)
AB(3)	C(4)	A(1)	A(7)
ABC(6)	C(1)	B(5)	B(5)
BC(2)	CD(3)	B(3)	C(3)
C(5)	DE(8)	C(2)	D(2)
D(8)	E(2)	D(8)	E(8)

- (1) Isochrysis galbana
- (2) Chaetoceros muelleri
- (3) Isochrysis galbana (T-Iso)
- (4) Isochrysis galbana; Chaetoceros muelleri
- (5) Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (7) Isochrysis galbana; Isochrysis galbana (T-Iso)
- (8) Ambient

A - E Diets in each column with the same letter do not differ significantly



#### III.4.iii. Organic Weight:

The relationship between organic weight, diet and time is shown in Figure 9. An analysis of variance was performed on the log transformed organic weight data, indicating a significant diet\*time interaction effect on organic weight ( $F=2.30$ ;  $df=21$ ;  $p=0.0058$ ).

The results of Duncan's means test indicate that no one diet produced significantly greater growth than the others throughout the experiment. By Day 60, the following four diets, although not significantly different from each other, had produced the greatest growth in terms of organic weight:

- (1) Isochrysis galbana; (2) Isochrysis galbana and T-Iso;
- (3) Isochrysis galbana and Chaetoceros muelleri; (4)

Isochrysis galbana, Chaetoceros muelleri and T-Iso. Growth was significantly lower in those scallops maintained in seawater alone. Of the foods tested, the following diets, although not significantly different from each other, showed the least growth: (1) T-Iso; (2) Chaetoceros muelleri; and (3) Chaetoceros muelleri and T-Iso. Similar trends were observed for the duration of the experiment.

#### III.5. GROWTH: EXPERIMENT 4

The experimental design of experiment 4 was similar to experiment 3, but a larger size class was used (2 - 4 mm shell height). In addition to algal diets consisting of

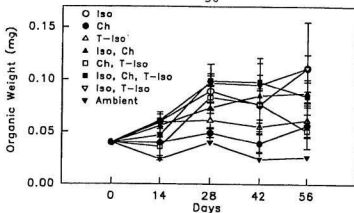


Figure 9. Placopecten macellanicus. Mean organic weights ( $\pm$  sd) for spat (1 - 2 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; Ch=Chaetoceros muelleri; T-Iso= Isochrysis galbana (T-Iso)). (Experiment 3)

Table 12. Placopecten macellanicus. Summary of results of Duncan's means test for differences in organic weights of spat (1 -2 mm) reared on various diets (Experiment 3).

Time (days)				
14	28	42	56	
A(7)	A(6)	A(6)	A(1)	
A(1)	A(7)	A(7)	A(7)	
A(3)	AB(1)	AB(4)	AB(4)	
AB(4)	AB(5)	AB(1)	AB(6)	
ABC(6)	BC(4)	AB(5)	BC(3)	
BC(2)	CD(3)	BC(3)	C(2)	
CD(5)	DE(2)	C(2)	C(5)	
D(8)	E(8)	D(8)	D(8)	

- (1) Isochrysis galbana
- (2) Chaetoceros muelleri
- (3) Isochrysis galbana (T-Iso)
- (4) Isochrysis galbana; Chaetoceros muelleri
- (5) Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (7) Isochrysis galbana; Isochrysis galbana (T-Iso)
- (8) Ambient

A - D Diets in each column with the same letter do not differ significantly

Isochrysis galbana, Isochrysis aff. galbana (T-Iso) and Chaetoceros muelleri, this experiment also included another Chaetoceros species, Chaetoceros calcitrans, and a commercial product known as ALGAL 161. Algal 161 was fed alone and was also combined with Isochrysis galbana in a ratio of 80% ALGAL 161 and 20% Isochrysis galbana.

#### III.5.i. Shell Height:

Growth in terms of shell height against time for scallops fed the different diets is shown in Figure 10. An analysis of variance was done on the log transformed shell height data, resulting in a significant diet\*time interaction on shell height ( $F=2.18$ ;  $df=21$ ;  $p=0.0058$ ).

Results of Duncan's means test show that at Day 7, there was no significant difference between any of the diets. By Day 29, both the ternary diets supported significantly greater growth than the other diets, although they were not significantly different from each other. However, on Day 36, these ternary diets, as well as the binary diets of T-Iso/Chaetoceros calcitrans and Isochrysis galbana/Chaetoceros calcitrans yielded the greatest growth, although these four diets were not significantly different from each other. The two diets containing ALGAL 161, as well as the unialgal Chaetoceros calcitrans diet, all supported the least growth and were not significantly

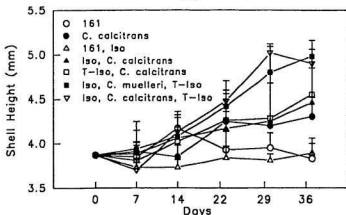


Figure 10. Placopecten magellanicus. Mean shell heights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; T-Iso=Isochrysis galbana (T-Iso); C.muelleri=Chaetoceros muelleri; C.calcitrans=Chaetoceros calcitrans; 161=ALGAL 161). (Experiment 4)

Table 13. Placopecten magellanicus. Summary of results of Duncan's means test for differences in shell heights of spat (2 - 4 mm) reared on various diets (Experiment 4).

	Time (days)				
	7	14	22	29	36
A(6)		^(1)	A(7)	A(7)	A(6)
A(2)		A(7)	A(6)	AB(6)	A(7)
A(4)		AB(6)	AB(5)	BC(5)	AB(5)
A(1)		AB(4)	AB(2)	BC(4)	AB(4)
A(5)		AB(5)	AB(4)	BC(2)	BC(2)
A(3)		AB(2)	B(1)	C(1)	C(3)
A(7)		B(3)	B(3)	C(3)	C(1)

(1) ALGAL 161

(2) Chaetoceros calcitrans

(3) ALGAL 161; Isochrysis galbana

(4) Isochrysis galbana; Chaetoceros calcitrans

(5) Isochrysis galbana (T-Iso); Chaetoceros calcitrans

(6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)

(7) Isochrysis galbana; Chaetoceros calcitrans; Isochrysis galbana (T-Iso)

A - C Diets in each column with the same letter do not differ significantly

different from each other.

#### III.5.ii. Dry Weight:

Changes in dry weight against time are shown in Figure 11. An analysis of variance was performed on the log transformed data, similar to that executed on the shell height data, with a similar significant diet\*time interaction ( $F=5.32$ ;  $df=24$ ;  $p=0.0001$ ).

Results of Duncan's means test indicate that on Day 7, there was no significant difference between any of the diets. By Day 29, the two ternary diets showed significantly greater growth than all the other diets, although these two diets were not significantly different from each other. The two diets containing ALGAL 161, although not significantly different from each other, both showed significantly less growth than the other diets examined. On Day 36, the same trends were observed except that the binary diet of T-Iso/Chaetoceros calcitrans was not significantly different from the two ternary diets.

#### III.5.iii. Organic Weight:

Changes in mean organic weight versus time for the animals on different diets is shown in Figure 12. An analysis of variance was performed on the log transformed

organic weight data, indicating a significant diet\*time interaction on organic weight ( $F=5.23$ ,  $df=24$ ;  $p=0.0001$ ).

Results from Duncan's means test indicate that on Day 7, there was no significant difference between any of the diets. By Day 29, the two ternary diets, although not significantly different from each other, both supported significantly greater growth than the other diets examined. The two diets containing ALGAL 161 were not significantly different from each other and showed significantly less growth than the other diets. By Day 36, the ternary diet consisting of Isochrysis galbana/T-Iso/Chaetoceros muelleri showed significantly greater growth than the other diets. The two diets containing ALGAL 161 showed the least growth and the other four diets examined did not show any significant difference in terms of organic weight.

### III.6. GROSS GROWTH EFFICIENCIES

The mean gross growth efficiencies (K1) for Experiment 1 are given in Figure 13. The K1 values for scallops fed the mixed algal diets remained reasonably consistent between 20 - 40% for the first three time periods whereas K1 values for animals fed unialgal diets decreased over time.

The mean gross growth efficiencies for Experiment 2 are given in Figure 14. Since this experiment is basically

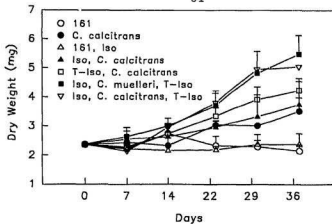


Figure 11. Placopecten magellanicus. Mean dry weights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; T-Iso=Isochrysis galbana (T-Iso); C. muelleri=Chaetoceros muelleri; C. calcitrans=Chaetoceros calcitrans; 161=ALGAL 161). (Experiment 4)

Table 14. Placopecten magellanicus. Summary of results of Duncan's means test for differences in dry weights of spat (2 - 4 mm) reared on various diets (Experiment 4).

		Time (days)				
		7	14	22	29	36
	A(6)	A(6)	A(6)	A(7)	A(7)	A(6)
	A(4)	A(4)	A(7)	A(6)	AB(6)	A(7)
	A(2)	AB(1)	AB(1)	A(5)	BC(5)	AB(5)
	A(5)	AB(5)	AB(5)	AB(4)	CD(4)	B(4)
	A(3)	AB(4)	AB(4)	AB(2)	D(2)	B(2)
	A(1)	BC(2)	BC(2)	BC(3)	E(1)	C(3)
	A(7)	C(3)	C(3)	BC(1)	E(3)	C(1)

- (1) ALGAL 161
- (2) Chaetoceros calcitrans
- (3) ALGAL 161; Isochrysis galbana
- (4) Isochrysis galbana; Chaetoceros calcitrans
- (5) Isochrysis galbana (T-Iso); Chaetoceros calcitrans
- (6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)
- (7) Isochrysis galbana; Chaetoceros calcitrans; Isochrysis galbana (T-Iso)

A - E Diets in each column with the same letter do not differ significantly

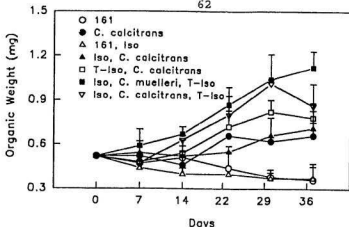


Figure 12. Placopecten magellanicus. Mean organic weights ( $\pm$  sd) for spat (2 - 4 mm) reared on various algal diets batch fed at 50 cells/ $\mu$ l. (Iso=Isochrysis galbana; T-Iso=Isochrysis galbana (T-Iso); C.muelleri=Chaetoceros muelleri; C.calcitrans=Chaetoceros calcitrans; 161=ALGAL 161). (Experiment 4)

Table 15. Placopecten magellanicus. Summary of results of Duncan's means test for differences in organic weights of spat (2 - 4 mm) reared on various diets (Experiment 4).

		Time (days)				
		7	14	22	29	36
A(6)	A(6)	A(6)	A(6)	A(6)	A(6)	A(6)
A(4)	AB(7)	AB(7)	AB(7)	AB(7)	AB(7)	B(7)
A(2)	BC(5)	AB(5)	AB(5)	BC(5)	BC(5)	B(5)
A(5)	BC(4)	BC(2)	BC(2)	CD(4)	CD(4)	B(4)
A(7)	BC(1)	C(4)	C(4)	D(2)	D(2)	B(2)
A(1)	CD(2)	D(1)	D(1)	E(1)	E(1)	C(3)
A(3)	D(3)	D(3)	D(3)	E(3)	E(3)	C(1)

(1) ALGAL 161

(2) Chaetoceros calcitrans

(3) ALGAL 161; Isochrysis galbana

(4) Isochrysis galbana; Chaetoceros calcitrans

(5) Isochrysis galbana (T-Iso); Chaetoceros calcitrans

(6) Isochrysis galbana; Chaetoceros muelleri; Isochrysis galbana (T-Iso)

(7) Isochrysis galbana; Chaetoceros calcitrans; Isochrysis galbana (T-Iso)

A - E Diets in each column with the same letter do not differ significantly



a replicate of Experiment 1, it is interesting to note that the Chaetoceros muelleri diet produced growth efficiencies in Experiment 2 which were generally higher than observed in Experiment 1. In Experiment 2, the second sampling period showed low efficiencies for all the diets examined. Although the ternary diet resulted in an excellent efficiency during the first time period, it seems to diminish as the experiment progressed. The binary diet of Isochrysis galbana/Chaetoceros muelleri showed relatively constant K1 values for the first and last time periods, although the two intermediate levels are very low. In general, there appears to be no specific trend in K1 values for the diets examined during the course of the experiment.

The mean gross growth efficiencies obtained for the different diets in Experiment 3 are shown in Figure 15. The animals used in the experiment were smaller than those used in the other experiments. In general, there is a trend of decreasing K1 values with time, with the exception of the unialgal Isochrysis galbana diet which shows an extremely high value during the last sampling period.

The mean gross growth efficiencies determined in Experiment 4 are given in Figure 16. Because ingestion rates could not be determined for ALGAL 161 using the

Table 16. Literature values for gross growth efficiencies of laboratory-reared juvenile bivalve molluscs fed algal diets.

Species	Size	Gross Growth Efficiency (%)	Source
<u>Mytilus edulis</u>	0.35-90.8mm	11 - 84	Jorgensen (1952)
<u>Crassostrea virginica</u>	225-552 mg (live wt.)	-37.7 - 22.6	Urban <u>et.al.</u> (1983)
<u>M. edulis</u>	100 mg (dry flesh wt.)	-35 - 53	Thompson and Bayne (1974)
<u>M. edulis</u>	16-20 mm	15 - 36	Riisgård and Randlov (1981)
<u>Tapes japonica</u>	14 mm	36.4-48.4	Langton <u>et.al.</u> (1977)
<u>C. virginica</u>	62.6 mg (soft tissue)	negative- 38.2	Romberger and Epifanio (1981)
<u>T. semidecussata</u>	0.3 mg (org. wt.)	0.07-0.32	Laing <u>et.al.</u> (1987)
<u>T. decussata</u>	0.3 mg (org. wt.)	0.04	Laing <u>et.al.</u> (1987)
<u>M. mercenaria</u>	0.3 mg (org. wt.)	0.08	Laing <u>et.al.</u> (1987)
<u>Q. edulis</u>	0.3 mg (org. wt.)	0.14	Laing and Millican (1986)

Coulter Counter, these values could not be considered. Again, the K1 values produced in this experiment do not show any clear trends among the diets or with time.

Values for growth efficiencies show considerable variation due to factors such as size and age of the animal, ration fed, temperature, salinity and species of bivalve used. As shown in Table 16, gross growth efficiency values vary widely in the literature.

Application of the two non-parametric tests, Friedman's method for randomized blocks and the Kruskal-Wallis test, used to test for significance in the gross growth efficiency data showed no significant differences between the K1 values when both tests were applied to each of the four growth experiments.

### III.7. EFFECT OF RATION ON FILTRATION AND INGESTION RATES

Filtration and ingestion rates were determined as outlined in the Materials and Methods section. Table 17 shows the treatments and the corresponding mean filtration and ingestion rates. Mean rates were used since individual tanks showed a wide variation in filtration rates per 30-minute sampling period, as can be observed in the standard deviation values. This is expected due to the varying rates observed in individual animals over a period of time. The

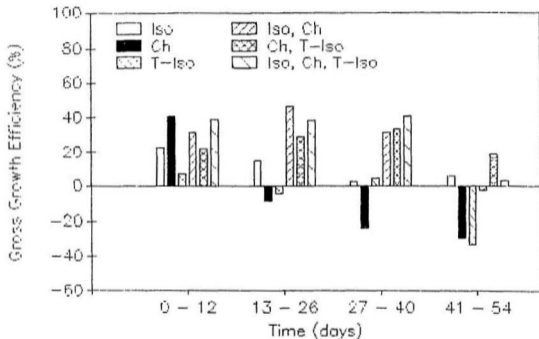


Figure 13. *Placopecten magellanicus*. Gross growth efficiencies (K1) of spat (2 - 4 mm) reared on various diets batch fed at 50 cells/ $\mu$ l. (Iso=*Isochrysis galbana*; Ch=*Chaetoceros muelleri*; T-Iso=*Isochrysis galbana* (T-Iso)). (Experiment 1)

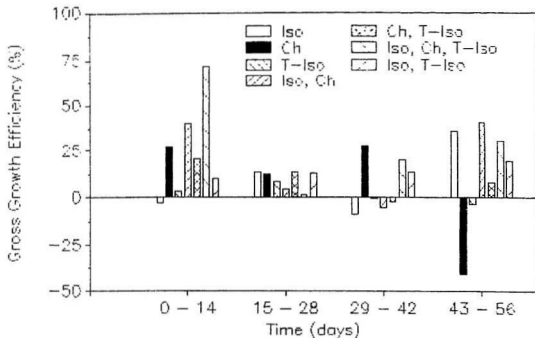


Figure 14. *Placopecten magellanicus*. Gross growth efficiencies (K1) of spat (2 - 4 mm) reared on various diets batch fed at 50 cells/ $\mu$ l. (Iso=*Isochrysis galbana*; Ch=*Chaetoceros muelleri*; T-Iso=*Isochrysis galbana* (T-Iso)). (Experiment 2)

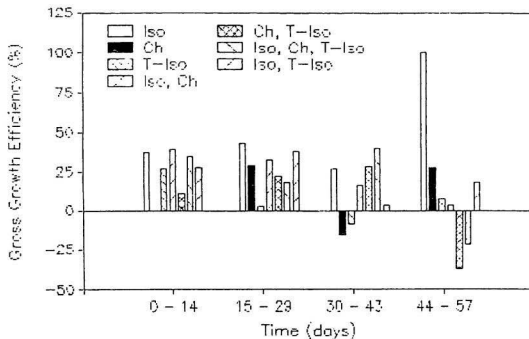


Figure 15. *Placopecten magellanicus*. Gross growth efficiencies (K1) of spat (1 - 2 mm) reared on various diets batch fed at 50 cells/ $\mu$ l. (Iso=*Isochrysis galbana*; Ch=*Chaetoceros muelleri*; T-Iso=*Isochrysis galbana* (T-Iso)). (Experiment 3)

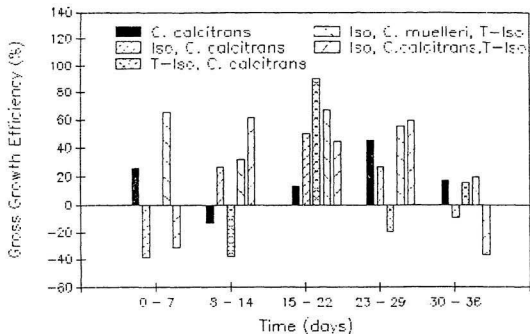


Figure 16. *Placopecten magellanicus*. Gross growth efficiencies (K1) of spat (2 - 4 mm) reared on various diets batch fed at 50 cells/ $\mu$ l. (Iso=*Isochrysis galbana*; T-Iso=*Isochrysis galbana* (T-Iso); C.muelleri=*Chaetoceros muelleri*; C.calcitrans=*Chaetoceros calcitrans*). (Experiment 4)

variation in published filtration rates for different bivalves can be noted in Table 18.

The appropriate inorganic and organic weight determinations for duplicate subsamples of animals from each tank are found in Table A-11. Scallops sized 4 - 6 mm and 6+ mm could be shucked so individual tissue and shell organic weights could be determined. In scallops sized 2 - 4 mm, total organic weights were determined.

The specific filtration and ingestion rates for each of the three size classes of scallops (2 - 4, 4 - 6 and >6 mm) at the three food rations studied (20, 40 and 80 cells/ $\mu$ l) are given in Table 19.

One of the replicates from treatment 7 was eliminated due to an operational problem and subsequently removed from the data set and ignored for the purposes of comparing effects of ration on filtration/ingestion rates.

Specific filtration rates, expressed as filtration rate per unit body weight, were determined using tissue organic weights. Such measurements are useful in comparing filtration rates at varying food rations in that the size variable is removed from the comparison.

Figure 17 depicts the specific filtration rates for the three size classes of scallops at varying rations. There were significant decreases in filtration rate with



Table 17. Mean filtration and ingestion rates for three size classes of scallops (2-4, 4-6 and >6 mm) at three food rations (20, 40 and 80 cells/ $\mu$ l).

Treat- ment	# animals	Size (mm)	Food conc. (cells/ $\mu$ l)	Filtration rate $\pm$ sd (ml/h)	Ingestion rate $\pm$ sd (cells $\times 10^3$ /h)
1	72	2 - 4	20	3.56 $\pm$ 2.80	83 $\pm$ 66
1	72	2 - 4	20	3.55 $\pm$ 3.05	84 $\pm$ 70
1	72	2 - 4	20	2.18 $\pm$ 1.88	53 $\pm$ 45
2	144	2 - 4	40	2.11 $\pm$ 0.44	101 $\pm$ 22
2	144	2 - 4	40	1.98 $\pm$ 0.52	95 $\pm$ 25
2	144	2 - 4	40	2.41 $\pm$ 0.67	114 $\pm$ 32
3	288	2 - 4	80	2.54 $\pm$ 2.01	206 $\pm$ 132
3	288	2 - 4	80	2.26 $\pm$ 1.68	184 $\pm$ 106
3	288	2 - 4	80	1.88 $\pm$ 1.26	161 $\pm$ 87
4	20	4 - 6	20	3.62 $\pm$ 6.00	93 $\pm$ 154
4	20	4 - 6	20	7.93 $\pm$ 5.31	193 $\pm$ 130
4	20	4 - 6	20	3.87 $\pm$ 9.83	97 $\pm$ 248
5	40	4 - 6	40	3.52 $\pm$ 2.57	167 $\pm$ 119
5	40	4 - 6	40	4.45 $\pm$ 2.58	214 $\pm$ 125
5	40	4 - 6	40	3.49 $\pm$ 3.71	170 $\pm$ 183
6	80	4 - 6	80	2.17 $\pm$ 0.94	210 $\pm$ 184
6	80	4 - 6	80	1.61 $\pm$ 0.65	156 $\pm$ 63
6	80	4 - 6	80	1.59 $\pm$ 0.61	160 $\pm$ 62
7	12	> 6	20	22.81 $\pm$ 7.35	537 $\pm$ 184
7	12	> 6	20	13.39 $\pm$ 5.63	317 $\pm$ 132
8	24	> 6	40	16.35 $\pm$ 10.34	735 $\pm$ 421
8	24	> 6	40	11.75 $\pm$ 5.75	549 $\pm$ 254
8	24	> 6	40	12.56 $\pm$ 5.52	587 $\pm$ 244
9	48	> 6	80	21.56 $\pm$ 9.33	1692 $\pm$ 539
9	48	> 6	80	12.04 $\pm$ 4.11	1051 $\pm$ 324
9	48	> 6	80	13.46 $\pm$ 6.85	1136 $\pm$ 459
10	0	-	20	(Control)	
11	0	-	40	..	
12	0	-	80	..	

Table 18. Literature values for filtration rates of various bivalve species.

Species	Length (mm)	Temp. (°C)	Rate (ml/h/animal)	Source
<u>Placopecten</u> <u>magellanicus</u>	2-4	7-7.5	1.88 - 3.56	Present Study
<u>P. macellanicus</u>	4-6	7-7.5	1.59 - 7.9	
<u>P. macellanicus</u>	6+	7-7.5	3.80 - 22.81	
<u>Mytilus edulis</u>	8.5	12	34	Winter, 1973
<u>M. edulis</u>	8.5	12	17	Winter, 1973
<u>M. edulis</u>	16.5	12	81	Winter, 1973
<u>M. edulis</u>	21.5	12	165	Winter, 1973
<u>M. edulis</u>	24-39	17	110	Owen, 1974
<u>M. edulis</u>	32	13-14	1500	Owen, 1974
<u>M. edulis</u>	48	12-15	1100	Owen, 1974
<u>Ostrea edulis</u>	19-39	17.5	1700	Owen, 1974
<u>O. edulis</u>	70-86	12-13	100-700	Owen, 1974
<u>Crassostrea</u> <u>angulata</u>	70-90	12-13	200-1680	Owen, 1974
<u>Pecten irradians</u>	38-44	22-26	3260	Owen, 1974
<u>Cardium edule</u>	30-40	17-19	500	Owen, 1974
<u>Venus striatula</u>	21-28	17	40	Owen, 1974
<u>Mya arenaria</u>	57-82	17.5	600-1300	Owen, 1974

## Larval Filtration Rates:

Species	Length (µm)	Temp. (°C)	Rate (ml/hr)	Source
<u>Ostrea edulis</u>	200	20-22	0.0271	Sprung, 1984
<u>O. edulis</u>	180-260	21	0.0125-0.025	Sprung, 1984
<u>Crassostrea gigas</u>	87-151	25	0.0028-0.007	Sprung, 1984
<u>C. gigas</u>	89-151	25	0.0023-0.0935	Sprung, 1984
<u>Mytilus edulis</u>	170-260	18	0.004-0.025	Sprung, 1984
<u>M. edulis</u>	260	16	0.0125	Sprung, 1984
<u>M. edulis</u>	260	11	0.002	Sprung, 1984
<u>M. edulis</u>	120-250	6	0.004-0.021	Sprung, 1984

increasing food ration for each size class studied: 2 - 4 mm ( $r=-0.75$ ); 4 - 6 mm ( $r=-0.88$ ) and 6+ ( $r=-0.72$ ).

Corresponding specific ingestion rates with increasing food rations for the three size classes of scallops studied are given in Figure 18. The 2 - 4 mm scallops showed a significant increase in ingestion rates with increasing rations ( $r=0.84$ ), whereas the 4 - 6 mm scallops did not show a significant increase ( $r=0.09$ ). The 6+ mm scallops, however, did show a significant increase ( $r=0.91$ ) in ingestion rates with increasing rations.

The relationship of filtration rates with organic weights at the three food rations studied is shown in Figure 19. Only the size classes, 4 - 6 mm and 6+ mm, were included in this figure since organic weights could be determined from shucked tissues. Filtration rates increased significantly with organic weight at all food rations: 20 cells/ $\mu$ l ( $r=0.98$ ); 40 cells/ $\mu$ l ( $r=0.99$ ) and 80 cells/ $\mu$ l ( $r=0.97$ ). This indicates that the animals may have been food limited at all rations tested since the filtration rates did not show signs of decline due to clogging of the filtering mechanism due to abnormally high ration.

Figure 20 compares scallop ingestion rates with organic weights. Again, there were significant increases in ingestion rates with increasing organic weight at all three

Table 19. Specific filtration and ingestion rates for three size classes of scallops (2-4, 4-6 and >6 mm) at three food rations (20, 40 and 80 cells/ $\mu$ l). Tissue organic weights used for calculations for 4-6 mm and >6 mm scallops and total organic weights used for 2-4 mm scallops.

Treat- ment	Size (mm)	Food conc. (cells/ $\mu$ l)	Organic weight (mg)	Filtration rate (ml/mg/hr)	Ingestion rate (cells x $10^3$ /mg/hr)
1	2 - 4	20	0.995	3.58	83
1	2 - 4	20	0.870	4.09	97
1	2 - 4	20	0.765	2.85	69
2	2 - 4	40	0.980	2.16	103
2	2 - 4	40	0.865	2.29	110
2	2 - 4	40	1.090	2.22	105
3	2 - 4	80	1.010	2.51	204
3	2 - 4	80	1.460	1.55	126
3	2 - 4	80	1.100	1.71	146
4	4 - 6	20	1.480	2.45	63
4	4 - 6	20	1.855	4.28	104
4	4 - 6	20	1.420	2.72	68
5	4 - 6	40	1.810	1.94	92
5	4 - 6	40	1.780	2.50	120
5	4 - 6	40	1.635	2.13	104
6	4 - 6	80	1.995	1.09	105
6	4 - 6	80	2.125	0.76	73
6	4 - 6	80	1.905	0.84	84
7	>6	20	4.020	5.67	134
7	>6	20	3.140	4.27	101
8	>6	40	4.445	3.68	165
8	>6	40	3.855	3.05	142
8	>6	40	3.725	3.37	158
9	>6	80	5.790	3.72	292
9	>6	80	4.940	2.44	213
9	>6	80	5.065	2.66	224
10	0	-	20	(Control)	
11	0	-	40	..	
12	0	-	80	..	

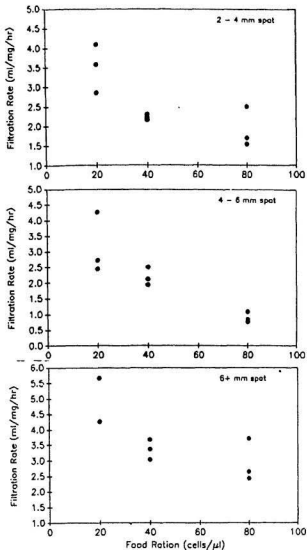


Figure 17. *Placopecten magellanicus*. Specific filtration rate for spat of various size classes when fed varying rations of a mixed algal diet.

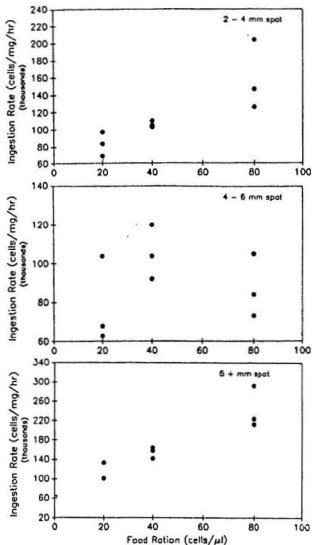


Figure 18. *Placopecten magellanicus*. Specific ingestion rate for spat of various size classes when fed varying rations of a mixed algal diet.

food rations studied: 20 cells/ $\mu$ l ( $r=0.98$ ); 40 cells/ $\mu$ l ( $r=0.99$ ) and 80 cells/ $\mu$ l ( $r=0.99$ ). This may have been due to the possibility that rations of 20, 40 and 80 cells/ $\mu$ l were not high enough to permit maximum ingestion for any of the sizes studied. There is no evidence that a threshold ration had been provided, above which no further increase in ingestion would have been expected.

The values for the parameters of the general allometric equation:  $F=aw^b$  which relates rate of physiological function (F) to growth rate (W) were obtained from the regression equations of the log-log plots of filtration and ingestion as a function of weight shown in Figures 19 and 20. The equations relating filtration to weight for scallops on the three rations were as follows:

Ration	20 cells/ $\mu$ l	$F = 2.18 W^{1.67}$	$r=.98$
..	40 cells/ $\mu$ l	$F = 1.62 W^{1.52}$	$r=.99$
..	80 cells/ $\mu$ l	$F = 0.37 W^{2.24}$	$r=.99$

and for ingestion:

Ration	20 cells/ $\mu$ l	$I = 55.08 W^{1.59}$	$r=.98$
..	40 cells/ $\mu$ l	$I = 81.25 W^{1.47}$	$r=.99$
..	80 cells/ $\mu$ l	$I = 40.78 W^{2.09}$	$r=.99$

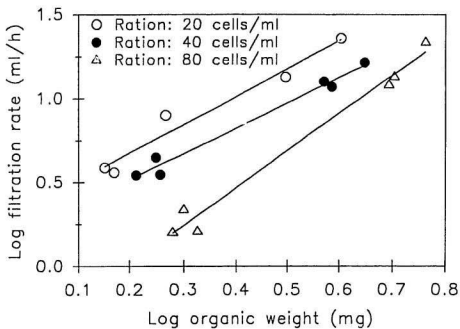


Figure 19. *Placopecten magellanicus*. Filtration rate per animal as a function of tissue organic weight at varying food rations.



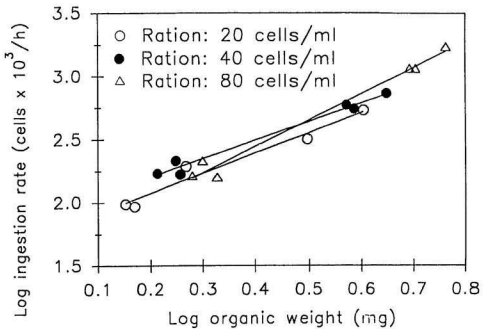


Figure 20. Placopecten magellanicus. Ingestion rate per animal as a function of tissue organic weight at varying food rations.

## IV DISCUSSION

## IV.1. EFFECT OF DIET

Experiments 1, 2 and 4 indicate that a mixed algal diet results in the greatest growth in scallops sized 2 to 4 mm. Such results have been observed by other researchers (Walne, 1974; Romberger and Epifanio, 1981; Enright et al., 1986; Davis and Guillard, 1958). The mixed diets studied in the present experiments consisted of combinations of Isochrysis galbana, Isochrysis aff. galbana (T-Iso) and either Chaetoceros muelleri or Chaetoceros calcitrans. The success of the mixed algal diets is expected considering that bivalves have specific nutritional requirements in order to maintain their metabolic functions and to grow. These conditions are more easily satisfied by a variety of algal species in the diet. Furthermore, a mixed algal diet would mask or dilute toxic metabolites produced by any one of the constituent algal species. For example, as Webb and Chu (1982) point out, such chlorophyte species as Chlorella sp., Chlamydomonas sp., Stichococcus sp. and one chrysophyte Prymesium parvum have been found to be toxic to oyster and clam larvae. However, Davis (1953) found that a mass culture of green phytoplankton consisting mainly of Chlorella sp. was good food for oyster larvae once they were larger than approximately 125 $\mu$ .

It may be expected that the diet which supplied the most organic weight to the animal would result in the greatest growth, but this has not been borne out in these experiments. The diet of T-Iso had the highest organic weight of the diets, with the ternary mixed diet ranking fourth. Therefore, the quantity of organic weight supplied is not directly correlated with the growth produced.

All the scallops used in these four experiments were juveniles. It has been observed (Holland, 1978) that bivalves require different amounts of organic nutrients at different stages of the life cycle. Bivalves 3 to 5 months old accumulate more glycogen than lipid (Holland and Hannant, 1974) whereas bivalves younger than this have more lipid reserves. This may partially explain why, in Experiment 3, scallops sized 1 - 2 mm seemed to grow as well on the unialgal Isochrysis galbana diet as on the ternary mixed diet. Both species of Isochrysis galbana and Isochrysis aff. galbana (T-Iso) have higher lipid and total nitrogen levels, while diatoms have higher levels of carbohydrates and mono-oligosaccharides (Whyte, 1987). The animals used in Experiment 3 were only 76 days old, whereas in Experiments 1, 2 and 4, the scallops were 230, 347 and 255 days old, respectively. The scallops in Experiment 3 may have been in the transition period between principally storing lipid or glycogen, and therefore showed good growth

for the following four diets: (1) the unialgal Isochrysis galbana diet; (2) the binary Isochrysis galbana/ Chaetoceros muelleri diet; (3) the binary Isochrysis galbana/ T-Iso diet and (4) the ternary mixed diet. This trend was noted in all the growth parameters examined in Experiment 3.

Isochrysis galbana was found to be more important than T-iso in the mixed diets (Whyte, 1987) although T-Iso was found to have the highest total energy from constituents, followed by Isochrysis galbana. In comparison, although both species had similar amounts of carotenoids and mono-oligosaccharides, Isochrysis galbana had slightly lower lipid levels and much lower polysaccharide levels.

The older scallops, sized 2 - 4 mm, in Experiments 1, 2 and 4 all showed increased growth when fed the ternary diets and, as can be seen in Experiment 4, both Chaetoceros muelleri and Chaetoceros calcitrans are satisfactory as diatom species. Although diatoms in the food are important as a source of carbohydrate for these larger scallops, the two phytoflagellates are important additions to the diet in that they provide high levels of energy from their constituents.

Both Isochrysis species are also important sources of lipids which are thought to have a direct relationship to growth in several bivalve species. Wikfors et al. (1984) found that the greater lipid content of Tetraselmis maculata

compared to Dunaliella tertiolecta, was correlated with greater growth for juvenile Crassostrea virginica . Furthermore, it was found that when both algal species were cultured in such a way as to produce a higher carbohydrate content, there was greater growth of the juvenile oysters. Enright et al. (1986) also suggest that once the caloric requirements of juvenile Ostrea edulis are met, the amounts of fatty acids, particularly the 22:6n3 variety, are associated with increased growth.

Laing et al. (1987) also point out the importance of the polyunsaturated fatty acids (PUFA), particularly eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3), in supporting good growth in clams. Their studies have shown that T-Iso, which has a high 22:6n3 content, and Skeletonema costatum, Chaetoceros calcitrans, and Thalassiosira pseudonana, which have a high 20:5n3 content, are all good foods for the clams. Although Phaeodactylum tricorputum has both 20:5n3 and 22:6n3 fatty acids, it is thought to be indigestible for many bivalves. It was pointed out that diatom diets are more difficult to digest and assimilate, resulting in less lipid in reserve, especially at higher temperatures.

Ukeles and Wikfors (1988), in comparing the food value to juvenile Crassostrea virginica of microalgae grown in the absence of vitamins, found that the amounts of carbohydrate

and lipid present in the algal species are directly related to the growth of the juvenile oysters. Again, the differences in levels of polyunsaturated fatty acids, especially 20:5n3 and 22:6n3 are suggested as possible reasons for the differing food values.

A comparison of the results of experiments 1 - 4 (Table A-9) indicates that there were differences in growth rates for similar sized animals among the experiments. To some extent these differences may be explained by the difficulties in experimenting with animals in situations where optimal conditions for growth are not known and experiments are subject to the availability of animals. The higher stocking densities of experiments 3 and 4 may account for the lower growth rates, although scallops of this size in the hatchery were routinely grown in 1000l tanks at densities of 125 scallops/l. Differences in growth rates in experiment 1 and 2 may be due to the age of the scallops.

It is possible that the volume of the tank influences growth, possibly due to the surface area to volume ratio and the possibility of biofouling on surfaces releasing inhibitory metabolites. The experimental tanks used in experiments 1 and 2 were of 60l volume whereas those in experiments 3 and 4 were 10l. The improved replication of treatments in experiments 3 and 4 was offset by the smaller tank volume.

These experiments however, demonstrate that the algal diet fed to juvenile scallops does influence long-term growth. Possible reasons for this include the presence of an algal cell wall, digestibility of the cells, cell size, chemical composition, and presence of toxic metabolites.

The presence of a rigid cell wall does not satisfactorily explain differences in food value (Walne, 1970). The 2 flagellate species used, Isochrysis galbana and Isochrysis galbana (T-Iso) have thin cellulose scales, whereas the diatom species used, Chaetoceros muelleri and Chaetoceros calcitrans, have rigid cell walls. If the presence or absence of a cell wall alone accounts for the success of a diet as a food, one would expect diets consisting solely of Isochrysis galbana and/or Isochrysis galbana (T-Iso) to result in greater growth of the scallops. However, this is not the case, as can be seen in Experiments 1 - 3. Furthermore, size of the cell alone cannot explain all the results obtained. Different bivalve species are able to retain different sizes of particles with different efficiencies depending on the filtering apparatus of the animal. Many species have been shown to retain particles above 3  $\mu\text{m}$  (Riisgård et al., 1980; Sprung, 1984; Haven and Morales-Alamo, 1970). Since the four algal species studied in these experiments all have diameters greater than 4  $\mu\text{m}$ , and are similar in size (Table 1), it can be assumed that

all can be retained with similar efficiency by P. magellanicus juveniles. Therefore, this factor cannot account for differences observed between the different diets.

The importance of artificial diets on the growth of bivalves has been viewed as a possible means of reducing the costs of growing food in a hatchery. Laing (1987) studied the food value of artificial diets for juvenile hatchery-produced oysters (Ostrea edulis, Crassostrea gigas and Crassostrea virginica) and clams (Tapes semidecussata and Mercenaria mercenaria). It was found that the artificial diet resulted in satisfactory growth of the spat when supplemented with 15% by weight of algae (Chaetoceros calcitrans in this case) for clams or 40% for oysters. On the other hand, Castell and Trider (1974) found that artificial diets did not support good growth in juvenile Chaetoceros virginica, oysters fed a natural diet showing 10-fold faster growth than those fed the artificial diets.

As can be seen in Experiment 4, the ALGAL 161 performed very poorly as a food for juvenile P. magellanicus. ALGAL 161 consists solely of spray dried heterotrophically grown Tetraselmis suecica, and although it has not been tested on the sea scallop, supported good growth in broodstock and spat for a number of bivalves, particularly oysters. In studying the biochemistry of certain live algal species,



Whyte (1987) found that T. suecica ranked lower than Chaetoceros calcitrans in energy from constituents, and of the six algal species studied showed the lowest levels of lipids and mono-oligosaccharides. It seems that ALGAL 161 is not a suitable diet for juvenile scallops, and even a ratio of 20% Isochrysis galbana to 80% ALGAL 161 results in extremely poor growth in scallops.

#### IV.2. GROSS GROWTH EFFICIENCIES

"Growth efficiencies" are important when considering physiological energetics of molluscs. Generally, high growth efficiencies can be maintained up to a critical body size, after which efficiency decreases with increasing body size, regardless of the diet fed (Jorgensen, 1976). Therefore, it may be expected that growth efficiencies would decline in these four experiments as time progressed. This trend is observed somewhat in Experiments 1 and 2 (Figures 14 and 15), although it is not as clear in the other experiments. However, Jorgensen (1976) found that growth efficiencies did not begin to decline for M. edulis juveniles until body weights of greater than 100 mg dry weight were reached.

Jorgensen (1976) suggests that it is not the growth efficiency obtained at a particular time that determines the size the bivalve will reach, but the amount of time high

growth efficiencies can be maintained.

There does not appear to be any clear trend in the K1 values observed. In several cases, the K1 values for the first time period were lower than those in the second time period. This is possibly due to a period of adjustment for the juveniles. They were taken from 800-l tanks, sampled and placed in the experimental set-up. This may have stressed the animals and affected their initial feeding.

In an environment with favourable growth conditions, shell height, dry weight and organic weight would be expected to rise continuously over time. Decreases in shell height over time probably indicate the effects of small sample size, since it is unlikely that shell size actually decreases. Decreases in dry and organic weight may indicate (in addition to the effects of sample size) weight loss due to shell secretion or to stressed conditions causing metabolism of stored compounds. Loss of organic weight would be manifested by a negative growth efficiency.

The estimation of growth efficiency requires an accurate measurement of ingestion. It is assumed in this study that the ration of 50 cells /ul is below that at which pseudofaeces production would occur. If algal quality is poor (which may happen occasionally in long term growth experiments) or if pseudofaeces are produced and resuspended by the bubbling in the experiments, then

estimates of ingestion are likely to be inaccurate.

In this study, executed using protocols which would normally be outside the scope of a hatchery manager, estimates of gross growth efficiency provided little useful insight into how the scallops were responding to diet, and hence it is premature to advocate that growth efficiencies should be measured in the operation of a hatchery for this species.

#### IV.3. EFFECT OF RATION ON FILTRATION AND INGESTION RATES

This experiment was of short duration, 30 hours, at stocking densities between 7 and 28 scallops per litre. Due to the availability of animals, the size classes selected (2 - 4 mm, 4 - 6 mm and >6 mm) are not necessarily very distinct. Weight specific rates were calculated to better represent the groups.

Regarding the effect of ration on three size classes of scallops, there is a great variation in the filtration and ingestion rates under identical conditions and treatments. This is expected due to a wide variation in filtering rates by individual animals. Such trends were also observed by Schulte (1975) and Winter (1973).

In general, filtration rates decrease with increasing food rations whereas the total amount of food filtered increases. Winter (1973) suggests that Mytilus edulis of a

given size filter out approximately the same amount of algal at high or low concentrations, with lower concentrations being compensated for by higher filtration rates.

In the present study, the specific filtration rates generally decreased with increasing food ration, and specific ingestion rates increased with increasing food ration which is consistent with the observations of Schulte (1975). Furthermore, Schulte (1975) reported that although absolute cell numbers removed increased, the percentage of cells available which were removed decreased with increasing algal concentrations. Owen (1974) suggested that this decrease in filtering activity at high algal concentrations is often due to overloading of the sorting and filtering mechanisms of the gill.

With respect to filtration rates and body size of the animal, Winter (1973) demonstrated how filtration rates and the amount of algae filtered increases with increasing body size. Regarding the filtration rates per animal in the three size classes studied in this study, the filtration rates increase as size increases, as does the ingestion rate.

Winter (1973) and Owen (1974) report that specific filtration rates decrease with increasing body size. However, in the present study with very young juvenile animals, specific filtration rates increased with increasing

organic weight of the tissues. This may be due to the metamorphosis of the developing gills from the larval velum. Benninger (1993) has reported that the gills of juvenile Pecten maximus increase efficiency at capturing particles as they develop during the post-larval phase where shell growth proceeds from 0.3mm to 6mm, the same general size range as reported in this study.

Filtration rates decrease with increasing food cell concentrations (Winter, 1978; Owen, 1974; Schulte, 1975; Foster-Smith, 1975). Ingestion rates increase with increasing particle concentration to a maximum value above which a further increase in particle concentration only serves to block the filtering mechanism. The threshold concentration has been reported at approximately 2 mg dry weight algae per litre (Palmer and Williams, 1980). A particle load of 2 mg/l corresponds to 60 - 100 cells / $\mu$ l for algae of 30 - 20 pg/cell respectively. The range of ration in this experiment, 20 - 80 cells/ $\mu$ l, is thus at or below the critical threshold, hence no decreases in ingestion due to increased particle load were observed. In terms of hatchery management, it appears that young scallop spat should be fed at least at an algal concentration of 80 cells / $\mu$ l.

There have been several studies with scallops relating filtration rate to body size using the allometric

relationship (for references see Bricelj and Shumway, 1991). The resulting weight exponents 'b' range from 0.606 to 0.943 for scallops ranging in weight between 0.05 - 7.0g flesh dry weight, compared to this study in which the weight exponent ranged from 1.52 - 2.24 for scallops 0.006 - 0.009 g flesh dry weight. The weight difference may explain the high values for the exponents, the hypothesis being that post larvae, spat or young juveniles have much higher weight specific physiological rates than adults of various sizes. Weight exponents greater than unity imply that the geometric rate of increase is greater for the physiological rate (F) than for weight (W) (Simpson et al. 1960). In this study, the fact that the weight exponents were greater than unity may be explained by the increasing efficiency of the developing gills of scallops over the size range studied as referenced above (Beninger et al. 1993).

The high values for the exponents in this study may also be due to the narrow size range used or to the small sample size.

The value of the allometric equation in terms of hatchery management is that it permits the predictive calculation of feeding rates for growing animals.

#### IV.4. SUMMARY

Results of this study indicate that scallop spat grow

better when fed mixed algal diets than when fed unialgal diets. In a hatchery situation, the effort of culturing several species of algae is worthwhile.

The results of the feeding rate study indicate that scallop spat can efficiently ingest algal cells from a suspension of 80 cells/ul, and this value may be below the optimum.

In order to have a successful aquaculture industry there must be a reliable source of spat, and often this is not the case with spat collected from the natural environment. This problem could be alleviated by hatchery-produced juveniles, and in order to ensure economic viability, it is necessary that the hatchery produce scallops which are large enough to suspend in the natural environment in the shortest possible amount of time. Studies such as those discussed here are useful in determining optimal diets. However, it is now necessary to further investigate more algal species in terms of their food value for the juvenile P. magellanicus.

Additional research should also be done on the culturing of the algae, and how different culturing methods may increase food value for the juveniles. Although the present study has shown the value of mixed algal diets for growth, more investigation should be done on what particular biochemical requirements are satisfied by the algae and what

particular needs the juveniles have. The presence or absence of toxic metabolites are also important in the effect an algal species has on bivalve growth. Metabolites produced by the algae can either inhibit or stimulate growth. Some of the algal species used in this experiment may have such an effect. Although the ration of 50 cells/ $\mu$ l has been found to be a good value for these juveniles, perhaps the equal ratios of each component species in mixed diets should be altered. This may prove to be more beneficial for growth.

It is interesting that the unialgal Isochrysis galbana diet produced as much growth as the ternary mixed diet for the juveniles sized 1 - 2 mm. This should be investigated further, in that a hatchery may need only feed the smaller juveniles only one species, which would save on costs and time.

The effects of tank size or surface area to volume effect on growth should be studied, particularly in batch culture not involving continuous water flow.

Such research as has been done here and the suggestions for further research will help ensure that a hatchery can produce large yields of healthy P. magellanicus juveniles which will aid in a successful scallop aquaculture industry in Newfoundland.



## IV.5. CONCLUSIONS

1. The ternary diet was shown to be the best in most cases, with the possible exception of the 1 - 2 mm scallops.
2. Chaetoceros calcitrans and C. muelleri were found to be about equal as components of mixed diets. C. calcitrans is harder to grow in bulk culture (requiring lower salinity) thus it is recommended that C. muelleri be used.
3. ALGAL 161 is not a suitable diet.
4. No significant differences in growth efficiencies between the diets observed over the time frame studied were found using Friedman's and Kruskal-Wallis tests.
5. Growth efficiencies may not be a useful management tool for shellfish hatcheries.
6. This research demonstrated that 40 - 50 day experiments were required to show significant growth differences related to the diet of juvenile scallops.
7. Filtration rates decrease with increasing food ration whereas ingestion rates (number of cells cleared from suspension) increased as food ration increased.
8. Filtration and ingestion rates increase with increasing body size in young juvenile scallops.
9. Specific filtration rates increased with increasing body size.
10. Maximum ingestion was observed from algal suspensions of 80 cells/ $\mu$ l.

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## APPENDIX

Table A-1 : Results for growth parameters of juvenile *P. macellanicus* in Experiment 1. Sample size was 30 scallops.

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
<b>TREATMENT 1</b>							
0	1	3.46		1.68		0.32	
14	1	3.52	3.53±.29	1.92	1.98±.39	0.39	.39±.06
	2	3.25		1.63		0.32	
	3	3.83		2.40		0.45	
28	1	4.09	3.99±.39	2.62	2.52±.67	0.48	.47±.13
	2	3.56		1.80		0.34	
	3	4.32		3.13		0.59	
42	1	4.20	4.31±.20	2.97	3.04±.38	0.39	.48±.10
	2	4.18		2.70		0.46	
	3	4.54		3.45		0.59	
56	1	4.06	4.21±.50	2.69	3.03±.96	0.46	.52±.16
	2	3.81		2.28		0.40	
	3	4.77		4.11		0.70	
<b>TREATMENT 2</b>							
0	1	3.46		1.68		0.32	
14	1	3.53	3.46±.26	2.11	1.95±.45	0.42	.39±.08
	2	3.68		2.30		0.46	
	3	3.17		1.45		0.30	
28	1	3.44	3.74±.07	1.97	1.93±.22	0.33	.33±.04
	2	3.38		1.70		0.30	
	3	3.47		2.13		0.37	
42	1	3.52	3.79±.12	1.87	2.11±.25	0.24	.29±.05
	2	3.25		2.10		0.29	
	3	3.47		2.36		0.34	
56	1	3.59	3.45±.07	1.38	1.48±.16	0.18	.19±.02
	2	3.27		1.40		0.18	

Table A-1: continued

Day	Sample	Shell Height (mm/scal)	Mean $\pm$ sd	Dry Weight (mg/scal)	Mean $\pm$ sd	Organic Weight (mg/scal)	Mean $\pm$ sd
<b>TREATMENT 3</b>							
	3	3.76		1.66		0.22	
0	1	3.46		1.68		0.32	
14	1	3.44	3.43 $\pm$ .04	1.80	1.81 $\pm$ .06	0.34	.35 $\pm$ .03
	2	3.38		1.75		0.32	
	3	3.47		1.88		0.38	
28	1	3.49	3.57 $\pm$ .14	1.71	1.82 $\pm$ .28	0.30	.32 $\pm$ .06
	2	3.49		1.61		0.28	
	3	3.74		2.14		0.39	
42	1	3.86	3.84 $\pm$ .11	2.24	2.23 $\pm$ .05	0.35	.34 $\pm$ .03
	2	3.73		2.17		0.36	
	3	3.94		2.28		0.31	
56	1	3.76	3.75 $\pm$ .01	2.20	2.14 $\pm$ .12	0.28	.28 $\pm$ .02
	2	3.73		2.23		0.30	
	3	3.75		2.01		0.27	
<b>TREATMENT 4</b>							
0	1	3.46		1.68		0.32	
14	1	3.52	3.41 $\pm$ .14	2.01	1.84 $\pm$ .19	0.45	.41 $\pm$ .04
	2	3.25		1.64		0.37	
	3	3.47		1.87		0.41	
28	1	4.48	4.45 $\pm$ .03	3.23	3.27 $\pm$ .05	0.66	.65 $\pm$ .02
	2	4.42		3.32		0.66	
	3	4.46		3.26		0.62	
42	1	4.84	4.77 $\pm$ .14	4.66	4.46 $\pm$ .50	0.88	.83 $\pm$ .08
	2	4.86		4.82		0.87	
	3	4.61		3.89		0.74	
56	1	4.60	4.79 $\pm$ .20	4.22	4.73 $\pm$ .45	0.69	.80 $\pm$ .10
	2	4.99		5.08		0.88	
	3	4.77		4.88		0.84	

Table A-1: continued

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
<b>TREATMENT 5</b>							
0	1	3.46		1.68		0.32	
14	1	3.59	3.54±.25	2.02	2.00±.30	0.44	.41±.05
	2	3.27		1.69		0.35	
	3	3.76		2.29		0.43	
28	1	4.12	4.34±.28	2.75	3.20±.65	0.57	.69±.15
	2	4.24		2.90		0.64	
	3	4.65		3.94		0.86	
42	1	5.15	4.98±.18	5.53	5.27±.56	1.10	1.09±.14
	2	4.79		4.62		0.95	
	3	5.00		5.65		1.23	
56	1	5.08	5.28±.18	6.45	7.25±.85	1.24	1.46±.19
	2	5.44		7.15		1.51	
	3	5.32		8.14		1.62	
<b>TREATMENT 6</b>							
0	1	3.46	3.46±0	1.68	1.68±0	0.32	.32±0
	2	3.46		1.68		0.32	
	3	3.46		1.68		0.32	
14	1	3.78	3.76±.10	2.43	2.51±.20	0.51	.50±.07
	2	3.85		2.74		0.57	
	3	3.65		2.36		0.43	
28	1	4.89	4.75±.13	4.65	4.26±.34	1.04	.96±.07
	2	4.63		4.10		0.94	
	3	4.72		4.04		0.90	
42	1	6.04	5.81±.20	9.62	8.39±1.12	2.00	1.65±.35
	2	5.71		7.42		1.29	
	3	5.67		8.12		1.67	
56	1	6.67	6.61±.23	12.24	12.0±1.16	1.63	1.73±.25
	2	6.36		10.93		1.54	
	3	6.80		13.24		2.01	

Table A-2. Ingestion values for P. magellanicus juveniles during the course of Experiment 1. Ingestion values based on cell counts using a Coulter Counter.

Time Period (days)	Treat- ment	Ingestion (#cells/ scal/2, wk) (x 10 <sup>7</sup> )	Ingestion (mg/scallop/2 wk)
0 - 14	1	1.571	.3109
15 - 28		2.737	.5416
29 - 42		2.103	.4162
43 - 56		3.582	.7089
0 - 14	2	1.033	.1712
15 - 28		4.122	.6832
29 - 42		1.018	.1687
43 - 56		2.071	.3433
0 - 14	3	1.710	.4468
15 - 28		2.599	.6791
29 - 42		1.737	.4539
43 - 56		6.957	1.818
0 - 14	4	1.564	.2843
15 - 28		2.838	.5159
29 - 42		3.129	.5688
43 - 56		5.598	1.018
0 - 14	5	1.909	.4076
15 - 28		4.531	.9674
29 - 42		5.618	1.199
43 - 56		9.211	1.966
0 - 14	6	2.225	.4635
15 - 28		5.791	1.206
29 - 42		8.133	1.694
43 - 56		1.233	2.568

Table A-3: Results for growth parameters of juvenile *P. magellanicus* in Experiment 2. Sample size was 50 scallops.

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
<b>TREATMENT 1</b>							
0	1	3.78		2.14		0.32	
14	1	3.66	3.90±.21	2.04	2.38±.30	0.32	0.31±.07
	2	3.99		2.52		0.24	
	3	4.06		2.59		0.38	
28	1	4.10	4.04±.49	2.80	2.62±.16	0.45	0.38±.06
	2	4.01		2.54		0.38	
	3	4.02		2.52		0.32	
42	1	4.60	4.47±.41	3.44	3.25±.87	0.35	0.32±.30
	2	4.79		4.00		0.60	
	3	4.01		2.30		0.68	
56	1	4.81	4.65±.37	4.53	4.10±.88	0.73	0.68±.07
	2	4.23		3.08		0.60	
	3	4.91		4.68		0.72	
<b>TREATMENT 2</b>							
0	1	3.78		2.14		0.32	
14	1	4.19	4.05±.21	2.95	2.65±.33	0.48	0.40±.07
	2	4.16		2.70		0.36	
	3	3.81		2.30		0.37	
28	1	4.23	4.15±.13	3.08	2.89±.34	0.43	0.43±.03
	2	4.21		3.09		0.46	
	3	4.00		2.49		0.39	
42	1	4.09	4.34±.21	2.37	2.77±.35	1.15	0.61±.47
	2	4.48		3.01		0.29	
	3	4.44		2.93		0.39	
56	1	3.92	4.16±.21	2.30	2.62±.28	0.26	.20±.04
	2	4.22		2.71		0.27	
	3	4.33		2.84		0.34	

Table A-3: continued

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
<b>TREATMENT 3</b>							
0	1	3.78		2.14		0.32	
14	1	3.82	3.92±.09	2.36	2.45±.10	0.43	.34±.20
	2	3.95		2.56		0.11	
	3	4.00		2.44		0.48	
28	1	4.12	4.13±.06	2.97	2.87±.16	0.53	.46±.06
	2	4.08		2.69		0.45	
	3	4.19		2.95		0.40	
42	1	4.18	4.42±.26	2.98	3.19±.26	0.43	.45±.02
	2	4.69		3.48		0.47	
	3	4.39		3.11		0.45	
56	1	4.12	4.10±.09	2.84	2.77±.28	0.41	.36±.04
	2	4.00		2.47		0.34	
	3	4.17		3.01		0.34	
<b>TREATMENT 4</b>							
0	1	3.78		2.14		0.32	
14	1	3.85	4.01±.15	2.39	2.68±.28	0.40	.47±.06
	2	4.02		2.71		0.50	
	3	4.16		2.94		0.52	
28	1	4.56	4.53±.30	3.60	3.62±.77	0.56	.49±.06
	2	4.21		2.86		0.45	
	3	4.81		4.40		0.46	
42	1	4.30	4.53±.21	2.87	3.25±.33	0.34	.46±.10
	2	4.70		3.44		0.53	
	3	4.59		3.45		0.50	
56	1	4.93	5.13±.21	5.23	6.29±1.06	0.99	1.02±.11
	2	5.12		6.29		1.14	
	3	5.35		7.35		0.92	

Table A-3: continued

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
<b>TREATMENT 5</b>							
0	1	3.78		2.14		0.32	
14	1	4.02	4.09±.07	2.50	2.69±.18	0.44	.67±.49
	2	4.08		2.85		0.33	
	3	4.17		2.71		1.23	
28	1	4.54	4.73±.21	3.81	4.18±.55	0.47	.68±.20
	2	4.96		4.81		0.88	
	3	4.69		3.91		0.68	
42	1	5.44	5.44±.22	6.17	6.14±.73	0.96	.94±.03
	2	5.66		6.86		0.95	
	3	5.22		5.39		0.90	
56	1	5.88	5.84±.54	8.65	9.25±2.17	1.27	1.54±.43
	2	6.36		11.66		2.04	
	3	5.29		7.44		1.32	
<b>TREATMENT 6</b>							
0	1	3.78		2.14		0.32	
14	1	4.07	3.95±.18	2.78	2.48±.27	0.35	.37±.02
	2	3.74		2.25		0.36	
	3	4.03		2.40		0.39	
28	1	4.55	4.38±.29	3.64	3.31±.58	0.61	.47±.12
	2	4.05		2.64		0.38	
	3	4.55		3.66		0.42	
42	1	4.78	4.70±.15	4.16	4.10±.34	0.71	.64±.07
	2	4.53		3.74		0.64	
	3	4.80		4.41		0.56	
56	1	5.68	5.40±.34	7.40	6.46±1.18	1.00	1.04±.15
	2	5.50		6.85		1.21	
	3	5.02		5.14		0.91	



Table A-3: continued

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
<b>TREATMENT 7</b>							
0	1	3.78		2.14		0.32	
14	1	4.09	4.12±.08	2.73	2.84±.18	0.25	.41±.19
	2	4.07		2.74		0.37	
	3	4.21		3.15		0.62	
28	1	4.26	4.23±.03	3.28	3.19±.20	0.48	.52±.05
	2	4.24		2.96		0.50	
	3	4.30		3.33		0.57	
42	1	4.39	4.47±.14	3.27	3.44±.51	0.40	.50±.12
	2	4.63		4.01		0.63	
	3	4.39		3.03		0.47	
56	1	4.57	4.41±.28	4.19	3.68±.57	0.66	.61±.06
	2	4.57		3.79		0.64	
	3	4.08		3.07		0.54	

Table A-4. Ingestion values for P. magellanicus juveniles during the course of Experiment 2. Ingestion values based on cell counts using a Coulter Counter.

Time Period (days)	Treat- ment	Ingestion (#cells/ scal/2 <sup>7</sup> wk) (x 10 <sup>7</sup> )	Ingestion (mg/scallop/2wk)
0 - 14	1	1.743	.345
15 - 28		2.585	.512
29 - 42		3.511	.695
43 - 56		4.978	.985
0 - 14	2	1.798	.298
15 - 28		1.479	.245
29 - 42		3.938	.653
43 - 56		4.713	.781
0 - 14	3	2.508	.655
15 - 28		5.309	.139
29 - 42		7.456	1.95
43 - 56		11.00	2.88
0 - 14	4	2.028	.369
15 - 28		2.468	.449
29 - 42		3.245	.590
43 - 56		7.548	1.37
0 - 14	5	2.332	.486
15 - 28		3.788	.789
29 - 42		6.268	1.31
43 - 56		9.532	1.98
0 - 14	6	2.195	.504
15 - 28		3.323	.763
29 - 42		5.500	1.26
43 - 56		9.052	2.08
0 - 14	7	2.033	.434
15 - 28		3.710	.792
29 - 42		4.760	1.02
43 - 56		6.534	1.40

Table A-5: Results for growth parameters of juvenile *P. magellanicus* in Experiment 3. Sample size was 50 scallops.

Day	Sample	Shell Height (mm/scal)	Mean $\pm$ sd	Dry Weight (mg/scal)	Mean $\pm$ sd	Organic Weight (mg/scal)	Mean $\pm$ sd
<b>TREATMENT 1</b>							
0	1			.161		.040	
14	1	1.34	1.35 $\pm$ .01	.249	.230 $\pm$ .024	.061	.060 $\pm$ .007
	2	1.35		.237		.067	
	3	1.36		.203		.053	
29	1	1.73	1.68 $\pm$ .05	.319	.287 $\pm$ .028	.084	.089 $\pm$ .017
	2	1.65		.268		.107	
	3	1.65		.275		.075	
43	1	1.70	1.70 $\pm$ .02	.380	.357 $\pm$ .020	.060	.076 $\pm$ .014
	2	1.72		.350		.082	
	3	1.67		.341		.085	
60	1	1.82	1.75 $\pm$ .06	.466	.409 $\pm$ .050	.111	.111 $\pm$ .013
	2	1.70		.374		.124	
	3	1.73		.388		.099	
<b>TREATMENT 2</b>							
0	1			.161		.040	
14	1	1.36	1.38 $\pm$ .04	.184	.188 $\pm$ .017	.046	.040 $\pm$ .013
	2	1.36		.173		.025	
	3	1.43		.207		.049	
29	1	1.49	1.50 $\pm$ .02	.205	.188 $\pm$ .019	.044	.049 $\pm$ .004
	2	1.49		.183		.051	
	3	1.52		.221		.051	
43	1	1.36	1.39 $\pm$ .02	.187	.260 $\pm$ .110	.028	.039 $\pm$ .009
	2	1.39		.386		.042	
	3	1.41		.206		.046	
60	1	1.34	1.35 $\pm$ .05	.201	.189 $\pm$ .022	.062	.056 $\pm$ .022
	2	1.30		.164		.031	
	3	1.40		.203		.074	

Table A-5: continued

Day Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd	
<b>TREATMENT 3</b>							
0	1		.161		.040		
14	1	1.39	1.41±.02	.220	.229±.007	.060	.059±.003
	2	1.42		.233		.056	
	3	1.43		.233		.062	
29	1	1.60	1.62±.01	.259	.258±.008	.061	.061±.007
	2	1.62		.250		.054	
	3	1.63		.266		.068	
43	1	1.44	1.48±.04	.238	.258±.018	.050	.055±.004
	2	1.49		.268		.055	
	3	1.51		.269		.059	
60	1	1.55	1.52±.07	.252	.257±.026	.055	.061±.015
	2	1.44		.234		.049	
	3	1.57		.286		.078	
<b>TREATMENT 4</b>							
0	1		.161		.040		
14	1	1.48	1.46±.02	.246	.245±.004	.044	.056±.011
	2	1.44		.241		.065	
	3	1.46		.249		.058	
29	1	1.44	1.65±.18	.286	.296±.009	.067	.073±.005
	2	1.77		.304		.077	
	3	1.73		.297		.074	
43	1	1.75	1.74±.01	.374	.357±.019	.088	.085±.007
	2	1.73		.336		.077	
	3	1.74		.362		.090	
60	1	1.69	1.72±.03	.371	.376±.013	.097	.087±.013
	2	1.72		.366		.072	
	3	1.75		.390		.092	
<b>TREATMENT 5</b>							
0	1		.161		.040		

Table A-5: continued

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
14	1	1.52	1.51±.01	.193	.200±.008	.037	.047±.008
	2	1.51		.198		.050	
	3	1.50		.208		.053	
29	1	1.60	1.60±.01	.414	.430±.018	.095	.099±.005
	2	1.61		.449		.105	
	3	1.60		.427		.098	
43	1	1.73	1.74±.03	.366	.374±.017	.123	.098±.023
	2	1.77		.393		.092	
	3	1.71		.363		.078	
60	1	1.66	1.66±0	.406	.393±.011	.089	.084±.005
	2	1.66		.386		.078	
	3	1.66		.388		.084	

## TREATMENT 6

0	1			.161		.040	
14	1	1.48	1.46±.02	.252	.234±.036	.063	.061±.008
	2	1.45		.258		.068	
	3	1.45		.192		.052	
29	1	1.72	1.72±.05	.305	.360±.078	.076	.097±.018
	2	1.78		.327		.109	
	3	1.67		.449		.105	
43	1	1.70	1.69±.10	.358	.357±.014	.078	.095±.021
	2	1.78		.371		.088	
	3	1.58		.343		.119	
60	1	1.72	1.66±.08	.372	.362±.015	.161	.111±.044
	2	1.70		.345		.085	
	3	1.57		.369		.086	

## TREATMENT 7

0	1			.161		.040	
14	1	1.51	1.42±.11	.215	.172±.039	.051	.036±.013
	2	1.45		.159		.025	

Table A-5: continued

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
	3	1.30		.141		.033	
29	1	1.45	1.45±.06	.396	.358±.043	.092	.083±.012
	2	1.51		.368		.087	
	3	1.38		.311		.069	
43	1	1.61	1.54±.09	.323	.286±.036	.108	.077±.028
	2	1.57		.284		.070	
	3	1.43		.252		.054	
60	1	1.55	1.48±.07	.327	.300±.024	.045	.051±.006
	2	1.49		.291		.050	
	3	1.40		.281		.058	
<b>TREATMENT 8</b>							
0	1			.161		.040	
14	1	1.36	1.34±.02	.121	.130±.009	.022	.024±.002
	2	1.33		.138		.026	
	3	1.33		.132		.025	
29	1	1.31	1.33±.01	.231	.238±.012	.035	.040±.005
	2	1.34		.252		.045	
	3	1.33		.232		.039	
43	1	1.30	1.34±.04	.144	.155±.011	.015	.024±.007
	2	1.35		.165		.029	
	3	1.37		.155		.027	
60	1	1.21	1.24±.03	.159	.156±.009	.027	.026±.002
	2	1.27		.163		.027	
	3	1.25		.146		.024	

Table A-6. Ingestion values for *P. magellanicus* juveniles during the course of Experiment 3. Ingestion values based on cell counts using a Coulter Counter.

Time Period (days)	Sample	Ingestion (# cells/ scal/2 wk) (millions)	Mean±sd (# cells/ scal/2 wk) (mg/ scal/2wk)	Mean Ingestion (millions)
<b>TREATMENT 1</b>				
0 - 14	1	3.269	2.726±.532	.054
	2	2.449		
	3	2.461		
15 - 29	1	3.849	3.269±.745	.065
	2	3.405		
	3	2.553		
30 - 43	1	3.492	3.109±.377	.048
	2	2.951		
	3	2.885		
44 - 60	1	3.329	3.025±.374	.033
	2	3.073		
	3	2.674		
<b>TREATMENT 2</b>				
0 - 14	1	1.222	1.269±.048	.020
	2	1.306		
	3	1.278		
15 - 29	1	1.621	1.912±.344	.031
	2	2.228		
	3	1.888		
30 - 43	1	1.627	1.723±.201	.063
	2	1.928		
	3	1.615		
44 - 60	1	1.853	1.703±.492	.062
	2	2.042		
	3	1.213		
<b>TREATMENT 3</b>				
0 - 14	1	2.852	2.707±.295	.071

Table A-6: continued

Time Period (days)	Sample	Ingestion (#cells/ scal/2 wk) (millions)	Mean±sd (# cells/ scal/2 wk) (millions)	Mean Ingestion (mg cells/ scal/2 wk)
	2	2.406		
	3	2.862		
15 - 29	1	3.849	3.131±.951	.082
	2	2.206		
	3	3.338		
30 - 43	1	3.117	2.815±.399	.071
	2	2.427		
	3	2.901		
44 - 60	1	2.884	2.731±.207	.082
	2	2.780		
	3	2.529		
<b>TREATMENT 4</b>				
0 - 14	1	2.297	2.268±.030	.041
	2	2.244		
	3	2.263		
15 - 29	1	3.192	2.910±.397	.052
	2	2.517		
	3	3.022		
30 - 43	1	2.909	2.392±.650	.074
	2	1.773		
	3	2.494		
44 - 60	1	1.988	1.676±.315	.068
	2	1.452		
	3	1.587		
<b>TREATMENT 5</b>				
0 - 14	1	1.930	2.305±.477	.049
	2	2.503		
	3	2.751		
15 - 29	1	3.521	3.682±.171	.076
	2	3.821		
	3	3.703		



Table A-6: continued

Time Period days)	Sample	Ingestion (#cells/ scal/2 wk) (millions)	Mean±sd (# cells/ scal/2 wk) (millions)	Mean Ingestion (mg cells/ scal/2 wk)
30 - 43	1	3.033	3.300±.516	.068
	2	3.040		
	3	3.826		
44 - 60	1	2.901	3.055±.617	.063
	2	2.604		
	3	3.661		
<b>TREATMENT 6</b>				
0 - 14	1	4.411	3.269±1.122	.075
	2	2.763		
	3	2.632		
15 - 29	1	5.007	4.134±.923	.095
	2	4.002		
	3	3.392		
30 - 43	1	3.529	3.024±.429	.069
	2	2.813		
	3	2.729		
44 - 60	1	3.943	2.931±.993	.067
	2	2.383		
	3	2.468		
<b>TREATMENT 7</b>				
0 - 14	1	2.501	1.797±.866	.038
	2	1.908		
	3	0.983		
15 - 29	1	3.759	3.181±.655	.067
	2	3.183		
	3	2.602		
30 - 43	1	2.953	2.968±.373	.063
	2	3.050		
	3	2.647		
44 - 60	1	2.542	3.350±.876	.071
	2	4.086		
	3	3.423		

Table A-7: Results for growth parameters of juvenile *P. magellanicus* in Experiment 4. Sample size was 20 scallops.

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
<b>TREATMENT 1</b>							
0	1	3.87		2.36		0.52	
7	1	3.77	3.85±.16	2.12	2.23±.19	0.43	0.47±.04
	2	3.74		2.12		0.46	
	3	4.03		2.45		0.51	
14	1	4.14	4.17±.16	2.68	2.74±.30	0.51	0.51±.04
	2	4.00		2.48		0.47	
	3	4.37		3.07		0.56	
22	1	3.75	3.93±.21	2.00	2.33±.33	0.34	0.44±.11
	2	3.88		2.33		0.41	
	3	4.16		2.66		0.56	
29	1	3.77	3.95±.17	2.07	2.30±.21	0.33	0.38±.05
	2	3.98		2.34		0.38	
	3	4.10		2.48		0.43	
36	1	3.86	3.83±.17	2.21	2.15±.21	0.35	0.36±.11
	2	3.65		1.91		0.35	
	3	3.99		2.32		0.37	
<b>TREATMENT 2</b>							
0	1	3.87		2.36		0.52	
71	1	3.55	3.91±.34	2.00	2.43±.40	0.41	0.52±10
	2	3.99		2.48		0.52	
	3	4.19		2.80		0.62	
14	1	4.00	3.85±.13	2.62	2.32±.27	0.51	0.46±.05
	2	3.77		2.10		0.40	
	3	3.78		2.23		0.47	
22	1	4.24	4.25±.11	3.00	3.04±.14	0.66	0.66±.01
	2	4.36		3.20		0.67	
	3	4.14		2.93		0.65	
29	1	4.08	4.20±.11	2.89	3.02±.11	0.59	0.62±.03

Table A-7: continued

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
	2	4.21		3.07		0.65	
	3	4.31		3.09		0.62	
36	1	4.06	4.30±.22	2.98	3.51±.47	0.56	0.66±.09
	2	4.34		3.67		0.69	
	3	4.49		3.89		0.73	
<b>TREATMENT 3</b>							
0	1	3.87		2.36		0.52	
7	1	3.72	3.73±.06	2.17	2.21±.16	0.40	0.44±.05
	2	3.67		2.08		0.43	
	3	3.80		2.39		0.50	
14	1	3.63	3.73±.09	2.08	2.16±.07	0.36	0.40±.04
	2	3.74		2.21		0.41	
	3	3.82		2.19		0.43	
22	1	3.70	3.84±.13	2.06	2.19±.12	0.39	0.40±.02
	2	3.87		2.19		0.39	
	3	3.96		2.31		0.43	
29	1	3.87	3.81±.07	2.22	2.37±.35	0.33	0.37±.04
	2	3.74		2.12		0.40	
	3	3.83		2.12		0.39	
36	1	3.76	3.89±.17	2.01	2.37±.38	0.29	0.37±.08
	2	4.09		2.77		0.44	
	3	3.83		2.33		0.39	
<b>TREATMENT 4</b>							
0	1	3.87		2.36		0.52	
7	1	3.81	3.89±.13	2.41	2.55±.12	0.56	0.54±.02
	2	3.82		2.62		0.53	
	3	4.04		2.63		0.53	
14	1	4.10	4.06±.12	2.78	2.63±.15	0.60	0.52±.07
	2	3.92		2.48		0.46	
	3	4.16		2.62		0.51	

Table A-7: continued

Day	Sample	Shell Height (mm/scal)	Mean±sd	Dry Weight (mg/scal)	Mean±sd	Organic Weight (mg/scal)	Mean±sd
22	1	4.40	4.17±.27	3.12	2.97±.23	0.59	0.55±.04
	2	3.87		2.71		0.52	
	3	4.24		3.09		0.54	
29	1	4.72	4.25±.43	4.16	3.33±.75	0.80	0.66±.12
	2	3.88		2.71		0.58	
	3	4.16		3.12		0.60	
36	1	4.84	4.46±.90	4.74	3.74±.90	0.85	0.71±.12
	2	4.05		3.49		0.66	
	3	4.48		3.49		0.66	
<b>TREATMENT 5</b>							
0	1	3.87		2.36		0.52	
7	1	3.77	3.81±.17	2.25	2.28±.15	0.49	0.48±.06
	2	3.67		2.44		0.53	
	3	4.00		2.15		0.41	
14	1	4.04	4.02±.27	2.92	2.73±.24	0.63	0.54±.09
	2	3.74		2.46		0.44	
	3	4.29		2.80		0.55	
22	1	4.39	4.26±.34	3.48	3.35±.29	0.78	0.72±.11
	2	3.87		3.55		0.79	
	3	4.51		3.02		0.60	
29	1	4.31	4.28±.52	3.45	3.92±.43	0.72	0.82±.08
	2	3.74		4.30		0.88	
	3	4.78		4.02		0.85	
36	1	4.45	4.55±.51	4.15	4.23±.31	0.70	0.78±.10
	2	4.09		4.57		0.89	
	3	5.10		3.97		0.76	
<b>TREATMENT 6</b>							
0	1	3.87		2.36		0.52	
7	1	3.67	3.94±.21	2.23	2.64±.31	0.44	0.59±.11
	2	3.91		2.61		0.60	
	3	4.00		2.78		0.62	

Table A-7: continued

Day	Sample	Shell Height (mm/scal)	Mean $\pm$ sd	Dry Weight (mg/scal)	Mean $\pm$ sd	Organic Weight (mg/scal)	Mean $\pm$ sd
	4	4.17		2.95		0.70	
14	1	4.02	4.08 $\pm$ .24	3.06	2.99 $\pm$ .28	0.70	0.67 $\pm$ .05
	2	3.77		2.57		0.60	
	3	4.29		3.14		0.67	
	4	4.24		3.18		0.70	
22	1	4.49	4.42 $\pm$ .14	4.00	3.72 $\pm$ .42	0.93	0.87 $\pm$ .12
	2	4.21		3.27		0.73	
	3	4.51		3.88		0.83	
	4	4.48		3.91		1.01	
29	1	4.63	4.80 $\pm$ .29	4.77	4.82 $\pm$ .76	1.06	1.04 $\pm$ .17
	2	4.57		4.06		0.89	
	3	4.78		4.58		0.95	
	4	5.22		5.87		1.27	
36	1	4.87	4.98 $\pm$ .18	5.47	5.48 $\pm$ .65	1.09	1.12 $\pm$ .11
	2	4.79		4.64		0.98	
	3	5.10		5.59		1.17	
	4	5.18		6.22		1.24	
<b>TREATMENT 7</b>							
0	1	3.87		2.36		0.52	
7	1	3.83	3.70 $\pm$ .18	2.28	2.14 $\pm$ .20	0.52	0.47 $\pm$ .06
	2	3.58		2.00		0.43	
14	1	4.02	4.13 $\pm$ .16	2.86	2.95 $\pm$ .13	0.61	0.63 $\pm$ .03
	2	4.25		3.05		0.65	
22	1	4.65	4.48 $\pm$ .23	4.11	3.81 $\pm$ .42	0.89	0.80 $\pm$ .13
	2	4.32		3.52		0.71	
29	1	5.09	5.02 $\pm$ .10	4.94	4.96 $\pm$ .03	0.99	1.01 $\pm$ .03
	2	4.95		4.99		1.03	
36	1	4.90	4.90 $\pm$ 0	5.02	5.04 $\pm$ .03	0.75	0.86 $\pm$ .15
	2	4.90		5.06		0.97	

Table A-8. Ingestion values for P. magellanicus juveniles during the course of Experiment 4. Ingestion values based on cell counts using a Coulter Counter.

Time Period (days)	Sample	Ingestion (#cells/ml/scal/wk)	Ingestion (mg cells/scal/wk)	Mean±sd (mg cells/scal/wk)
<b>TREATMENT 2</b>				
0 - 7	1	614	.064	.072±.007
	2	690	.072	
	3	750	.079	
8 - 14	1	1492	.157	.157±0
	2	1492	.157	
	3	1495	.157	
15 - 22	1	2124	.223	.223±.001
	2	2148	.225	
	3	2117	.222	
23 - 29	1	1941	.204	.205±.004
	2	1927	.202	
	3	1987	.209	
30 - 36	1	2385	.250	.255±.009
	2	2377	.249	
	3	2534	.266	
<b>TREATMENT 4</b>				
0 - 7	1	682	.103	.079±.021
	2	434	.065	
	3	456	.069	
8 - 14	1	1304	.197	.149±.043
	2	757	.114	
	3	902	.136	
15 - 22	1	1719	.259	.228±.029
	2	1344	.203	
	3	1467	.221	
23 - 29	1	1709	.258	.245±.011
	2	1573	.237	
	3	1589	.240	

Table A-8: continued

Time Period (days)	Sample	Ingestion (#cells/ml/ scal/wk)	Ingestion (mg cells/ scal/wk)	Mean $\pm$ sd (mg cells/ scal/wk)
30 - 36	1	2324	.351	.289 $\pm$ .055
	2	1790	.270	
	3	1630	.246	
<b>TREATMENT 5</b>				
0 - 7	1	675	.123	.104 $\pm$ .056
	2	816	.149	
	3	223	.041	
8 - 14	1	1474	.270	.230 $\pm$ .069
	2	1475	.270	
	3	824	.151	
15 - 22	1	2106	.385	.363 $\pm$ .056
	2	2209	.404	
	3	1633	.299	
23 - 29	1	1959	.358	.375 $\pm$ .028
	2	1967	.360	
	3	2226	.407	
30 - 36	1	2574	.471	.448 $\pm$ .020
	2	2402	.439	
	3	2375	.435	
<b>TREATMENT 6</b>				
0 - 7	1	486	.100	.107 $\pm$ .021
	2	434	.090	
	3	628	.130	
	4	586	.121	
8 - 14	1	1315	.272	.254 $\pm$ .041
	2	1001	.207	
	3	1373	.284	
	4	1241	.257	
15 - 22	1	1572	.325	.300 $\pm$ .031
	2	1284	.266	
	3	1493	.309	
	4	1511	.313	

Table A-8: continued

Time Period (days)	Sample	Ingestion (#cells/ml/ scal/wk)	Ingestion (mg cells/ scal/wk)	Mean $\pm$ sd (mg cells/ scal/wk)
23 - 29	1	1617	.335	.309 $\pm$ .029
	2	1339	.277	
	3	1522	.315	
	4	1463	.303	
30 - 36	1	2379	.065	.404 $\pm$ .077
	2	1685	.349	
	3	1792	.371	
	4	1897	.393	
<b>TREATMENT 7</b>				
0 - 7	1	871	.164	.162 $\pm$ .002
	2	856	.161	
8 - 14	1	1474	.277	.262 $\pm$ .021
	2	1316	.247	
15 - 22	1	2201	.414	.385 $\pm$ .042
	2	1888	.355	
23 - 29	1	1859	.349	.355 $\pm$ .008
	2	1915	.360	
30 - 36	1	2347	.441	.413 $\pm$ .040
	2	2051	.385	



Table A-9. Summary of conditions yielding highest growth rates of *P. magellanicus* spat from experiments 1-4.

	Exp1.			Exp2.			Exp3.			Exp4.		
shell ht. ( $\mu\text{m}/\text{d}$ )	53.0			37.5			5.4			27.8		
dry wt. ( $\text{mg}/\text{d}$ )	0.17			0.13			0.004			0.083		
organic wt. ( $\text{mg}/\text{d}$ )	0.026			0.021			0.001			0.017		
diet	Iso	Ch	TIso	Iso	Ch	TIso	Iso, Iso/TIso	Iso, Iso/TIso	Iso	Ch	TIso	
Stocking density/l	10			10			100			16		
Age (mos)	7			11			3			9		
Temp °C	10			10			7			10		

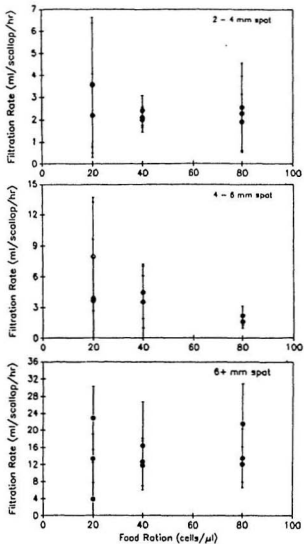


Figure A-1. *Placopecten magellanicus*. Filtration rate per animal for spat of various size classes when fed varying rations of a mixed algal diet.

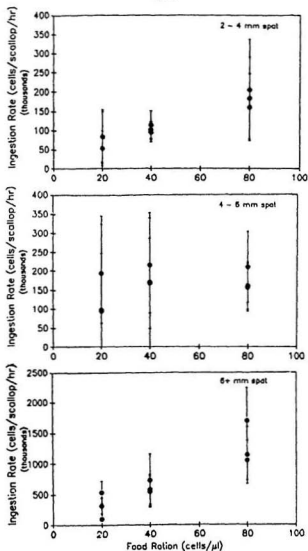


Figure A-2. *Placopecten magellanicus*. Ingestion rate per animal for spat of various size classes when fed varying rations of a mixed algal diet.

Table A-10. Inorganic dry weights and organic dry weights of scallops for each treatment studied (number of animals sampled in brackets following treatment).

Treatment	Inorganic Weights (g)			Organic Weights (g)		
	Shell	Meat	Total Animal	Shell	Meat	Total Animal
1 (20)			.00483			.00089
1 (20)			.00398			.00110
1 (20)			.00431			.00085
1 (20)			.00421			.00089
1 (20)			.00434			.00077
1 (20)			.00440			.00076
2 (25)			.00545			.00101
2 (25)			.00497			.00095
2 (25)			.00413			.00079
2 (25)			.00484			.00094
2 (25)			.00588			.00108
2 (25)			.00576			.00110
3 (25)			.00672			.00117
3 (25)			.00662			.00092
3 (25)			.00561			.00093
3 (25)			.00594			.00127
3 (25)			.00602			.00130
3 (25)			.00490			.00180
3 (25)			.00531			.00127
3 (25)			.00519			.00108
3 (25)			.00468			.00094
4 (10)	.01091	.00026		.00035	.00153	
4 (10)	.01073	.00028		.00061	.00143	
4 (10)	.01117	.00032		.00030	.00181	
4 (10)	.01138	.00030		.00046	.00190	
4 (10)	.00855	.00026		.00031	.00136	
4 (9)	.01038	.00024		.00031	.00148	
5 (20)	.01033	.00027		.00043	.00193	
5 (20)	.00914	.00022		.00038	.00169	
5 (20)	.01058	.00025		.00044	.00194	
5 (20)	.00916	.00025		.00039	.00162	
5 (20)	.00838	.00019		.00036	.00150	
5 (20)	.00991	.00019		.00041	.00177	
6 (25)	.01038	.00036		.00049	.00190	
6 (25)	.01178	.00032		.00058	.00209	
6 (25)	.01098	.00110		.00059	.00263	
6 (25)	.00992	.00030		.00055	.00162	
6 (25)	.01248	.00030		.00053	.00207	
6 (25)	.00977	.00029		.00047	.00174	
7 (10)	.02241	.00102		.00048	.00397	
7 (10)	.02560	.00062		.00073	.00407	

Table A-10 con't

Tank	Inorganic Weights (g)			Organic Weights (g)		
	Shell	Meat	Total Animal	Shell	Meat	Total Animal
7 (10)	.01944	.00051		.00042	.00294	
7 (10)	.01497	.00044		.00273	.00273	
7 (10)	.01924	.00062		.00057	.00307	
7 (9)	.02289	.00047		-.00125	.00321	
8 (12)	.02695	.00085		0	.00490	
8 (12)	.02238	.00058		.00074	.00399	
8 (12)	.02046	.00062		.00056	.00393	
8 (12)	.02169	.00052		.00063	.00378	
8 (12)	.02121	.00054		0	.00367	
8 (12)	.02108	.00045		.00074	.00378	
9 (15)	.03621	.00095		.00122	.00593	
9 (15)	.03192	.00105		.00095	.00565	
9 (15)	.02736	.00081		.00089	.00498	
9 (15)	.02786	.00073		.00098	.00490	
9 (15)	.02957	.00096		.00095	.00540	
9 (15)	.02736	.00076		.00095	.00473	





